Division of Pathology
Department of Laboratory Medicine
Karolinska Institutet
Huddinge University Hospital
SE-146 86 Stockholm, Sweden

In vitro studies on the biosynthesis and reduction of ubiquinone

Tomas Nordman

Stockholm 2003

All previously published papers were reproduced with permission from the publisher.

Published and printed by US-AB
Stockholm, Sweden
© Tomas Nordman, 2003
ISBN 91-7349-475-5

To Veronica
& Tobias
with love

ABSTRACT

This thesis concerns the role of ubiquinone, the only endogenously synthesised lipid soluble antioxidant, in the cellular defence against peroxidation of proteins and lipids.

The aims of the present investigations were to study the biosynthesis of ubiquinone in two different organelle fractions, i.e. microsomes and peroxisomes and to characterise the enzyme reactions of the three flavoenzymes, lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase in the reduction of ubiquinone.

A semipreparative HPLC method was established to rapidly isolate different polyprenols with high purity. The isolated compounds could be used for studies of different enzyme reactions in the mevalonate pathway, and as standards for quantitative HPLC-analysis. Compared to conventional chromatographic methods this new technique was much more rapid and polyprenols with higher purity was isolated.

It was demonstrated that both peroxisomes and microsomes were involved in the biosynthesis of ubiquinone. Two enzymes involved in the synthesis of ubiquinone, *trans*-prenyltransferase and nonaprenyl-4-hydroxybenzoate (NPHB)-transferase, were investigated. The results clearly showed differences in the regulation of the synthesis of ubiquinone in those organelles. The specific activity of *trans*-prenyltransferase in peroxisomes was 30% of the total activity found in both organelles.

The characteristics of the regeneration of ubiquinol by the flavoenzymes, lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase were investigated. These enzymes belong to the same family of enzymes and are defined as homo-dimeric pyridine nucleotide-disulfide oxidoreductases. The reduction of ubiquinone by lipoamide dehydrogenase and glutathione reductase was shown to be highly elevated by addition of zinc to the reaction mixture, whereas this reaction by thioredoxin reductase was inhibited by zinc. For lipoamide dehydrogenase and glutathione reductase the pH optimum for the reaction was found at acidic pH, but at physiological pH for thioredoxin reductase.

The reduction of ubiquinone by thioredoxin reductase was confirmed to be selenium dependent by use of full-length bovine and rat, *E. coli* (lacking selenocysteine), recombinant human (selenocysteine replaced by alanine), and truncated rat thioredoxin reductases, as well as with stable cell lines overexpressing thioredoxin reductase.

Altogether, the novel biological findings in this thesis are that; ubiquinone is not only synthesised in microsomes but also to a high extent in peroxisomes; ubiquinone is efficiently reduced by glutathione reductase and thioredoxin reductase; the reduction of ubiquinone by thioredoxin reductase is entirely selenium dependent.

LIST OF PUBLICATIONS

This thesis is based on the following publications, which will be referred to by their Roman numerals:

I. **Tomas Carlson**, Karolina Skorupinska-Tudek, Jozefina Hertel, Tadeuz Chojnacki, Jerker M Olsson and Ewa Swiezewska. Single polyprenol and dolichol isolation by semipreparative high-performance liquid chromatography technique. *Journal of Lipid Research* 2000, 41: 1177-1180

II. Michael Tekle, Magnus Bentinger, **Tomas Nordman,** Eeva-Liisa Appelkvist, Tadeusz Chojnacki And Jerker M. Olsson. Ubiquinone biosynthesis in rat liver peroxisomes. *Biochem Biophys Res Commun.* 2002, 291 (5): 1128-1133

III. Ling Xia, Mikael Björnstedt, **Tomas Nordman**, Lennart C. Eriksson and Jerker M. Olsson. Reduction of ubiquinone by lipoamide dehydrogenase: an antioxidant regenerating pathway. *European Journal of Biochemistry* 2001, 268: 1486-1490

IV. Ling Xia*, **Tomas Nordman***, Jerker M. Olsson, Anastassios Damdimopoulos, Linda Björkhem-Bergman, Ivan Nalvarte, Lennart C. Eriksson, Elias S.J. Arnér, Giannis Spyrou and Mikael Björnstedt. The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone. A novel mechanism for defense against oxidative stress. *Journal of Biological Chemistry* 2003, 278: 2141-2146

V. **Tomas Nordman**, Mikael Björnstedt and Jerker M. Olsson. Reduction of ubiquinone by glutathione reductase. Submitted for publication, 2003.

* The authors contributed equally to this article.

All papers are reprinted by permission from the copyright owners.

CONTENTS

Abstract……………………………………………………………………..i
List of publications…………………………………………………………ii
Contents……………………………………………………………………iii
List of abbreviations………………………………………………………..iv
1 Introduction 1
  1.1 General introduction 1
  1.2 The mevalonate pathway 5
  1.3 Ubiquinone 9
    1.3.1 Biosynthesis of ubiquinones 11
    1.3.2 Uptake and distribution of ubiquinone 13
    1.3.3 Catabolism of ubiquinone 14
    1.3.4 The functions of ubiquinone 15
    1.3.5 Clinical relevance of ubiquinone 17
  1.4 Regenerating enzymes systems 19
    1.4.1 Lipoamide dehydrogenase 19
    1.4.2 Glutathione reductase 20
    1.4.3 Thioredoxin reductase 21
2 Present investigation 25
  2.1 Aims 25
  2.2 Results 25
    2.2.1 Development of a rapid HPLC method for separation of polyprenols (Paper I) 25
    2.2.2 The biosynthesis of ubiquinone in peroxisomes (paper II) 27
    2.2.3 Regeneration of ubiquinol by flavoenzymes (Paper III, IV and V) 29
    2.2.4 The role of selenium in the reduction of ubiquinone by thioredoxin reductase (paper IV) 31
3 General discussion and future perspectives 32
4 Acknowledgements 35
5 References 37

LIST OF ABBREVIATIONS

DMAPP	Dimethylallyl pyrophosphate
FPP	Farnesyl pyrophosphate
GPP	Geranyl pyrophosphate
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
IPP	Isopentenyl pyrophosphate
LDL	Low density lipoproteins
LipDH	Lipoamide dehydrogenase
Prx	Peroxiredoxin
ROS	Reactive oxygen species
Sec	Selenocysteine
Ser	Serine
SREBP	Sterol regulatory element binding protein
Trx	Thioredoxin
Trx-$(SH)_2$	Reduced thioredoxin
TrxR	Thioredoxin reductase
TrxR2	Mitochondrial TrxR
Trx-S_2	Oxidized thioredoxin
VLDL	Very low density lipoproteins

1 INTRODUCTION

1.1 General introduction

The biomembranes of mammalian cells consists of a hydrophobic lipid bilayer that is able to keep apart the interior of the organelle from the exterior and uphold transmembrane gradients for different kinds of ions, like protons, calcium, sodium and potassium but also of other organic molecules like carbohydrates and proteins. The membrane is not only separating the different environments but actually contains components that build up the gradients. The cellular metabolism, that constitutes cell function, consists of a constant interaction between reactive, polarized molecules that are able to form new combinations and build up new constitutive components of the cell or form and store energy necessary for the metabolism.

Well aware of the necessity for molecular interaction and metabolism of formation and handling of reactive molecules I will, in this theses, deal with the potential hazard for the cell with the reactive intermediates. Any molecule with a reactive center or a free radical may form a covalent binding to a structural part of the cell or to its DNA or RNA and disturb cellular function. In the worst scenario the cell may be injured and collapse. It is obvious that the cell needs systems to protect it self from these oxidative attacks and systems that repair or regenerate damaged components of the cell. The role of ubiquinol for this cellular defence is the topic of this thesis.

Reactive molecules that may cause an oxidative hazard to the cell is constantly formed. Five percent of the oxygen used in cell respiration form oxygen radicals and other metabolic processes like cytochrome P450 catalyse hydroxylations that produces an excess of these reactive oxygen

species (ROS). Oxidative stress is a common term used to describe a state caused by reactive oxygen species. ROS formation, e.g. superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) (Cadenas 1989; Cadenas & Davies 2000; DiGuiseppi & Fridovich 1984), are seen in many different locations i.e. the mitochondrial respiratory chain, reduction by superoxide dismutase (SOD), the Fenton reaction, and reduction by the cytochrome P450 systems (Dröge 2002). ROS are not only harmful for the cell but also crucial for normal metabolism playing an essential role in the biosynthesis of complex organic molecules, detoxification of xenobiotic chemicals, and defence against pathogens such as virus, bacteria or other microorganism (Dröge 2002; Finkel 1998; Kamata & Hirata 1999; Moran et al 2001; Rhee 1999).

Macromolecules such as DNA, proteins, and lipids that are damaged by high oxidative stress may eventually lead to apoptosis or cell death. ROS can affect proteins and lipids by peroxidation (Ernster 1993). Lipid peroxidation is a threat to the integrity of the biomembrane and to the structure and function of the membranous organelle itself. Rupture of lysosomal compartments is a direct threat to the existence of the cell. The lipid peroxidation in cellular membranes is a chain reaction that is self-propagating once initiated by different ROS (Fig. 1).

The target of ROS in the initial step is the unsaturated fatty acids of the phospholipids in the biomembrane. In this first step a carbon centered lipid radical ($L^{\cdot}$) is formed that immediately can react with oxygen and form a lipid peroxyl radical ($LOO^{\cdot}$). In the phase called propagation this lipid peroxyl radical can react with unsaturated fatty acids of other, closely located phospholipids in the membrane.

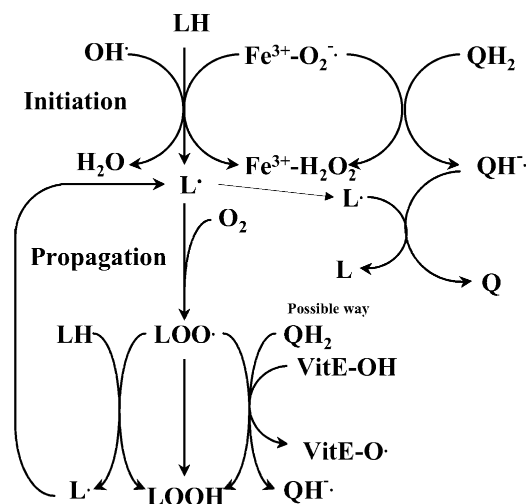

Figure 1. A simplified scheme of the lipid peroxidation. Fe^{3+}-$O_2^{-\cdot}$, perferryl radical; OH·, hydroxyl radical; L·, carbon centred lipid radical; LH, unsaturated fatty acid; LOO·, lipid peroxyl radical; LOOH, lipid hydroperoxide; QH_2, ubiquinol; QH·, semiquinones; Q, ubiquinone; VitE-OH, vitamin E or α-tocopherol; VitE-O·, α-tocopheryl radical.

The final product of each chain reaction of lipid peroxidation is lipid hydroperoxides (LOOH). The breakdown of the lipid membrane may lead to increased fluidity as well as inactivation of membrane proteins and receptors. The permeability of the membrane is also hampered making the maintenance of cellular compartments impossible. Unless the cell can protect itself against these reactions and maintain, an appropriate cellular life would be impossible. It has to balance the formation of radical from different sources by regulation of the defence systems.

Cellular defence aims to trap and neutralise (reduce) the free radicals formed. This can be done by either scavenging of the radicals by molecules that can participate in a redox reaction or by enzymatic conjugation and excretion of the reduced and conjugated molecules. To

protect the cell against the cellular damages caused by lipid peroxidation defence mechanisms scavenging free radicals and lipid hydroperoxides are of great importance. As an example the initiation of the lipid peroxidation was inhibited by the reduced form of ubiquinone (Q), ubiquinol (QH_2) (Fig. 1) (Ernster 1993; Forsmark-Andree et al 1997). Furthermore, the lipid peroxyl radical formed, was in these investigations, trapped by another lipid soluble antioxidant, vitamin E (α-tocopherol) that terminated the propagation phase. It was also proposed that ubiquinol is a scavenger of this radical (Ernster & Dallner 1995). The lipid soluble antioxidant, Vitamin E, is easily taken up via the intestinal system and transported to the liver and from this organ transported in VLDL via the blood to different organs in the body (Herrera & Barbas 2001; Traber 1999). There are today no enzymatic systems known, that can regenerate vitamin E to its reduced form from α-tocopheryl radical, but both vitamin C (ascorbate) and ubiquinol have been shown to efficiently do this (Beyer 1994; Ernster & Dallner 1995). Several factors affect the function and the levels of these lipid soluble antioxidants. One important factor is selenium which has been suggested to have functional connections to vitamin E and ubiquinol since selenium deficiency is associated with lower concentrations of vitamin E and ubiquinol (Chen & Tappel 1995; Scholz et al 1997; Vadhanavikit & Ganther 1993; Vadhanavikit & Ganther 1994).

To maintain a high capacity of this very important antioxidative function of ubiquinol it is required that this molecule is either synthesised in sufficient amounts or that it is regenerated by other enzyme systems.

1.2 The mevalonate pathway

The mevalonate pathway is a sequence of enzyme catalysed events that besides cholesterol, that is the main known lipid product generated in this pathway, also contributes to the production of several other neutral lipids i.e. ubiquinone, dolichol and dolichyl phosphate (Fig. 2). Furthermore, this pathway is also involved in the regulation of features such as signal transduction by isoprenylation of proteins, isopentenylation of tRNA for efficient selenoprotein synthesis, and N-linked glycosylation of proteins (Diamond et al 1996; Faust & Dice 1991; Goldstein & Brown 1990; Schroepfer 1981).

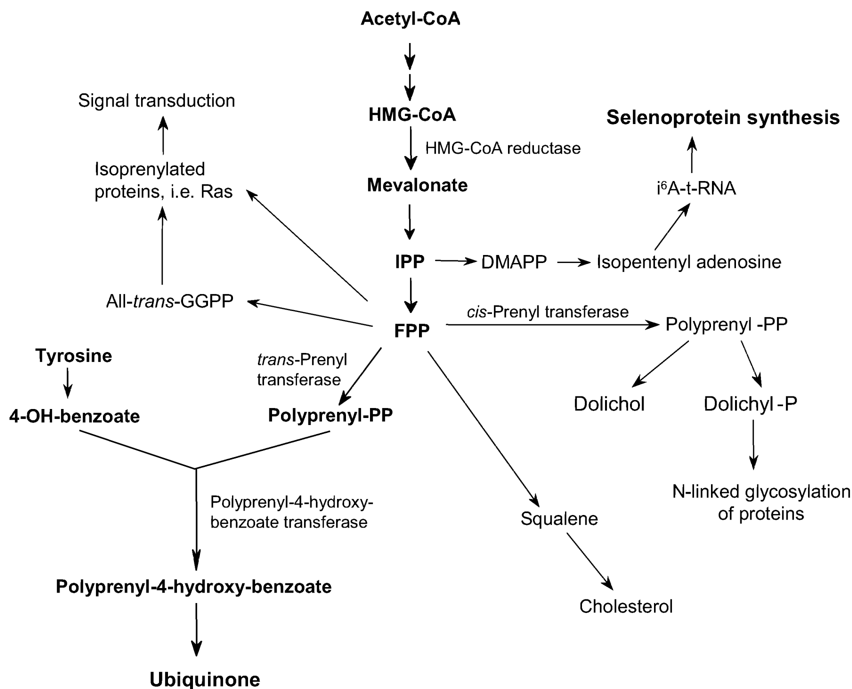

Figure 2. Schematic scheme of the mevalonate pathway.

In the initial part of the mevalonate pathway the formation of the branch-point product, farnesyl pyrophosphate (FPP), is mainly regulated by the enzyme HMG-CoA reductase (Goldstein & Brown 1990). Although, this pathway has been one of the most intensely studied there are still many unsolved questions that further need to be clarified.

The first reaction in this pathway, that was demonstrated in cytosol, was a condensation of two acetyl-CoA catalysed by the enzyme acetoacetyl-CoA thiolase, also accepted as the committed step in the isoprenoid biosynthesis (Schroepfer 1981). Thompson and Krisans demonstrated also that this reaction was catalysed by a peroxisomal thiolase and suggested that this organelle may be involved in the isoprenoid production (Thompson & Krisans 1990).

The next reaction in this pathway is the formation of HMG-CoA catalysed by HMG-CoA synthase, present mainly in cytosol but also in mitochondria (Ayte et al 1990; Clarke et al 1987; Clinkenbeard et al 1975; Goldstein & Brown 1990). HMG-CoA is the substrate for the most studied enzyme in the mevalonate pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase). This enzyme catalyses a two step reduction of HMG-CoA to the formation of mevalonate. The enzyme was primarily found to be located in the endoplasmic reticulum, integrated in the membrane with its catalytic site facing the cytosol but later on also discovered in peroxisomes (Keller et al 1985; Keller et al 1986; Kovacs et al 2001). HMG-CoA reductase is well investigated and is regarded to be the major rate-limiting enzyme in the biosynthesis of cholesterol, highly feedback regulated by its metabolites i.e. oxycholesterols (Goldstein & Brown 1990; Ness & Chambers 2000; Åkerlund & Björkhem 1990). The amounts and the activation of this enzyme are regulated by different

mechanisms e.g. phosphorylation/dephosporylation causing inactivation/activation (Beg et al 1987; Clarke & Hardie 1990), transcriptionally by regulation of the Sterol Regulatory Element Binding Protein (SREBP) (Ericsson & Edwards 1998; Hua et al 1996; Kawabe et al 1994), and by proteolytic degradation (McGee et al 1996; Miller et al 1989; Parker et al 1986). Pharmaceutical drugs called statins are used to efficiently inhibit the enzyme HMG-CoA reductase in order to regulate the high levels of cholesterol in persons with hypercholesterolemia causing atherosclerosis, one of the most common diseases in the western world (Alberts 1988; Vaughan et al 2000). Although, these drugs are lowering the cholesterol levels in blood it may also have other unexpected effects that today is not completely evaluated. Thus, some investigations have shown that the incidence of cardiac incompensation is increased among people on statins. Furthermore, other investigators have reported a lower incidence of colon cancer in patients supplemented with statins (Agarwal et al 2002; Viner et al 2002).

The mevalonate is further phosphorylated in two steps forming the mevalonate-5-pyrophosphate catalysed by mevalonate kinase and phosphomevalonate kinase a reaction that requires both ATP and Mg^{2+} for optimal activity. These enzymes were purified from liver cytosol, but high levels of the mevalonate kinase activity were also demonstrated in peroxisomes (Biardi et al 1994; Stamellos et al 1992; Tanaka et al 1990).

The mevalonate pyrophosphate decarboxylase catalyse the formation of the product isopentenyl pyrophosphate (IPP) that is, the five-carbon compound, known to be the main substrate for FPP synthesis (Alvear et al 1982; Chiew et al 1987)

The enzyme, isopentenyl pyrophosphate isomerase (IPP isomerase) is mainly associated with the cytosol, but later studies have also indicated enzyme activities in mitochondria and peroxisomes (Ericsson et al 1992; Runquist et al 1994). The isomerisation reaction of IPP to dimetylallyl pyrophosphate (DMAPP) requires the presence of either Mg^{2+} or Mn^{2+}. The condensation of DMAPP and one IPP lead to the formation of geranyl pyrophosphate (GPP). A further condensation of one IPP to GPP forms FPP. These reactions are catalysed by farnesyl pyrophosphate synthase (FPP synthase) (Poulter & Rilling 1981). Both GPP and FPP were shown to feedback down regulate the IPP isomerase activity *in vitro* (Rilling & Chayet 1985). Another possibly important function of the synthesis of the DMAPP is the isopentenylation of $tRNA^{Ser/Sec}$ necessary for effective synthesis of selenoproteins i.e. glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) (Diamond et al 1996; Faust et al 1980; Moustafa et al 2001; Warner et al 2000).

1.3 Ubiquinone

Ubiquinone was discovered 1955 by Morton and co-workers (Festenstein et al 1955). By investigation of the physiological and chemical properties, Crane *et al.* proved that this compound contain a quinoid part and they were also able to isolate this lipid from mitochondria of bovine hearts (Crane et al 1957). At the same time Morton *et al.* introduced the name ubiquinone, based on that this compound is ubiquitously present in all biological systems (Morton et al 1957). In 1958, Folkers and his colleagues synthesised the complete structure; 2,3-dimetoxy-5-methyl-6-all-trans-polyprenyl-1,4-benzoquinone, and produced it by fermentation (Wolf et al 1958). Later it was shown that ubiquinone was an essential component in the mitochondrial respiratory chain (Ernster et al 1969; Mitchell 1975). However, it has been established that ubiquinone is present also in all extramitochondrial membranes including the plasma membrane with other vital functions for the cell (Kalén et al 1987; Zhang et al 1996). The distribution of ubiquinone in different subcellular fractions of rat liver cells is shown in table 1.

Table 1. Subcellular distribution of ubiquinone-9 in rat liver cells.

Fraction	Q9
Nuclear fraction	0.2
Mitochondria	1.4
Microsomes	0.15
Lysosomes	1.9
Golgi vesicles	2.6
Peroxisomes	0.3
Plasma membranes	0.7

(Kalén et al 1987; Zhang et al 1996) The values are given in µg/mg protein.

Ubiquinone consists of a redox active quinone ring that in different species has a condensed all-*trans*-isoprenoid side chain with various lengths (Fig. 3).

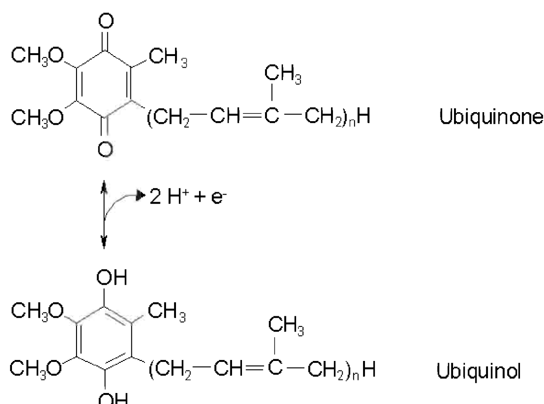

Figure 3. The chemical structures of the oxidized and reduced form of ubiquinone.

The predominant form of ubiquinone in humans has the longest known isoprene side chain with ten isoprene units (Q10) (Olson & Rudney 1983). Thus, other species show different homologues with various chain lengths indicating not only species variations but also functional varieties of ubiquinone depending on the length of the hydrophobic side chain (Table 2).

Table 2. Different ubiquinone homologues in various species.

Homologue	Occurrence
Q0	African Millipede
Q5	E. Coli
Q6	E. Coli, S. cerevisiae
Q7	E. Coli, Torula
Q8	E. Coli, Algae
Q9	Mouse, Rat
Q10	Human, Pig, Tobacco leaf

1.3.1 Biosynthesis of ubiquinones

The intracellular biosynthesis of ubiquinone, illustrated in figure 4, involves several events i.e. synthesis of the quinoid ring from aromatic amino acids and the synthesis of the isoprenoid side-chain.

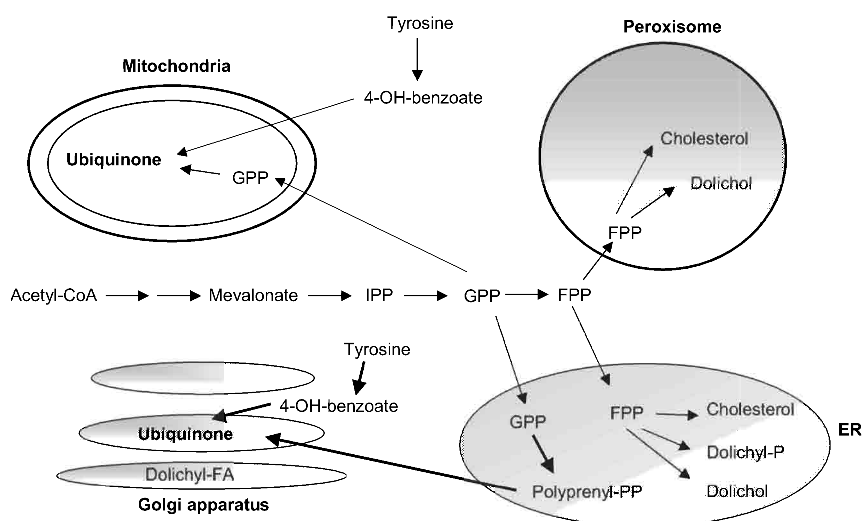

Figure 4. Schematic illustration of where mevalonate pathway enzyme reactions are located in the cell.

The condensation of the isoprenoid moiety with the ring, 4-hydroxybenzoate, is known predominantly to occur in the endoplasmatic reticulum/Golgi system (Kalén et al 1990), but total biosynthesis was also demonstrated in mitochondria (Trumpower et al 1974). After prenylation of the ring, several modifications such as decarboxylation, hydroxylations, O-metylations, and a C-metylation have to take place in order to yield a functional ubiquinone (Fig. 5).

In mammals the aromatic amino acids tyrosine and phenylalanine are proposed to be the precursors required for the synthesis of 4-

hydroxybenzoate, necessary for ubiquinone biosynthesis (Booth et al 1960; Olson & Rudney 1983).

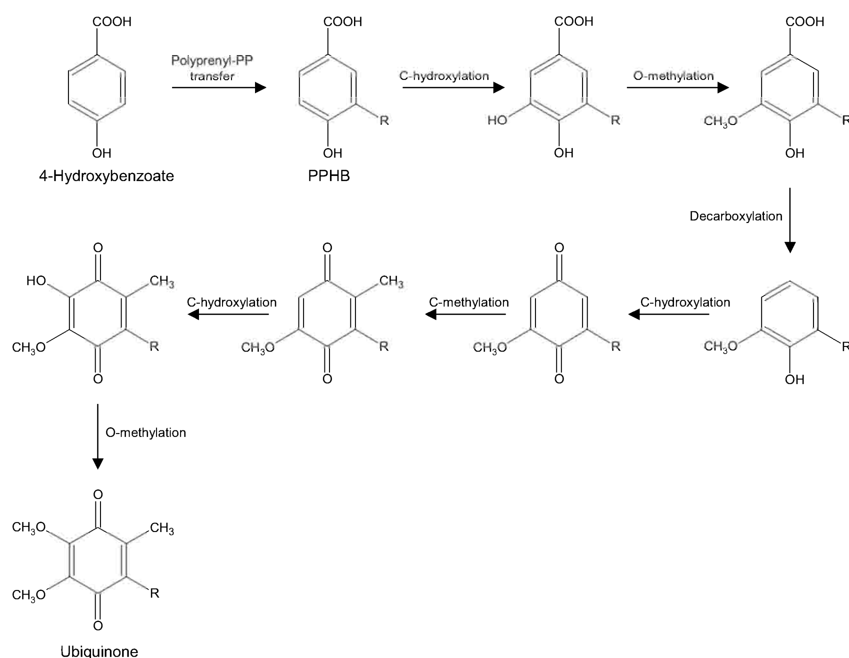

Figure 5. The eight last terminal steps in the biosynthesis of ubiquinone. R=polyisoprenoid chain; PPHB=polyprenyl-4-hydroxybenzoate.

The synthesis of the isoprenoid side-chain in mammals is catalysed by *trans*-polyprenyltransferase, an enzyme that has different intracellular locations (Grünler et al 1994). *In vitro* studies showed that the substrate for this reaction was unexpectedly GPP and not FPP (Teclebrhan et al 1993). This enzyme condenses isoprene units in *trans*-configuration to its final chain length. The polyprenyl-4-OH-benzoate transferase catalyses the condensation of the polyprenyl side chain to the ring structure, 4-hydroxybenzoate (Gupta & Rudney 1985; Kalén et al 1990).

1.3.2 Uptake and distribution of ubiquinone

Different studies when radioactively labelled ubiquinone was used showed that the dietary uptake of ubiquinone is limited and only a small amount of exogenous ubiquinone was detected in various tissues (Ramasarma 1985; Zhang et al 1995). Although, ubiquinone is administrated in its oxidised form the major part (80-90%) is found in reduced antioxidant form, ubiquinol (Zhang et al 1995; Åberg et al 1992). The mechanism for the reduction of ubiquinone is so far not completely evaluated.

The predominant form of ubiquinone is in rat Q9 and in human Q10 (Dallner & Sindelar 2000). The distribution of those ubiquinones in different organs is shown in table 3.

Table 3 Amounts of Q9 and Q10 in tissues of rats and humans.

	Q9		**Q10**	
Tissue	**Rat**	**Human**	**Rat**	**Human**
Heart	202.0	2.5	16.9	114.0
Kidney	123.9	3.3	22.0	66.5
Liver	130.9	1.8	21.3	54.9
Muscle	42.6	1.0	3.1	39.7
Brain	37.4	1.0	18.9	13.4
Pancreas	37.2	1.6	2.8	32.7
Spleen	22.9	0.7	9.2	24.6
Lung	16.9	0.6	2.4	7.9
Thyroidea	43.5	1.2	6.6	24.7
Testis	32.4	0.4	4.7	10.5
Intestine	50.9	0.5	19.0	11.5
Colon	47.5	0.4	8.4	10.7
Ventricle	55.6	n.d	5.3	11.8

(Åberg et al 1992) The values are given in µg/g tissue, n.d = not determinated

1.3.3 Catabolism of ubiquinone

The endogenous catabolism of ubiquinone varies in different rat tissues with half-life times from 49 to 125 hours (Table 4) (Andersson et al 1990; Thelin et al 1992).

Table 4. Half-life time (hours) of ubiquinone in rat tissues.

Tissue	Ubiquinone
Kidney	125
Thymus	104
Pancreas	94
Brain	90
Liver	79
Stomach	72
Spleen	64
Heart	59
Colon	54
Intestine	54
Muscle	50
Testis	50
Thyroid	49

(Andersson et al 1990; Thelin et al 1992)

Interestingly, the half-life times for cholesterol and dolichol in the liver is almost the same as for ubiquinone but is 40- and 10-fold longer, respectively, in brain (Andersson et al 1990). These findings indicate that the biosynthesis and metabolism of these lipids are regulated in different ways.

The catabolism of ubiquinone has only to a limited extent been studied. The breakdown products of ubiquinone that so far have been identified have the same ring structure but the side-chain was shown to be much shorter and carboxylated (Imada et al 1970; Nakamura et al 1999). By

these changes of the side-chain the product will be more hydrophilic and, thereby, easier to excrete from the cell.

1.3.4 The functions of ubiquinone

Ubiquinone possesses many essential functions depending on its localisation (Nohl et al 2001). Besides its most known function as an electron carrier in the mitochondrial respiratory chain it is also an antioxidant, involved in extramitochondrial electron transport, regulate mitochondrial permeability pores, and destabilise membranes and participate in plasma membrane electron transport necessary for the control of intracellular pH among other things (Crane & Navas 1997; Fontaine & Bernardi 1999; Gille & Nohl 2000; Villalba et al 2001)

1.3.4.1 The respiratory chain

As a component of the respiratory chain ubiquinone plays a vital role in oxidative phosphorylation. Located in the inner mitochondrial membrane ubiquinone shuffles electrons from complex I and II to complex III (Crane 2001; Mitchell 1975; Nohl et al 2001). The unique function of ubiquinone in the mitochondria where it is the only non-protein component involved in cell respiration, enable it to freely participate and transfer electrons between cytochromes and flavoproteins. Therefore, ubiquinone plays an essential role in the adenosine triphosphate (ATP) production (Schultz & Chan 2001).

1.3.4.2 The function as an antioxidant

More than 30 years ago reduced ubiquinone-6 was proposed to possess antioxidant properties (Mellors & Tappel 1966a; Mellors & Tappel

1966b). An effective role of ubiquinol as antioxidant was later demonstrated to prevent lipid peroxidation in liposomes, lipid emulsions, phospholipids, and LDL (Fiorentini et al 1993; Frei et al 1990; Landi et al 1990; Pobezhimova & Voinikov 2000; Takahashi et al 1995; Thomas et al 1997; Yamamoto et al 1990). Furthermore, supplemented ubiquinone-10 was also shown to protect DNA and membrane proteins (Ernster & Dallner 1995; Tomasetti et al 2001; Tomasetti et al 1999).

The protective role of ubiquinol as an inhibitor of lipid peroxidation has been widely discussed. According to some investigator ubiquinone was shown to prevent lipid peroxidation at the same rate as vitamin E (Yamamoto et al 1990). Other studies have shown an even higher effectiveness of ubiquinol in prevention of LDL oxidation than vitamin E (Frei et al 1990; Stocker et al 1991). This inhibition of lipid peroxidation by ubiquinol was further confirmed by the use of submitochodrial particles in which lipid peroxidation was initiated by ascorbate and ADP-Fe^{3+} (Forsmark et al 1991). Furthermore, ubiquinol was shown to be required in order to maintain vitamin E in its reduced active form (Fig. 1) (Frei et al 1990; Kagan et al 1990; Mukai et al 1990; Nohl et al 1999).

For the antioxidant function of ubiquinol and because it is widely spread in all membranes it is of high importance that the reduced form can be regenerated at all these locations. Many investigators have so far studied the regeneration of ubiquinone and different quinone reductases have been proposed as reduction enzymes (Kishi et al 1999; Takahashi et al 1996; Takahashi et al 1995). The most studied enzyme is DT-diaphorase, a cytosolic homodimeric enzyme (Beyer et al 1996; Beyer et al 1997; Landi et al 1997). Recently, it was shown *in vitro* that the homodimeric enzyme

lipoamide dehydrogenase efficiently could reduce ubiquinone (Olsson et al 1999).

1.3.5 Clinical relevance of ubiquinone

Several studies have shown that the ubiquinone concentrations are decreased during ageing (Kalén et al 1989; Lönnrot et al 1995). In addition, it has been shown that deficiency of ubiquinone is associated with several disease conditions, i.e. cardiomyopathies, muscle degenerative and neurogenerative diseases (Littarru 1995; Rosenfeldt et al 1999; Shults et al 1998). The lowered concentration of ubiquinone found in these different diseases may suggest a very important function of this compound in different biological systems.

The uptake of supplemented ubiquinone, under normal conditions, is restricted and it is obvious that under normal conditions the endogenous synthesis of ubiquinone is enough to provide all the cells with this compound necessary for its membrane functions. However, under conditions were ubiquinone is not synthesised or regenerated in adequate levels a supplementation is probably required for the maintenance of its functional properties.

One very severe disease, mitochondrial encephalomyopathy, caused by a gene mutation leads to deficiency in synthesis of ubiquinone and thereby dysfunction of the electron transfers in the respiratory chain (Rötig et al 2000). In a clinical trail supplementation with ubiquinone-10 was shown to mostly restore the ubiquinol levels and to reduce the symptoms of the disease (Rötig et al 2000).

It was shown that oral supplementation to healthy subjects with 30 mg Q10/day for 3 to 9 months increased the blood concentration form 1mg Q10/l to 2mg Q10/l (Folkers et al 1994). This dietary uptake of ubiquinone was suggested to only have a primary role in the blood but lower importance in (other) different tissues. These elevated concentrations in the blood may serve several important functions, i.e. an enhanced protection of LDL from oxidation, a prevention of free radical damage caused by neutrophils in inflammatory diseases, and prevention of oxidative injury by endothelial cells resulting from ischemia-reperfusion (Ernster & Dallner 1995; Kontush et al 1995). These and possibly other protective functions against free-radical damage in the circulation may account for the majority of the beneficial effects of ubiquinone supplementation in experimental and clinical medicine. Furthermore, supplementation of ubiquinone has shown to have beneficial effects on diseases in the cardiovascular system (Langsjoen et al 1994; Langsjoen & Langsjoen 1999).

1.4 Regenerating enzymes systems

The redox status of the cell is of vital importance for cellular activities. Many enzymes and transcription factors have to be kept in reduced form to be functionally active and able to remain in the right configuration in the cell. Several redox systems, such as the thioredoxin system, the glutathione system and also many other systems participate in this mission of keeping vital biological molecules in an optimal redox state.

Although this field has by now been insufficiently investigated we know that several enzymes are involved in the redox processes. One important group is the FAD-containing enzymes lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase, each enzyme catalysing electron transfer between pyridine nucleotide, FAD and a disulfide moiety. These enzymes are homodimeric enzymes with subunits that have a molecular weight of about 55 kDa and belong to a family of pyridine nucleotide disulfide oxidoreductases sharing both structural and functional similarities (Burleigh & Williams 1972; Jones & Williams 1975; Krohne-Ehrich et al 1977; Ronchi & Williams 1972; Williams 1992).

1.4.1 Lipoamide dehydrogenase

The first known function of lipoamide dehydrogenase was its participation in the three α-ketoacid complexes located in the inner mitochondrial membrane (Hayakawa et al 1969; Hirashima et al 1967). Figure 6 demonstrate the function of this enzyme (E_3) in the pyruvate dehydrogenase complex. By oxidation of the lipoic acid linked to a lysine at E_2 it transfers electrons to NAD^+, generating NADH (Spencer et al 1984). Lipoic acid in its free form can be reduced to dihydrolipoic acid by lipoamide dehydrogenase with NADH as cofactor (Biewenga et al 1996;

Kamata & Akiyama 1990; Podda et al 1994). Two other enzymes of the same family, thioredoxin reductase and glutathione reductase, can also reduce this compound, but with NADPH as cofactor (Arnér et al 1996; Pick et al 1995). In turn dihydrolipoic acid was demonstrated to be able to reduce ubiquinone (Kozlov et al 1999). Lipoamide dehydrogenase was *in vitro* also shown to effectively reduce ubiquinone (Olsson et al 1999). It is obvious that these enzyme systems are connected and contribute to an elevated level of resistance against oxidative stress in the cell.

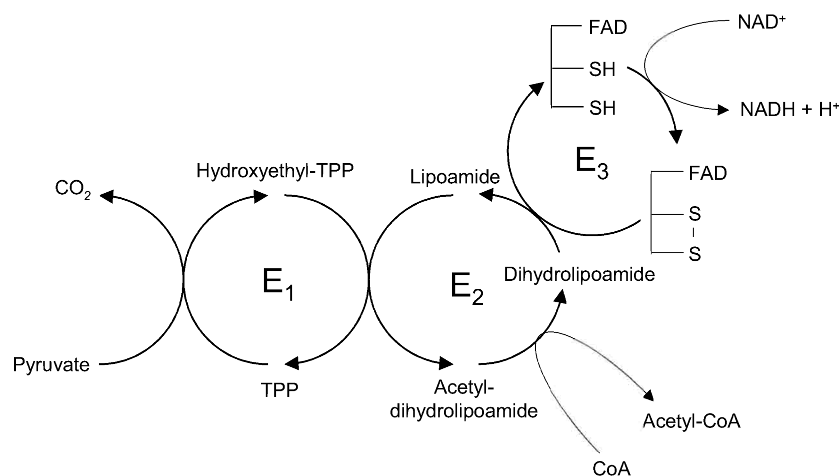

Figure 6. Schematic illustration of the pyruvate dehydrogenase complex. E1 = Pyruvate dehydrogenase; E2 = Dihydrolipoyl transacetylase; E3 = Lipoamide dehydrogenase.

1.4.2 Glutathione reductase

Glutathione reductase is known to effectively reduce oxidised glutathione (GSSG) with NADPH as cofactor and thereby maintain the essential pool of reduced glutathione (GSH) (Meister 1995; Williams 1992). This enzyme is located mainly in the cytosol. Comparisons between enzymes isolated from various species showed high degree of similarities both

structurally and functionally with lipoamide dehydrogenase (Greer & Perham 1986; Krauth-Siegel et al 1982; Stephens et al 1983; Williams et al 1982). The homology of these enzymes was demonstrated to be associated with their redox active disulfide sites (Jones & Williams 1975; Williams 1992).

GSH is a water-soluble compound consisting of the three amino acids, glutamate, cysteine and glycine, that is involved in both non-enzymatic and enzymatic reactions (Meister 1995). The main non-enzymatic functions are to effectively reduce disulfides, serve as a thiol buffer and conjugate hydrophobic compounds to facilitate an efficient excretion (Hathcock 1985; Lu 1999; Sharma et al 2000; Suzuki & Sugiyama 1998). Enzymatically GSH serves as an electron donor in several reactions catalysed by i.e. glutathione transferases (GST) and glutathione peroxidases (GPx) (Brigelius-Flohe 1999; Mannervik 1985; Mannervik & Danielson 1988; Rinaldi et al 2002; Ursini et al 1995).

1.4.3 Thioredoxin reductase

Although, thioredoxin reductase belongs to the same family as lipoamide dehydrogenase and glutathione reductase there are crucial structural differences affecting the functions of this enzyme (Ronchi & Williams 1972; Thelander 1970; Williams 1992). The C-terminal part of mammalian thioredoxin reductase is in comparison with the two other enzymes elongated with about twenty amino acids and containing cysteine and selenocysteine adjacent to each other (Williams 1992; Zhong et al 2000). The incorporation of selenium as selenocysteine in proteins has been suggested to be facilitated by isopentenylation of the $tRNA^{ser/sec}$ via the mevalonate pathway (Ding et al 1998; Warner et al 2000). The

exceptionally broad substrate specificity is connected to this selenenylsulfide as it is located closely to the active disulfide site consisting of the conserved sequence Cys-Val-Asn-Val-Gly-Cys at the N-terminal of the other subunit (Fig. 7) (Williams 1992; Zhong et al 2000).

The thioredoxin system consists of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH (Fig. 7). Trx are small ubiquitous dithiol proteins that exist in both mammalian cells and in prokaryotes (Holmgren 1985). Examples of three distinct thioredoxins are the classical Trx (cytosol), Trx2 (mitochondria) and SpTrx (testis) (Masutani et al 1996; Miranda-Vizuete et al 2001; Spyrou et al 1997). The most studied thioredoxin is the 12 kDa cytosolic form (Eklund et al 1991; Holmgren 1985). The functional role of Trx is depending on the essential and well-characterized redox-active disulfide/dithiol moiety, -Cys-Gly-Pro-Cys- (Gleason & Holmgren 1988; Holmgren 1989).

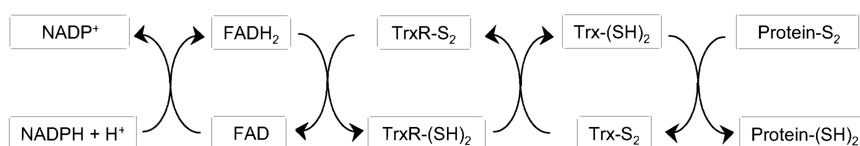

Figure 7. A simplified scheme of the thioredoxin system.

All thioredoxins are reactivated by TrxR with NADPH as cofactor. One specific function for Trx is as an electron donor to ribonucleotide reductases (Miranda-Vizuete et al 1996; Padovani et al 2001; Reichard 1993). However, Trx is also important in a variety of other cellular

activities, such as thiol redox control of enzymes, receptors and transcription factors, redox signalling, and cellular growth including tumor cell proliferation (Holmgren et al 1998). More interestingly, thioredoxins were also shown to be involved in apoptosis, by regulation of the apoptosis signal-regulating kinase-1 (ASK-1), in a redox-controlled manner (Saitoh et al 1998). Additionally, thioredoxins can reduce disulfides in proteins that have been formed by hydrogen peroxide as well as directly scavenge free radicals (Nordberg & Arnér 2001). Other more recently discovered functions of thioredoxins are the reactivation of peroxiredoxins (Prx) (Chae et al 1994). Peroxiredoxins are a relatively recently discovered family of antioxidant proteins. These proteins are peroxidases and by its location in the cytosol, they catalyse the degradation of hydroperoxides to either water or alcohol, depending on the substrate (Chae et al 1994; Chae et al 1999; Rhee et al 1994).

TrxR was originally purified and characterised from *E. coli* (Thelander 1967; Williams et al 1967). Later on this enzyme has also been cloned and sequenced (Russel & Model 1988). The molecular weight of the dimer was shown to be 70 kDa and each of the subunits contain a NADPH and a FAD binding domain in the active site specific for its homologues Trx, but have also reactivity with other prokaryotic Trx, i.e. *Anabena* (Gleason & Holmgren 1988).

Thioredoxin reductases have also been purified from different mammalian species, i.e. calf, rat and man (Holmgren 1977; Oblong et al 1993). The mammalian TrxR has very broad substrate specificity and reduce not only its homologous Trx, but also Trx from other species and several low molecular weight compounds including selenium compounds (Björnstedt et al 1997; Holmgren et al 1998), hydroperoxides (Björnstedt et al 1995)

and NK-lysin (Andersson et al 1996). Furthermore, TrxR1 is also an electron donor to human plasma GPx in the detoxification of hydroperoxides (Björnstedt et al 1994). The most studied TrxR in mammalian is the cytosolic form, but a mitochondrial TrxR have also been found (Miranda-Vizuete et al 1999).

2 PRESENT INVESTIGATION

2.1 Aims

- To develop a new method for rapid semipreparative isolation of polyprenols with very high purity that could be used as substrates and standards in studies of the biosynthesis of specific compounds of the mevalonate pathway, i.e. ubiquinone.

- To study and characterise the reduction of ubiquinone by the flavoenzymes lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase.

- To study the role of selenium in the regeneration of ubiquinol by thioredoxin reductase.

2.2 Results

2.2.1 Development of a rapid HPLC method for separation of polyprenols (Paper I)

In this paper, a semi preparative HPLC method was established in order to rapidly isolate different polyprenols with high purity. The conventional chromatographic methods are time consuming and the purity of the polyprenols isolated is quite poor. By this new HPLC method we could reduce the time for isolation and collect fractions of polyprenols with very high purity (Fig. 8).

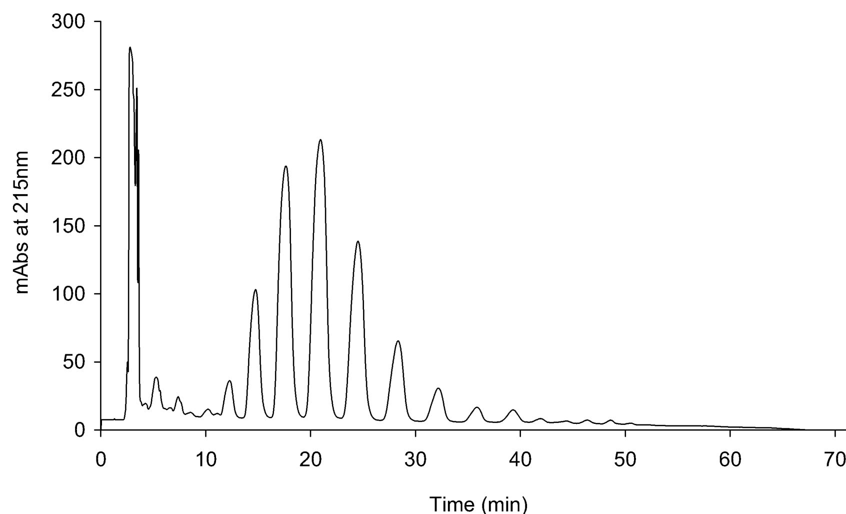

Figure 8. Chromatogram of semipreparative HPLC isolation of polyprenols.

Due to the high hydrophobicity of isoprenoid alcohols with significant chain lengths they are difficult to isolate in sufficient amounts and purity. In conventional chromatography time saving results in overlapping of the different polyprenols with decreased recovery and purity as a consequence. The results obtained by this new semipreparative HPLC method showed that 100 mg of material could easily be isolated within days in contrast to several weeks to months with old techniques.

In this paper we separated products synthesized via *cis*-prenyltransferase but, of course, products synthesized via *trans*-prenyltransferase can also be isolated.

The substances isolated have been used for development of radioactive substrates for the purpose to study different enzyme reactions in the

mevalonate pathway. They were also used as standards for quantitative HPLC-analysis.

2.2.2 The biosynthesis of ubiquinone in peroxisomes (paper II)

Since several enzymes from the mevalonate pathway, involved in both the initial and the terminal phases have been detected in peroxisomes (Grünler et al 1994; Thompson & Krisans 1990) the question was to presume whether peroxisomes also might contribute to the biosynthesis of the terminal part of ubiquinone (Fig 9).

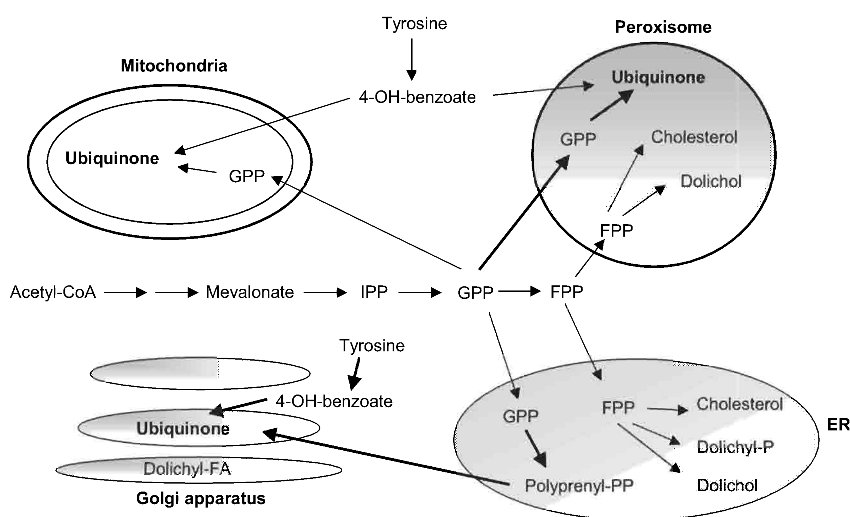

Figure 9. A schematic illustration of the locations of the mevalonate pathway reactions in the cell. The biosynthesis of ubiquinone in peroxisomes is bolded.

Many investigators have during decades shown that the biosynthesis of ubiquinone is mainly located in the endoplasmic reticulum/Golgi system.
In this investigation we analysed two enzymes, one that is involved in the synthesis of the isoprenoid side chain, *trans*-prenyltransferase, and the other enzyme that mediates the transfer of the isoprenoid side chain to the precursor ring, nonaprenyl-4-hydroxybenzoate (NPHB)-transferase.
These studies were performed in both peroxisomal and microsomal fractions isolated from normal rat liver.

The *trans*-prenyltransferases in both of these organelles required *trans*-geranyl-PP as substrate and not *trans*-farnesyl-PP. However, the microsomal *trans*-prenyltransferase activity was highly activated in the presence of Mg^{2+}, whereas the peroxisomal *trans*-prenyltransferase activity reached the highest activity with Mn^{2+}. Moreover, in the presence of detergents, like digitonin, the microsomal *trans*-prenyltransferase activity was activated, while the peroxisomal enzyme was inactivated. These observations together with observations made after mechanical treatment such as sonication or freezing/thawing indicated that the location and the regulation of these isoenzymes differ. On a protein basis, the specific activity of the total *trans*-prenyltransferase, 30 % was found in the peroxisomal and 70% in the microsomal fraction. The effect of treatment with the HMG-CoA reductase inhibitor, mevinolin was also investigated and the enzyme activity in both of these locations was enhanced by this compound. Treatment with clofibrate, a peroxisomal inducer, enhanced the microsomal activity, whereas the peroxisomal activity was decreased.

NPHB-transferase activity was also found in the peroxisomes, with an equal catalytic rate as in the microsomal fraction. Different regulatory patterns of the two enzymes were shown after treatment of the rats with

chemical compounds such as clofibrate and DEHP. By these treatments only the peroxisomal activity was affected, by decrease of the activity. Altogether, the results presented suggest that at least two enzymes involved in the terminal part of the biosynthesis of ubiquinone are located in the peroxisomes.

2.2.3 Regeneration of ubiquinol by flavoenzymes (Paper III, IV and V)

The antioxidant function of ubiquinol is predominately to protect against lipid and protein peroxidation. Ubiquinone is synthesised in all cells and is integrated in all membranes. Its reduced form, ubiquinol, can inhibit the initiation of the peroxidation reaction by reducing the perferryl radical. It can also terminate the propagation phase by regeneration of vitamin E or, maybe act by itself to scavenge the lipidperoxyl radical formed in the process. Although the antioxidant function requires that ubiquinol is continuously regenerated the non-mitochondrial enzymatic systems involved are only characterised to a limited extent. The aim of these investigations was to characterise the reduction of ubiquinol by the three flavoenzymes, lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase.

The reduction of ubiquinone by all three enzymes characterised was shown to have similar rates of reduction of ubiquinone with both NADH and NADPH as cofactors. However, the addition of zinc in the reaction mixture containing NADPH as cofactor, enhanced both LipDH and GR activity. Among other divalent ions tested only cadmium had the same effect on the reduction rate of ubiquinone by LipDH and GR. In contrast,

the reduction of ubiquinone by thioredoxin reductase was inhibited by zinc.

The pH optimum of the rate of reduction of ubiquinone was for lipoamide dehydrogenase and glutathione reductase acidic, pH 6 and 4.5, respectively. Under these acidic conditions the activities were almost the same for both enzymes as in the presence of zinc. This remarkable behaviour may be physiologically relevant during ROS generating conditions like ischemia/reperfusion. On the other hand, the pH optimum for thioredoxin reductase was found to be at physiological pH. All together, these observations indicate that ubiquinol-regeneration can operate during various intracellular conditions, maybe at different intracellular locations. Furthermore, the highest rate of reduction under physiological conditions was achieved by TrxR1.

By changing the ratio between $NAD(P)H/NAD(P)^+$, regulatory mechanisms were studied using lipoamide dehydrogenase as enzyme. An inhibitory effect on the reduction of ubiquinone by this enzyme was only achieved when the $NADPH/NADP^+$ ratio was low. When GR was used in the reaction mixture it was shown that the reduction of ubiquinone was competitively inhibited by the traditionally known substrate, oxidised glutathione, but only at very high concentrations. The reduction of ubiquinone was also studied using FAD alone. It was found that a very low activity could be achieved. Thus, these results indicate that FAD plays an important role as a part of the enzymes studied.

2.2.4 The role of selenium in the reduction of ubiquinone by thioredoxin reductase (paper IV)

We also demonstrated the importance of selenium for the reduction of ubiquinone by TrxR1. In the study HEK293 cell lines overexpressing TrxR1, normal bovine and rat TrxR1, non-selenium containing *E. coli* enzyme, mutant human TrxR (selenocysteine replaced by alanine) and recombinant truncated rat TrxR lacking the two amino acids Sec and Gly at the C-terminal part were used. The results showed that there was almost no reduction of ubiquinone when the enzymes lacking selenocysteine was used in the reaction mixture. By incubation of the overexpressing HEK293 cell lines with selenite we also showed that the increase in TrxR1 activity in homogenates was accompanied by an increase in reduction of ubiquinone. In conclusion, those results strongly suggest selenium to be essential for reduction of ubiquinone by TrxR1.

3 GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In this work the biosynthesis and reduction of ubiquinone with focus on the extra mitochondrial fraction of the compound was studied. It is obvious that the mevalonate pathway is complex in several aspects. First of all it is divided into several pathways that ends up in different products. The regulation of the different pathways is not fully discovered, but the fact that parts of the synthetic pathways are located in different subcellular compartments indicate a necessity of compartmentalization to guaranty the supply of ubiquinone in the different locations where it is needed. In this thesis we have shown that ubiquinone biosynthesis also occur in the peroxisomes suggesting a specific protecting role of this compound at this location. As many other enzymes of the mevalonate pathway have been discovered in this organelle it is reasonable to ask the question if not all of the enzymes of this pathway are located here.

Although the amounts of ubiquinol in the membranes are considerable it will not be enough for effective antioxidant function unless it can't be regenerated. The turnover of ubiquinol is much faster than can be explained by replacement via *de novo* synthesis. It has been shown that oxidative stress increases the amount of the oxidised ubiquinone in the cell and, thereby, also possibly enhances the degradation of this compound. To compensate for this depletion increased synthesis of ubiquinone, regeneration of ubiquinol and uptake from the blood or all three functions may occur.

In this thesis we investigated, as mentioned, other locations of ubiquinone biosynthesis but more intensively the characteristics of the reactions of

regeneration of ubiquinol by the flavoenzymes, lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase.

The results obtained when the characteristics of the reduction of ubiquinone by glutathione reductase were investigated may suggest other functions of this enzyme. This proposal is based on that the highest rate of reduction of ubiquinone was shown at acidic pH and that zinc at physiological pH increased the reduction rate almost 3-fold.

In the studies with thioredoxin reductase we also used transfected stable cell lines overexpressing this enzyme. By this system together with enzymes lacking selenocysteine, we showed that the reduction of ubiquinone by this enzyme was selenium dependent. These results support the findings that deficiency of selenium decrease the concentrations of ubiquinone and vitamin E. The conclusion may be that if thioredoxin reductase is not sufficiently saturated with selenocysteine the reduction of ubiquinone is harmed and, thereby, the regeneration of vitamin E.

In the future it would be of great interest to also study cell lines overexpressing lipoamide dehydrogenase or glutathione reductase. As those latter enzymes are not selenium dependent these studies may add more physiological and molecular aspects onto the regulatory mechanisms of the reduction of ubiquinone. Other *in vitro* cell studies that could be interesting are the effect of oxidative stress on the biosynthesis and reduction of ubiquinone.

Supplementation of ubiquinone to different populations and species is today widely studied. In the future one target is to find a model to study

the effects of supplementation on both biosynthesis of ubiquinone and regeneration of ubiquinol.

4 ACKNOWLEDGEMENTS

The investigations presented in this thesis were done at the Division of Pathology, Department of Laboratory Medicine (former Dept. of Microbiology, Pathology and Immunology), Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden.

It all started 1996 when I as a student was located/adapted into Lennart Eriksson's research group with Jerker Olsson as my supervisor. He thought me a lot about HPLC and along the time I soon realized that even machines had their own life, Jerker could actually "talk" to machines. After my 8 weeks of practical experience, he for some reason wanted me back as a graduated student, and so I did and Jerker became my fulltime supervisor. Patience was another of many things that I also learned from him. How important it is when you try to establish a new HPLC method that every second, minute even every hour is worth waiting for, even if 5 hours in a row is a very long time to wait for something to happen! Jerker is a person with many "irons in the fire", he knows a lot about lot of things. As a person, Jerker is a very nice, generous and warm and for all of you that have tasted his food, a very good chef. Thank you Jerker, for your friendship, for teaching, guiding and supporting me in the field of biochemistry and finally, for all the help I got from you during my time as a graduate student.

I would also like to express my warmest gratitude to my Co-supervisors, Prof. Lennart Eriksson and Assoc. Prof. Mikael Björnstedt for their support and help and above all for sharing their broad knowledge in pathology and biochemistry with me.

A special tanks to Prof. Göran Andersson, head of the Division of Pathology, for providing a nice research environment.

I would also like to thank all the other members in our group Ulla-Britta Torndal, Agneta Söderstedt, Katarina Stråhle, Linda Björkhem-Bergman, Servet Eken for their personalities and friendship. A special thanks to Agneta Söderstedt for all your help, knowledge and expertise in the cell culturing area and to Ulla-Britta Torndal for your kindness as a person, knowledge and skilful expertise in different subcellular fractionation techniques.

I would also like to thank all the former members in LE´s research group, Per Ståhl, Per Rhissler, Gen-Sheng, Guo, Ling Xia, Michael Tekle and

Henrik von Eulér. A special tanks to Ling Xia for all your help and kindness during your PhD time.

My polish colleagues from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Assoc. Prof. Ewa Swiezewska, Prof. Tadeuz Chojnacki for our collaboration and for your hospitality and kindness during my stay in Warsaw, Poland.

A special tanks to my other polish colleagues, Drs. Zbyzek and Jola, Dept. of Cell Biology, Faculty of Biotechnology, Jagiellonian University, Krakow, Poland for our long and fruitful conversations and for showing me how a cockroach looks like (hope that you didn't find any in your luggage!).

I would like to thank all my other collaborators, Giannis Spyrou and Ivan Nalvarte at the Dept. of Biosciences at Novum, Center for Biotechnology, Karolinska Institutet and Elias Arnér at Department of Medical Biochemistry and Biophysics, Karolinska Institutet.

Thank you, all the personnel at the div. of Pathology for your contribution to an enjoyable family atmosphere. Especially thanks to Pernilla Lång, Karin Hollberg and Daphne Vassiliou for nice collaboration during our teaching.

I would also like to thank my mother, Lena, my mother in law, Key, and all my brothers and sisters Ulf, Magnus, Anneli, and Annett, and their families. Thank you for being my family.

Finally, I would like to thank my wonderful and beloved wife, Veronica, for always being there and supporting me during all these years, and for giving me a son, Tobias, a real miracle. You are everything for me, without your inspiration and understanding this work had never been done.

5 REFERENCES

Agarwal, B., Halmos, B., Feoktistov, A. S., Protiva, P., Ramey, W. G., et al. 2002. Mechanism of lovastatin-induced apoptosis in intestinal epithelial cells. *Carcinogenesis* 23:521-8.

Alberts, A. W. 1988. Discovery, biochemistry and biology of lovastatin. *Am J Cardiol* 62:10J-15J.

Alvear, M., Jabalquinto, A. M., Eyzaguirre, J., Cardemil, E. 1982. Purification and characterization of avian liver mevalonate-5- pyrophosphate decarboxylase. *Biochemistry* 21:4646-50.

Andersson, M., Elmberger, P. G., Edlund, C., Kristensson, K., Dallner, G. 1990. Rates of cholesterol, ubiquinone, dolichol and dolichyl-P biosynthesis in rat brain slices. *FEBS Lett* 269:15-8.

Andersson, M., Holmgren, A., Spyrou, G. 1996. NK-lysin, a disulfide-containing effector peptide of T-lymphocytes, is reduced and inactivated by human thioredoxin reductase. Implication for a protective mechanism against NK-lysin cytotoxicity. *J Biol Chem* 271:10116-20.

Arnér, E. S., Nordberg, J., Holmgren, A. 1996. Efficient reduction of lipoamide and lipoic acid by mammalian thioredoxin reductase. *Biochem Biophys Res Commun* 225:268-74.

Ayte, J., Gil-Gomez, G., Haro, D., Marrero, P. F., Hegardt, F. G. 1990. Rat mitochondrial and cytosolic 3-hydroxy-3-methylglutaryl-CoA synthases are encoded by two different genes. *Proc Natl Acad Sci U S A* 87:3874-8.

Beg, Z. H., Stonik, J. A., Brewer, H. B., Jr. 1987. Phosphorylation and modulation of the enzymic activity of native and protease-cleaved purified hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase by a calcium/calmodulin-dependent protein kinase. *J Biol Chem* 262:13228-40.

Beyer, R. E. 1994. The role of ascorbate in antioxidant protection of biomembranes: interaction with vitamin E and coenzyme Q. *J Bioenerg Biomembr* 26:349-58.

Beyer, R. E., Segura-Aguilar, J., Di Bernardo, S., Cavazzoni, M., Fato, R., et al. 1996. The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane systems. *Proc Natl Acad Sci U S A* 93:2528-32.

Beyer, R. E., Segura-Aguilar, J., di Bernardo, S., Cavazzoni, M., Fato, R., et al. 1997. The two-electron quinone reductase DT-diaphorase generates and

maintains the antioxidant (reduced) form of coenzyme Q in membranes. *Mol Aspects Med* 18:S15-23.

Biardi, L., Sreedhar, A., Zokaei, A., Vartak, N. B., Bozeat, R. L., et al. 1994. Mevalonate kinase is predominantly localized in peroxisomes and is defective in patients with peroxisome deficiency disorders. *J Biol Chem* 269:1197-205.

Biewenga, G. P., Dorstijn, M. A., Verhagen, J. V., Haenen, G. R., Bast, A. 1996. Reduction of lipoic acid by lipoamide dehydrogenase. *Biochem Pharmacol* 51:233-8.

Björnstedt, M., Hamberg, M., Kumar, S., Xue, J., Holmgren, A. 1995. Human thioredoxin reductase directly reduces lipid hydroperoxides by NADPH and selenocystine strongly stimulates the reaction via catalytically generated selenols. *J Biol Chem* 270:11761-4.

Björnstedt, M., Kumar, S., Björkhem, L., Spyrou, G., Holmgren, A. 1997. Selenium and the thioredoxin and glutaredoxin systems. *Biomed Environ Sci* 10:271-9.

Björnstedt, M., Xue, J., Huang, W., Åkesson, B., Holmgren, A. 1994. The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. *J Biol Chem* 269:29382-4.

Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., Jones, F. T., DeEds, F. 1960. Urinary phenolic acid metabolites of tyrosine. *j Biol Chem* 235:2649-2652

Brigelius-Flohe, R. 1999. Tissue-specific functions of individual glutathione peroxidases. *Free Radic Biol Med* 27:951-65.

Burleigh, B. D., Jr., Williams, C. H., Jr. 1972. The isolation and primary structure of a paptide containing the oxidation-reduction active cystine of Escherichia coli lipoamide dehydrogenase. *J Biol Chem* 247:2077-82.

Cadenas, E. 1989. Biochemistry of oxygen toxicity. *Annu Rev Biochem* 58:79-110

Cadenas, E., Davies, K. J. 2000. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 29:222-30.

Chae, H. Z., Chung, S. J., Rhee, S. G. 1994. Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem* 269:27670-8.

Chae, H. Z., Kang, S. W., Rhee, S. G. 1999. Isoforms of mammalian peroxiredoxin that reduce peroxides in presence of thioredoxin. *Methods Enzymol* 300:219-26

Chen, H., Tappel, A. L. 1995. Protection of vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q0, coenzyme Q10, beta-

carotene, canthaxanthin, and (+)-catechin against oxidative damage to rat blood and tissues in vivo. *Free Radic Biol Med* 18:949-53.

Chiew, Y. E., O'Sullivan, W. J., Lee, C. S. 1987. Studies on pig liver mevalonate-5-diphosphate decarboxylase. *Biochim Biophys Acta* 916:271-8.

Clarke, C. F., Edwards, P. A., Fogelman, A. M. 1987. In *Plasma lipoproteins*, ed. J. A.M. Gotto. pp. 261-276. Amsterdam: Elsevier Science Publisher

Clarke, P. R., Hardie, D. G. 1990. Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase in vitro and in intact rat liver. *Embo J* 9:2439-46.

Clinkenbeard, K. D., Reed, W. D., Mooney, R. A., Lane, M. D. 1975. Intracellular localization of the 3-hydroxy-3-methylglutaryl coenzme A cycle enzymes in liver. Separate cytoplasmic and mitochondrial 3- hydroxy-3-methylglutaryl coenzyme A generating systems for cholesterogenesis and ketogenesis. *J Biol Chem* 250:3108-16.

Crane, F. L. 2001. Biochemical functions of coenzyme Q10. *J Am Coll Nutr* 20:591-8.

Crane, F. L., Hatefi, Y., Lester, R. L., Widmer, C. 1957. Isolation of a quinone from beef heart mitochondria. *Science* 25:220-221

Crane, F. L., Navas, P. 1997. The diversity of coenzyme Q function. *Mol Aspects Med* 18:S1-6.

Dallner, G., Sindelar, P. J. 2000. Regulation of ubiquinone metabolism. *Free Radic Biol Med* 29:285-94.

Diamond, A. M., Jaffe, D., Murray, J. L., Safa, A. R., Samuels, B. L., Hatfield, D. L. 1996. Lovastatin effects on human breast carcinoma cells. Differential toxicity of an adriamycin-resistant derivative and influence on selenocysteine tRNAS. *Biochem Mol Biol Int* 38:345-55.

DiGuiseppi, J., Fridovich, I. 1984. The toxicology of molecular oxygen. *Crit Rev Toxicol* 12:315-42

Ding, L., Liu, Z., Zhu, Z., Luo, G., Zhao, D., Ni, J. 1998. Biochemical characterization of selenium-containing catalytic antibody as a cytosolic glutathione peroxidase mimic. *Biochem J* 332:251-5.

Dröge, W. 2002. Free radicals in the physiological control of cell function. *Physiol Rev* 82:47-95.

Eklund, H., Gleason, F. K., Holmgren, A. 1991. Structural and functional relations among thioredoxins of different species. *Proteins* 11:13-28

Ericsson, J., Appelkvist, E. L., Thelin, A., Chojnacki, T., Dallner, G. 1992. Isoprenoid biosynthesis in rat liver peroxisomes. Characterization of

cis-prenyltransferase and squalene synthetase. *J Biol Chem* 267:18708-14.

Ericsson, J., Edwards, P. A. 1998. CBP is required for sterol-regulated and sterol regulatory element- binding protein-regulated transcription. *J Biol Chem* 273:17865-70.

Ernster, L. 1993. Lipid peroxidation in biological membranes: mechanism and implications. In *Active Oxygens, Lipid Peroxides, and Antioxidants.*, ed. K. Yagi. pp. 1-38. New York: CRC Press

Ernster, L., Dallner, G. 1995. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta* 1271:195-204.

Ernster, L., Lee, I. Y., Norling, B., Persson, B. 1969. Studies with ubiquinone-depleted submitochondrial particles. Essentiality of ubiquinone for the interaction of succinate dehydrogenase, NADH dehydrogenase, and cytochrome b. *Eur J Biochem* 9:299-310.

Faust, J. R., Brown, M. S., Goldstein, J. L. 1980. Synthesis of delta 2-isopentenyl tRNA from mevalonate in cultured human fibroblasts. *J Biol Chem* 255:6546-8.

Faust, J. R., Dice, J. F. 1991. Evidence for isopentenyladenine modification on a cell cycle-regulated protein. *J Biol Chem* 266:9961-70.

Festenstein, G. N., Heaton, F., S, L. J., A., M. R. 1955. A constituent of the unsaponifiable portion of animal tissue lipids. *Biochem J* 59:558-566

Finkel, T. 1998. Oxygen radicals and signaling. *Curr Opin Cell Biol* 10:248-53.

Fiorentini, D., Cabrini, L., Landi, L. 1993. Ubiquinol-3 and ubiquinol-7 exhibit similar antioxidant activity in model membranes. *Free Radic Res Commun* 18:201-9

Folkers, K., Moesgaard, S., Morita, M. 1994. A one year bioavailability study of coenzyme Q10 with 3 months withdrawal period. *Mol Aspects Med* 15:s281-5.

Fontaine, E., Bernardi, P. 1999. Progress on the mitochondrial permeability transition pore: regulation by complex I and ubiquinone analogs. *J Bioenerg Biomembr* 31:335-45.

Forsmark, P., Åberg, F., Norling, B., Nordenbrand, K., Dallner, G., Ernster, L. 1991. Inhibition of lipid peroxidation by ubiquinol in submitochondrial particles in the absence of vitamin E. *FEBS Lett* 285:39-43.

Forsmark-Andree, P., Lee, C. P., Dallner, G., Ernster, L. 1997. Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles. *Free Radic Biol Med* 22:391-400

Frei, B., Kim, M. C., Ames, B. N. 1990. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci U S A* 87:4879-83.

Gille, L., Nohl, H. 2000. The existence of a lysosomal redox chain and the role of ubiquinone. *Arch Biochem Biophys* 375:347-54.

Gleason, F. K., Holmgren, A. 1988. Thioredoxin and related proteins in procaryotes. *FEMS Microbiol Rev* 4:271-97.

Goldstein, J. L., Brown, M. S. 1990. Regulation of the mevalonate pathway. *Nature* 343:425-30.

Greer, S., Perham, R. N. 1986. Glutathione reductase from Escherichia coli: cloning and sequence analysis of the gene and relationship to other flavoprotein disulfide oxidoreductases. *Biochemistry* 25:2736-42.

Grünler, J., Ericsson, J., Dallner, G. 1994. Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. *Biochim Biophys Acta* 1212:259-77.

Gupta, A., Rudney, H. 1985. 4-Hydroxybenzoate polyprenyltransferase from rat liver. *Methods Enzymol* 110:327-34

Hathcock, J. N. 1985. Metabolic mechanisms of drug-nutrient interactions. *Fed Proc* 44:124-9.

Hayakawa, T., Kanzaki, T., Kitamura, T., Fukuyoshi, Y., Sakurai, Y., et al. 1969. Mammalian alpha-keto acid dehydrogenase complexes. V. Resolution and reconstitution studies of the pig heart pyruvate dehydrogenase complex. *J Biol Chem* 244:3660-70.

Herrera, E., Barbas, C. 2001. Vitamin E: action, metabolism and perspectives. *J Physiol Biochem* 57:43-56.

Hirashima, M., Hayakawa, T., Koike, M. 1967. Mammalian alpha-keto acid dehydrogenase complexes. II. An improved procedure for the preparation of 2-oxoglutarate dehydrogenase complex from pig heart muscle. *J Biol Chem* 242:902-7.

Holmgren, A. 1977. Bovine thioredoxin system. Purification of thioredoxin reductase from calf liver and thymus and studies of its function in disulfide reduction. *J Biol Chem* 252:4600-6.

Holmgren, A. 1985. Thioredoxin. *Annu Rev Biochem* 54:237-71

Holmgren, A. 1989. Thioredoxin and glutaredoxin systems. *J Biol Chem* 264:13963-6.

Holmgren, A., Arnér, E., Åslund, F., Björnstedt, M., Liangwei, Z., et al. 1998. Redox regulation by the thioredoxin and glutaredoxin systems. In *Oxidative*

Stress, Cancer, AIDS, and Neurodegenerative Diseases, ed. O. Montagnier, Pasquier. pp. 229-246. New York: Marcel Dekker, Inc

Hua, X., Nohturfft, A., Goldstein, J. L., Brown, M. S. 1996. Sterol resistance in CHO cells traced to point mutation in SREBP cleavage-activating protein. *Cell* 87:415-26.

Imada, I., Watanabe, M., Matsumoto, N., Morimoto, H. 1970. Metabolism of ubiquinone-7. *Biochemistry* 9:2870-8.

Jones, E. T., Williams, C. H., Jr. 1975. The sequence of amino acid residues around the oxidation-reduction active disulfide in yeast glutathione reductase. *J Biol Chem* 250:3779-84.

Kagan, V., Serbinova, E., Packer, L. 1990. Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. *Biochem Biophys Res Commun* 169:851-7.

Kalén, A., Appelkvist, E. L., Chojnacki, T., Dallner, G. 1990. Nonaprenyl-4-hydroxybenzoate transferase, an enzyme involved in ubiquinone biosynthesis, in the endoplasmic reticulum-Golgi system of rat liver. *J Biol Chem* 265:1158-64.

Kalén, A., Appelkvist, E. L., Dallner, G. 1989. Age-related changes in the lipid compositions of rat and human tissues. *Lipids* 24:579-84.

Kalén, A., Norling, B., Appelkvist, E. L., Dallner, G. 1987. Ubiquinone biosynthesis by the microsomal fraction from rat liver. *Biochim Biophys Acta* 926:70-8.

Kamata, H., Hirata, H. 1999. Redox regulation of cellular signalling. *Cell Signal* 11:1-14.

Kamata, K., Akiyama, K. 1990. High-performance liquid chromatography with electrochemical detection for the determination of thioctic acid and thioctic acid amide. *J Pharm Biomed Anal* 8:453-6

Kawabe, Y., Honda, M., Wada, Y., Yazaki, Y., Suzuki, T., et al. 1994. Sterol mediated regulation of SREBP-1a,1b,1c and SREBP-2 in cultured human cells. *Biochem Biophys Res Commun* 202:1460-7.

Keller, G. A., Barton, M. C., Shapiro, D. J., Singer, S. J. 1985. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase is present in peroxisomes in normal rat liver cells. *Proc Natl Acad Sci U S A* 82:770-4.

Keller, G. A., Pazirandeh, M., Krisans, S. 1986. 3-Hydroxy-3-methylglutaryl coenzyme A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine-treated animals: quantitative biochemical and immunoelectron microscopical analyses. *J Cell Biol* 103:875-86.

Kishi, T., Takahashi, T., Usui, A., Hashizume, N., Okamoto, T. 1999. Cytosolic NADPH-UQ reductase, the enzyme responsible for cellular ubiquinone redox cycle as an endogenous antioxidant in the rat liver. *Biofactors* 9:189-97

Kontush, A., Hubner, C., Finckh, B., Kohlschutter, A., Beisiegel, U. 1995. Antioxidative activity of ubiquinol-10 at physiologic concentrations in human low density lipoprotein. *Biochim Biophys Acta* 1258:177-87.

Kovacs, W. J., Faust, P. L., Keller, G. A., Krisans, S. K. 2001. Purification of brain peroxisomes and localization of 3-hydroxy-3- methylglutaryl coenzyme A reductase. *Eur J Biochem* 268:4850-9.

Kozlov, A. V., Gille, L., Staniek, K., Nohl, H. 1999. Dihydrolipoic acid maintains ubiquinone in the antioxidant active form by two-electron reduction of ubiquinone and one-electron reduction of ubisemiquinone. *Arch Biochem Biophys* 363:148-54.

Krauth-Siegel, R. L., Blatterspiel, R., Saleh, M., Schiltz, E., Schirmer, R. H., Untucht-Grau, R. 1982. Glutathione reductase from human erythrocytes. The sequences of the NADPH domain and of the interface domain. *Eur J Biochem* 121:259-67.

Krohne-Ehrich, G., Schirmer, R. H., Untucht-Grau, R. 1977. Glutathione reductase from human erythrocytes. Isolation of the enzyme and sequence analysis of the redox-active peptide. *Eur J Biochem* 80:65-71.

Landi, L., Fiorentini, D., Galli, M. C., Segura-Aguilar, J., Beyer, R. E. 1997. DT-Diaphorase maintains the reduced state of ubiquinones in lipid vesicles thereby promoting their antioxidant function. *Free Radic Biol Med* 22:329-35

Landi, L., Fiorentini, D., Stefanelli, C., Pasquali, P., Pedulli, G. F. 1990. Inhibition of autoxidation of egg yolk phosphatidylcholine in homogenous solution and in liposomes by oxidized ubiquinone. *Biochim Biophys Acta* 1028:223-8.

Langsjoen, H., Langsjoen, P., Willis, R., Folkers, K. 1994. Usefulness of coenzyme Q10 in clinical cardiology: a long-term study. *Mol Aspects Med* 15:s165-75.

Langsjoen, P. H., Langsjoen, A. M. 1999. Overview of the use of CoQ10 in cardiovascular disease. *Biofactors* 9:273-84

Littarru, G. P. 1995. *Energy and Defence* Roma: C.E.S.I., Litografica IRIDE. 91 pp.

Lu, S. C. 1999. Regulation of hepatic glutathione synthesis: current concepts and controversies. *Faseb J* 13:1169-83.

Lönnrot, K., Metsa-Ketela, T., Alho, H. 1995. The role of coenzyme Q-10 in aging: a follow-up study on life-long oral supplementation Q-10 in rats. *Gerontology* 41:109-20.

Mannervik, B. 1985. The isoenzymes of glutathione transferase. *Adv Enzymol Relat Areas Mol Biol* 57:357-417

Mannervik, B., Danielson, U. H. 1988. Glutathione transferases--structure and catalytic activity. *CRC Crit Rev Biochem* 23:283-337

Masutani, H., Hirota, K., Sasada, T., Ueda-Taniguchi, Y., Taniguchi, Y., et al. 1996. Transactivation of an inducible anti-oxidative stress protein, human thioredoxin by HTLV-I Tax. *Immunol Lett* 54:67-71.

McGee, T. P., Cheng, H. H., Kumagai, H., Omura, S., Simoni, R. D. 1996. Degradation of 3-hydroxy-3-methylglutaryl-CoA reductase in endoplasmic reticulum membranes is accelerated as a result of increased susceptibility to proteolysis. *J Biol Chem* 271:25630-8.

Meister, A. 1995. Glutathione metabolism. *Methods Enzymol* 251:3-7

Mellors, A., Tappel, A. L. 1966a. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J Biol Chem* 241:4353-6.

Mellors, A., Tappel, A. L. 1966b. Quinones and quinols as inhibitors of lipid peroxidation. *Lipids* 1:282-284

Miller, S. J., Parker, R. A., Gibson, D. M. 1989. Phosphorylation and degradation of HMG CoA reductase. *Adv Enzyme Regul* 28:65-77

Miranda-Vizuete, A., Damdimopoulos, A. E., Pedrajas, J. R., Gustafsson, J. A., Spyrou, G. 1999. Human mitochondrial thioredoxin reductase cDNA cloning, expression and genomic organization. *Eur J Biochem* 261:405-12.

Miranda-Vizuete, A., Ljung, J., Damdimopoulos, A. E., Gustafsson, J. A., Oko, R., et al. 2001. Characterization of Sptrx, a novel member of the thioredoxin family specifically expressed in human spermatozoa. *J Biol Chem* 276:31567-74.

Miranda-Vizuete, A., Rodriguez-Ariza, A., Toribio, F., Holmgren, A., Lopez-Barea, J., Pueyo, C. 1996. The levels of ribonucleotide reductase, thioredoxin, glutaredoxin 1, and GSH are balanced in Escherichia coli K12. *J Biol Chem* 271:19099-103.

Mitchell, P. 1975. The protonmotive Q cycle: a general formulation. *FEBS Lett* 59:137-9.

Moran, L. K., Gutteridge, J. M., Quinlan, G. J. 2001. Thiols in cellular redox signalling and control. *Curr Med Chem* 8:763-72.

Morton, R. A., Wilson, G. M., Lowe, J. S., Leat, W. M. F. 1957. Ubiquinone. *Chemical Industry*:1649

Moustafa, M. E., Carlson, B. A., El-Saadani, M. A., Kryukov, G. V., Sun, Q. A., et al. 2001. Selective inhibition of selenocysteine tRNA maturation and selenoprotein synthesis in transgenic mice expressing isopentenyladenosine-deficient selenocysteine tRNA. *Mol Cell Biol* 21:3840-52.

Mukai, K., Kikuchi, S., Urano, S. 1990. Stopped-flow kinetic study of the regeneration reaction of tocopheroxyl radical by reduced ubiquinone-10 in solution. *Biochim Biophys Acta* 1035:77-82.

Nakamura, T., Ohno, T., Hamamura, K., Sato, T. 1999. Metabolism of coenzyme Q10: biliary and urinary excretion study in guinea pigs. *Biofactors* 9:111-9

Ness, G. C., Chambers, C. M. 2000. Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: the concept of cholesterol buffering capacity. *Proc Soc Exp Biol Med* 224:8-19.

Nohl, H., Gille, L., Kozlov, A. V. 1999. Critical aspects of the antioxidant function of coenzyme Q in biomembranes. *Biofactors* 9:155-61

Nohl, H., Kozlov, A. V., Staniek, K., Gille, L. 2001. The multiple functions of coenzyme Q. *Bioorg Chem* 29:1-13.

Nordberg, J., Arnér, E. S. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31:1287-312.

Oblong, J. E., Gasdaska, P. Y., Sherrill, K., Powis, G. 1993. Purification of human thioredoxin reductase: properties and characterization by absorption and circular dichroism spectroscopy. *Biochemistry* 32:7271-7.

Olson, R. E., Rudney, H. 1983. Biosynthesis of ubiquinone. *Vitam Horm* 40:1-43

Olsson, J. M., Xia, L., Eriksson, L. C., Björnstedt, M. 1999. Ubiquinone is reduced by lipoamide dehydrogenase and this reaction is potently stimulated by zinc. *FEBS Lett* 448:190-2.

Padovani, D., Mulliez, E., Fontecave, M. 2001. Activation of class III ribonucleotide reductase by thioredoxin. *J Biol Chem* 276:9587-9.

Parker, R. A., Miller, S. J., Gibson, D. M. 1986. Phosphorylation state of HMG CoA reductase affects its catalytic activity and degradation. *Adv Enzyme Regul* 25:329-43

Pick, U., Haramaki, N., Constantinescu, A., Handelman, G. J., Tritschler, H. J., Packer, L. 1995. Glutathione reductase and lipoamide dehydrogenase have opposite stereospecificities for alpha-lipoic acid enantiomers. *Biochem Biophys Res Commun* 206:724-30.

Pobezhimova, T. P., Voinikov, V. K. 2000. Biochemical and physiological aspects of ubiquinone function. *Membr Cell Biol* 13:595-602

Podda, M., Tritschler, H. J., Ulrich, H., Packer, L. 1994. Alpha-lipoic acid supplementation prevents symptoms of vitamin E deficiency. *Biochem Biophys Res Commun* 204:98-104.

Poulter, C. D., Rilling, H. C. 1981. prenyl transferases and isomerases. In *Biosynthesis of Isoprenoid Compounds*, ed. J. P. a. S. Spurgeon. pp. 161-224. Vol. 2. New York: John Wiley and Sons

Ramasarma, T. 1985. Natural occurrence and distribution of coenzyme Q. In *Coenzyme Q*, ed. G. Lenaz. pp. 67-81. Chichester: John Wiley

Reichard, P. 1993. From RNA to DNA, why so many ribonucleotide reductases? *Science* 260:1773-7.

Rhee, S. G. 1999. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med* 31:53-9.

Rhee, S. G., Kim, K. H., Chae, H. Z., Yim, M. B., Uchida, K., et al. 1994. Antioxidant defense mechanisms: a new thiol-specific antioxidant enzyme. *Ann N Y Acad Sci* 738:86-92.

Rilling, H., Chayet, L. 1985. Biosynthesis of Cholesterol. In *Sterols and Bile Acids.*, ed. D. H. a. S. J. pp. 1-39. Amsterdam: Elsevier

Rinaldi, R., Eliasson, E., Swedmark, S., Morgenstern, R. 2002. Reactive intermediates and the dynamics of glutathione transferases. *Drug Metab Dispos* 30:1053-8.

Ronchi, S., Williams, C. H., Jr. 1972. The isolation and primary structure of a peptide containing the oxidation-reduction active cystine of Escherichia coli thioredoxin reductase. *J Biol Chem* 247:2083-6.

Rosenfeldt, F. L., Pepe, S., Ou, R., Mariani, J. A., Rowland, M. A., et al. 1999. Coenzyme Q10 improves the tolerance of the senescent myocardium to aerobic and ischemic stress: studies in rats and in human atrial tissue. *Biofactors* 9:291-9

Runquist, M., Ericsson, J., Thelin, A., Chojnacki, T., Dallner, G. 1994. Isoprenoid biosynthesis in rat liver mitochondria. Studies on farnesyl pyrophosphate synthase and trans-prenyltransferase. *J Biol Chem* 269:5804-9.

Russel, M., Model, P. 1988. Sequence of thioredoxin reductase from Escherichia coli. Relationship to other flavoprotein disulfide oxidoreductases. *J Biol Chem* 263:9015-9.

Rötig, A., Appelkvist, E. L., Geromel, V., Chretien, D., Kadhom, N., et al. 2000. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. *Lancet* 356:391-5.

Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., et al. 1998. Mammalian thioredoxin is a direct inhibitor of apoptosis signal- regulating kinase (ASK) 1. *Embo J* 17:2596-606.

Scholz, R. W., Minicucci, L. A., Reddy, C. C. 1997. Effects of vitamin E and selenium on antioxidant defense in rat heart. *Biochem Mol Biol Int* 42:997-1006.

Schroepfer, G. J., Jr. 1981. Sterol biosynthesis. *Annu Rev Biochem* 50:585-621

Schultz, B. E., Chan, S. I. 2001. Structures and proton-pumping strategies of mitochondrial respiratory enzymes. *Annu Rev Biophys Biomol Struct* 30:23-65

Sharma, R., Awasthi, S., Zimniak, P., Awasthi, Y. C. 2000. Transport of glutathione-conjugates in human erythrocytes. *Acta Biochim Pol* 47:751-62

Shults, C. W., Beal, M. F., Fontaine, D., Nakano, K., Haas, R. H. 1998. Absorption, tolerability, and effects on mitochondrial activity of oral coenzyme Q10 in parkinsonian patients. *Neurology* 50:793-5.

Spencer, M. E., Darlison, M. G., Stephens, P. E., Duckenfield, I. K., Guest, J. R. 1984. Nucleotide sequence of the sucB gene encoding the dihydrolipoamide succinyltransferase of Escherichia coli K12 and homology with the corresponding acetyltransferase. *Eur J Biochem* 141:361-74.

Spyrou, G., Enmark, E., Miranda-Vizuete, A., Gustafsson, J. 1997. Cloning and expression of a novel mammalian thioredoxin. *J Biol Chem* 272:2936-41.

Stamellos, K. D., Shackelford, J. E., Tanaka, R. D., Krisans, S. K. 1992. Mevalonate kinase is localized in rat liver peroxisomes. *J Biol Chem* 267:5560-8.

Stephens, P. E., Lewis, H. M., Darlison, M. G., Guest, J. R. 1983. Nucleotide sequence of the lipoamide dehydrogenase gene of Escherichia coli K12. *Eur J Biochem* 135:519-27.

Stocker, R., Bowry, V. W., Frei, B. 1991. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. *Proc Natl Acad Sci U S A* 88:1646-50.

Suzuki, H., Sugiyama, Y. 1998. Excretion of GSSG and glutathione conjugates mediated by MRP1 and cMOAT/MRP2. *Semin Liver Dis* 18:359-76

Takahashi, T., Sugimoto, N., Takahata, K., Okamoto, T., Kishi, T. 1996. Cellular antioxidant defense by a ubiquinol-regenerating system coupled with

cytosolic NADPH-dependent ubiquinone reductase: protective effect against carbon tetrachloride-induced hepatotoxicity in the rat. *Biol Pharm Bull* 19:1005-12.

Takahashi, T., Yamaguchi, T., Shitashige, M., Okamoto, T., Kishi, T. 1995. Reduction of ubiquinone in membrane lipids by rat liver cytosol and its involvement in the cellular defence system against lipid peroxidation. *Biochem J* 309:883-90.

Tanaka, R. D., Lee, L. Y., Schafer, B. L., Kratunis, V. J., Mohler, W. A., et al. 1990. Molecular cloning of mevalonate kinase and regulation of its mRNA levels in rat liver. *Proc Natl Acad Sci U S A* 87:2872-6.

Teclebrhan, H., Olsson, J., Swiezewska, E., Dallner, G. 1993. Biosynthesis of the side chain of ubiquinone:trans-prenyltransferase in rat liver microsomes. *J Biol Chem* 268:23081-6.

Thelander, L. 1967. Thioredoxin reductase. Characterization of a homogenous preparation from Escherichia coli B. *J Biol Chem* 242:852-9.

Thelander, L. 1970. The amino acid sequence of a peptide containing the active center disulfide of thioredoxin reductase from Escherichia coli. *J Biol Chem* 245:6026-9.

Thelin, A., Schedin, S., Dallner, G. 1992. Half-life of ubiquinone-9 in rat tissues. *FEBS Lett* 313:118-20.

Thomas, S. R., Neuzil, J., Stocker, R. 1997. Inhibition of LDL oxidation by ubiquinol-10. A protective mechanism for coenzyme Q in atherogenesis? *Mol Aspects Med* 18:S85-103.

Thompson, S. L., Krisans, S. K. 1990. Rat liver peroxisomes catalyze the initial step in cholesterol synthesis. The condensation of acetyl-CoA units into acetoacetyl-CoA. *J Biol Chem* 265:5731-5.

Tomasetti, M., Alleva, R., Borghi, B., Collins, A. R. 2001. In vivo supplementation with coenzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage. *Faseb J* 15:1425-7.

Tomasetti, M., Littarru, G. P., Stocker, R., Alleva, R. 1999. Coenzyme Q10 enrichment decreases oxidative DNA damage in human lymphocytes. *Free Radic Biol Med* 27:1027-32.

Traber, M. G. 1999. Utilization of vitamin E. *Biofactors* 10:115-20

Trumpower, B. L., Houser, R. M., Olson, R. E. 1974. Studies on ubiquinone. Demonstration of the total biosynthesis of ubiquinone-9 in rat liver mitochondria. *J Biol Chem* 249:3041-8.

Ursini, F., Maiorino, M., Brigelius-Flohe, R., Aumann, K. D., Roveri, A., et al. 1995. Diversity of glutathione peroxidases. *Methods Enzymol* 252:38-53

Vadhanavikit, S., Ganther, H. E. 1993. Decreased ubiquinone levels in tissues of rats deficient in selenium. *Biochem Biophys Res Commun* 190:921-6.

Vadhanavikit, S., Ganther, H. E. 1994. Selenium deficiency and decreased coenzyme Q levels. *Mol Aspects Med* 15:s103-7.

Warner, G. J., Berry, M. J., Moustafa, M. E., Carlson, B. A., Hatfield, D. L., Faust, J. R. 2000. Inhibition of selenoprotein synthesis by selenocysteine tRNA[Ser]Sec lacking isopentenyladenosine. *J Biol Chem* 275:28110-9.

Vaughan, C. J., Gotto, A. M., Jr., Basson, C. T. 2000. The evolving role of statins in the management of atherosclerosis. *J Am Coll Cardiol* 35:1-10.

Villalba, J. M., López-Liuch, G., Santos-Ocana, C., Rodríguez-Aguilera, J. C., Navas, P. 2001. Extramitochondrial functions of coenzyme Q. In *Coenzyme Q: Molecular Mechanism in Health and Disease*, ed. V. a. Q. Kagan, PJ. pp. 83-98. Boca Raton: CRC Press

Williams, C. H., Jr., Arscott, L. D., Schulz, G. E. 1982. Amino acid sequence homology between pig heart lipoamide dehydrogenase and human erythrocyte glutathione reductase. *Proc Natl Acad Sci U S A* 79:2199-201.

Williams, C. H., Jr., Zanetti, G., Arscott, L. D., McAllister, J. K. 1967. Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, and thioredoxin. *J Biol Chem* 242:5226-31.

Williams, C. H. J., ed. 1992. *Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase and mercuric ion reductase - A family of flavoenzyme transhydrogenase.* Vol. 3, 121-211. Boca, Raton: CRC Press

Viner, J. L., Umar, A., Hawk, E. T. 2002. Chemoprevention of colorectal cancer: problems, progress, and prospects. *Gastroenterol Clin North Am* 31:971-99.

Wolf, D. E., Hoffman, C. H., Trenner, N. R., Arison, B. H., Shunk, C. H., et al. 1958. Structure studies on the coenzyme Q group. *J. Am. Chem Soc.* 80:4748-4752

Yamamoto, Y., Komuro, E., Niki, E. 1990. Antioxidant activity of ubiquinol in solution and phosphatidylcholine liposome. *J Nutr Sci Vitaminol (Tokyo)* 36:505-11.

Zhang, Y., Turunen, M., Appelkvist, E. L. 1996. Restricted uptake of dietary coenzyme Q is in contrast to the unrestricted uptake of alpha-tocopherol into rat organs and cells. *J Nutr* 126:2089-97.

Zhang, Y., Åberg, F., Appelkvist, E. L., Dallner, G., Ernster, L. 1995. Uptake of dietary coenzyme Q supplement is limited in rats. *J Nutr* 125:446-53.

Zhong, L., Arner, E. S., Holmgren, A. 2000. Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. *Proc Natl Acad Sci U S A* 97:5854-9.

Åberg, F., Appelkvist, E. L., Dallner, G., Ernster, L. 1992. Distribution and redox state of ubiquinones in rat and human tissues. *Arch Biochem Biophys* 295:230-4.

Åkerlund, J. E., Björkhem, I. 1990. Studies on the regulation of cholesterol 7 alpha-hydroxylase and HMG- CoA reductase in rat liver: effects of lymphatic drainage and ligation of the lymph duct. *J Lipid Res* 31:2159-66.