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Institutionen för medicinsk biokemi och biofysik

Development and biomedical applications of highly sensitive enzyme activity assays of thioredoxin, thioredoxin reductase and glutaredoxin

AKADEMISK AVHANDLING

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ABSTRACT

Oxidative stress is implicated in the pathogenesis of many human diseases, thus it is interesting to study the relationship between antioxidants, the development and progression of diseases. The thioredoxin and glutaredoxin systems are ubiquitous redox-active proteins known to be induced in chronic inflammatory related processes, such as cancer and diabetes, to protect against oxidative stress. The thioredoxin system is composed of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH. The glutaredoxin system comprises glutathione reductase (GR), glutathione (GSSG/GSH), glutaredoxin (Grx) and NADPH. Trx is an important antioxidant and a redox regulator, crucial for the activation of transcription factors and modulation of intra- and extracellular signaling pathways. While, Grx is another crucial redox regulator with an important role in maintaining intracellular GSH-disulfide-dithiol exchange, apoptosis and cell differentiation. In fact, Trx, Grx and TrxR have been suggested biomarkers for disease monitoring. Thus, there is a current need for new techniques to detect and monitor Trx, Grx and TrxR activities in human patient samples; since the traditional methods showed several limitations related to background, specificity and sensitivity. The aim of this thesis was to develop highly sensitive and reproducible assays to enable analysis of Trx, TrxR and Grx activities in clinical patient samples. Thus, we optimized the synthesis of two fluorescent substrates, diethionyl-glutathione (Di-E-GSSG) and fluorescein labelled insulin (FITC-insulin), which both gave higher fluorescence upon disulfide reduction. The latter, FITC-insulin, was used to develop highly sensitive microplate assays for Trx (≥ 0.4 picomoles) and TrxR (≥ 40 femtomole). Moreover, this method allowed reproducible measurements of re-activated Trx, commonly present in frozen or over-oxidized samples from cell, tissue (biopsies) and blood plasma origin (manuscript I). The former Di-E-GSSG, was an excellent substrate of Grx and could be used to glutathionylate proteins (such as BSA, yielding E-GS-BSA), thus becoming a useful fluorescent marker for glutathionylated proteins in gel electrophoresis. This mixed disulfide substrate, E-GS-BSA, allowed measurements of Grx1 and Grx2 activities in picomole concentrations (manuscript II). E-GS-BSA was further a key substrate for reverse-S-glutathionylation catalysis studies, which facilitated the characterization and study of the catalytic properties of human recombinant Grx5 (paper IV). In addition, we applied these optimized methods to the study of relevant clinical samples from patients showing a mutation in the selenocysteine insertion sequence-binding protein 2 gene, which lead to a multisystem selenoprotein deficiency disorder. Our measurements of TrxR activity in skin biopsies and PBMCs from these patients, showed significant decreased TrxR activity and concomitant increased of ROS levels when compared to healthy controls (paper III). In conclusion, we present novel sensitive tools to study Trx, TrxR and Grx activities in complex samples from biological origin. Since these redox enzymes have been suggested as potential biomarkers for several diseases; the actual relevance of our newly developed methodologies, goes beyond the enzymatic measurements performed, as these methods might assist in detecting and/or analyzing disease progression with clear biomedical applications.