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ALTERING HIV-1 ENVELOPE GLYCOPROTEIN MATURATION AND ITS EFFECTS ON VIRAL INFECTIVITY

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ABSTRACT

HIV-1 is dependent on its envelope glycoprotein (Env) to initiate infection. Env binds to cellular receptors and mediate the following fusion of the viral envelope with the cell plasma membrane. In an attempt to inhibit these events the tri-peptide glycyl-prolyl-glycine amide (GPG-NH₂) was designed to block the interaction of Env with its secondary co-receptor. Although the GPG-NH₂ was shown to have antiviral properties, its mode of action was found to be other than the intended. It was observed that GPG-NH₂ acted late in the viral replication cycle and that it affected the cellular expression of Env, but its antiviral mechanism remained unclear. Therefore, the main objectives of this thesis were:

1) To elucidate the effect of GPG-NH₂ on Env and determine if this affected virus infectivity. **2)** To examine if the antiviral mechanism and the specific effect on Env was owing to GPG-NH₂ or its metabolites G-NH₂ or α HGA. **3)** To examine the regulatory importance of the native Env signal sequence for cellular Env expression, viral particle incorporation of Env and viral replication.

In this thesis it is shown that treatment of HIV-1 infected cells with GPG-NH₂ results in production of viral particles with dramatically reduced infectivity. This is in part a consequence of reduced viral incorporation of Env, which disables the viral entry into cells. The mechanism was uncovered by examining Env expression in GPG-NH₂ treated cells, which revealed a significant reduction in Env steady-state levels and its processing to gp120/gp41 but also a decrease in its molecular mass as a result of glycan removal. Taken together the results show that GPG-NH₂ impairs Env maturation, which targets it for endoplasmic reticulum-associated protein degradation (ERAD), where Env is deglycosylated en route to its destruction. This effect of GPG-NH₂ was further shown to be a result of its metabolizing via the intermediate G-NH₂ into the active metabolite α HGA, by enzymes in the fetal bovine serum (FBS) added to the cell culture medium. It was further shown that in the presence of human serum or in the absence of any serum only the final metabolite α HGA was capable of directing Env for destruction. These observed effects were all found to be dependent on the native Env signal sequence and the proteasome.

The 30 residue long Env signal sequence of the precursor Env, gp160, targets it for co-translational translocation into the endoplasmic reticulum (ER). We found that the ER targeting function of the signal sequence was remarkably tolerant to large N-terminal truncations. Its first 8 N-terminal residues were entirely dispensable for adequate gp160 expression levels. However, they provide the signal sequence with regulatory functions detected first when examining the viral particles. The wild type virus incorporated ~80 % more of the precursor gp160 and 20 % less of its processed form, gp120/gp41, compared to the 8 residue truncated signal sequence virus. By promoting viral incorporation of the inactive precursor gp160 over the fusogenic gp120/gp41 the wt signal sequence down regulate the viral particle infectivity by ~40 %. This indicates that the signal sequence may have post ER targeting functions that permit significant amounts of gp160 trafficking through Golgi without being processed and become incorporated into the viral particles. Interestingly, the intra cellular capsid protein levels were initially lower and the viral particle release was initiated later in the presence of the native Env signal sequence than in its absence or in the presence of truncated Env signal sequences.

In conclusion these data illustrate that changes in the viral particle Env content and composition has a profound effect on the HIV-1 infectivity, which can be achieved by targeting selective steps in its biosynthesis and that small molecules may be utilized therapeutically to target unwanted pathogenic proteins for degradation by the existing cellular machinery.

Keywords: HIV-1, Env, gp160, gp120, gp41, signal sequence, ERAD, GPG-NH₂, G-NH₂, α HGA