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Ligand-induced structural changes in HIV-1 envelope glycoprotein

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ABSTRACT

The first step in HIV-1 entry is the interaction between viral envelope glycoprotein gp120 and CD4 receptor on permissive host cells. The interaction initiates a series of sequential conformational rearrangements that exposes epitopes involved in interaction with co-receptors. Using x-ray crystallography, the tertiary structural rearrangements are elucidated. However, the envelope glycoprotein on the virus surface is arranged as a trimer consisting of three gp120 and three gp41 subunits (Env) and the tertiary conformational change observed in gp120 has not been characterised in the context of a quaternary structure with sufficient conformity.

In this thesis, the envelope glycoprotein responsible for initiating virus entry, Env, was studied using cryo-electron microscopy. The quaternary structure of Env, in the pre-entry and entry intermediate, provided evidence of conformational shifts upon receptor or ligand-binding. This method was further used to analyse the quaternary structure of another ligand-bound Env, the Tat protein-bound Env. The Tat protein-bound Env was studied due to the postulation that Tat protein contributes, in part, to disease progression. This stems from the observations that combination vaccine trials encompassing both Tat and Env proteins protected macaques from mucosal SHIV challenge, that monoclonal anti-Tat antibodies inhibited HIV-1 replication and that HIV-1 infected individuals who are slow progressors of the disease (have a longer clinical asymptomatic phase) show an inverse relationship in their anti-Tat antibody levels and viral load magnitude. In addition, the combined Tat-Env vaccine induced antibodies that recognised more regions in Env and had antibodies with higher reactivity for epitopes in the V3 loop of Env.

Comparative analysis of the structures obtained showed that after HIV-1 binding to its receptor CD4, the tertiary conformational arrangement of gp120 is translated to the following quaternary changes: gp120 subunit tilt from the z-axis, gp120 subunit rotation along its own axis and rotation of CD4 epitopes with concomitant exposure of co-receptor epitopes. Including the Tat protein-bound Env in the comparison showed that Tat-bound Env has a conformation intermediate between the native and CD4-bound Env, and hence, optimal for virus entry. This structure change could explain the increased disease progression in patients with low anti-Tat antibody to viral load magnitude and slower disease progressions in those patients with high anti-Tat antibody. In addition, the intermediate structure of Tat-gp120 complex could expose epitopes on gp120 that provide better immune protection in slow progressors.

This thesis further evaluated the effect of Tat protein treatment of HIV-1 on infectivity and spread *in vitro*, showing that Tat protein increases infection in target cells and increases spread between infected and non-infected cells through cell-cell contacts. The increased infectivity and spread was proposed to be due to interaction of Tat protein with the V3 loop of gp120, which potentially alters the co-receptor recognition. In this way, HIV-1 of a particular tropism, for example an X4-tropic HIV-1, could infect by using CCR5 as a co-receptor instead. Moreover, the low concentration of circulating Tat protein observed in the serum of HIV-1 patients could be a result of regulation by the virus as Tat protein at high concentration was found to have a negative impact on infectivity rates.

Key words: HIV-1, Env, CD4, Tat, quaternary structure, infectivity

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