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Preparation of HIV-1 founder virus envelope glycoprotein core crystals, and computational characterisation of covalent and non-covalent complexes it forms with 2dCD4

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Abstract

The envelope glycoprotein (Env) is responsible for human immunodeficiency virus type 1 (HIV-1) entry into CD4+ T cells through the binding of CD4 and represents an ideal target for therapeutics as well as vaccine development strategies. During transmission, only a limited number of founder viruses (FV) are capable of establishing infection in a new host, thus an enhanced understanding of the FV Env structure is critical in vaccine immunogen development. Previous findings showed that immunizations of small animals with a novel Env–2dCD4^{S60C} complex generated broadly neutralizing antibodies against clinically relevant HIV-1. The mechanism behind this enhanced immune response is attributed to the targeted disulphide bond between the gp120 component of Env and 2dCD4^{S60C} but has not yet been elucidated. This study aimed to optimize the crystallization conditions of a modified unliganded gp120 core FV sequence (gp120$_{FVC}$ExC), as well as compare gp120$_{FVC}$ExC to a similarly modified gp120 core subtype C (gp120Core$_e$) sequence. This allowed for a better understanding of the structure and conformational dynamics of gp120 FV protein compared to a non-founder virus variant. MD analyses were also performed on the gp120$_{FVC}$ExC–2dCD4^{S60C} and gp120$_{FVC}$ExC – 2dCD4^{WT} complexes to identify potential structural changes that may be responsible for the enhanced immunogenicity produced by the S60C mutation in the Env – 2dCD4^{S60C} immunogen.

The gp120 FV sequence was designed and modified to allow for unliganded crystallization through the truncation of the V1, V2, and V3 loops. The modified gp120 was expressed in modified GnTi-deficient cells, purified using lectin affinity chromatography, and deglycosylated with Endoglycosidase H to enhance homogeneity. The purified and homogenate protein was successfully crystallized using a Morpheus Index kit in an under-oil diffusion method, validating the chosen modifications for crystallization. Homology models of gp120$_{FVC}$ExC, gp120Core$_e$, 2dCD4^{WT}, 2dCD4^{S60C}, and the gp120$_{FVC}$ExC – 2dCD4$^{S60C/WT}$ complexes were generated using the SWISS-MODEL platform. Generated homology models were used for 50 ns MD simulations on the Maestro platform at a 1 ns timestep to investigate structural and dynamic differences between the proteins. Further analyses of the MD simulated proteins were performed on the Schrodinger platform. Comparative MD analyses of gp120$_{FVC}$ExC and gp120Core$_e$ showed marked differences in the variable loop regions (V1-V5) as well as overall less fluctuations of gp120$_{FVC}$ExC residues. The V1 and V4 loops show increased flexibility while the V3 loop exhibited a drastic decrease of approximately 4 Å in

fluctuations in $gp120_{FVC}ExC$ when compared to $gp120Core_{e.}$ MD simulations of the $gp120_{FVC}ExC$ – $2dCD4^{S60C}$ complex shows a decrease in fluctuation near the N-terminus at residues 38-43 and marked increase of 2 Å in fluctuations of residues in the V3 loop comparative to $gp120_{FVC}ExC$ – $2dCD4^{WT}$. These investigations show distinct structural and conformational differences between the $gp120_{FVC}ExC$ and the $gp120Core_e$ protein that may be characteristic of a gp120 FV sequence. Additionally, the differences in fluctuations observed in the comparative simulation of $gp120_{FVC}ExC$ – $2dCD4^{S60C/WT}$ complexes of residues 38-43 (reduced in $gp120_{FVC}ExC$ – $2dCD4^{S60C}$) and residues 214-224 (increased in $gp120_{FVC}ExC$ – $2dCD4^{S60C}$) provided insight and an initiation point for further investigation into the enhanced immunogenicity of the novel Env–$2dCD4^{S60C}$ vaccine immunogens.