Structure and dynamics of the native HIV-1 Env trimer

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ABSTRACT

HIV-1/AIDS remains one of the worst pandemics in human history. Despite tremendous efforts, no effective vaccine has been found. Two recent reports give new insights into the structure and dynamics of the HIV-1 Env trimer and renew hopes that a better understanding of Env will translate into new vaccine candidates and more effective antiretroviral therapies.

HIV-1 is the etiological agent of the AIDS pandemic. Over 70 million people have been infected by the virus and ~39 million have died from AIDS-related illness. Antiretroviral therapy has reached ~11 million people and allows many HIV-1-infected people a long lifespan. However, the life-long chemotherapy is not without side effects and the cancer burden among HIV-1-infected people remains elevated. Despite recent hopes that a cure might be possible (1-3), the more we learn about the viral reservoir, the more we realize how difficult it will be to eradicate HIV-1 in an infected patient (4-6). Given this situation, a low-cost preventative such as a vaccine or an adeno-associated virus-delivered prophylactic might be the preferred response to protect the human population (7). However, despite tremendous efforts, no effective vaccine has been found. This is largely due to specific features of the envelope glycoprotein (Env), which is uniquely exposed on the surface of the virion, and as such is the primary target of antibodies.

HIV-1 Env is synthesized as a gp160 precursor and processed into a trimer of a heterodimers containing gp120 and gp41 subunits. HIV-1 Env promotes entry into target cells by recognizing cellular receptors and fusing viral and cellular membranes. The gp120 receptor-binding domain
of Env first engages cellular CD4. This interaction leads to a conformational rearrangement in
Env that results in presentation of the coreceptor-binding site. Interaction with the coreceptor
triggers the gp41 membrane-fusion domain to mediate virus entry.

Why is it so hard to generate a vaccine against HIV-1? In addition to recognizing cellular
receptors, Env has also evolved effective means of concealing functional centers from attack by
antibodies. The Env trimer has three distinct features that make it an evasive machine that
escapes neutralizing antibodies. First, Env is covered by a dense glycan layer that makes up
half of its total molecular weight. This glycan shield restricts access of immunoglobulins to 97%
of the Env surface (8). Second, the protein surface undergoes unusually rapid sequence
variation. Approximately 50% of the Env surface has genetic variability greater than 10%. Taken
together, these two factors result in only 2% of the Env surface being accessible to
immunoglobulins with genetic variability less than 10% (8). Third, the Env trimer has significant
structural flexibility. Env can adopt a closed conformation, in which functional centers are
masked, while still responding to interaction with receptor and coreceptor. The high
glycosylation and the conformationally dynamic nature of Env has for many years impeded its
structural characterization. Recent advances give new insights into the structure and dynamics
of the HIV-1 Env trimer (8-11) and renew hopes that a better understanding of the HIV-1 Env
trimer will translate into new vaccine candidates and more effective antiretroviral therapies.

We set out to advance the understanding of the conformational dynamics of the native Env
trimer. Available structural data on the intact trimer at low resolution indicated large-scale
rearrangements, in which the V1V2 loop located at the tip of the trimer opens in response to
CD4 and coreceptor mimics (12). Given the scale of this conformational change, and the known
timescale of HIV-1 entry, we therefore expected dynamics in the milliseconds to seconds range.
One method that provides access to conformational changes on this timescale is single
molecule fluorescence resonance energy transfer (smFRET). We therefore developed smFRET
imaging methods to elucidate the conformational changes of HIV-1 Env on the surface of native
HIV-1 virions (9). The application of smFRET to HIV-1 Env required the site-specific
incorporation of fluorophores into the native trimer. To this end, we inserted two 6-12 amino-acid
peptides into variable loops of the gp120 domain of Env, which allowed enzymatic labelling with
donor and acceptor fluorophores. Peptides were placed into the V1 loop of gp120 known to
open in response to CD4 (12), and into V4 or V5, which served as points of reference from
which to observe V1 repositioning. Labeling sites were identified that did not result in significant
loss of infectivity or neutralization sensitivity as compared to wild type Env. To ensure that only
a single fluorescently labeled gp120 molecule was present on the surface of the virus, wild-type  
HIV-1 was co-transfected at a ratio of 40:1 over the dually-tagged plasmid during generation of  
the virus. Virions were dually labeled enzymatically, purified, surface immobilized, and imaged  
via prism-based total internal reflection fluorescence (TIRF) microscopy, which allows for the  
observation of conformational transitions in hundreds of individual molecules simultaneously  
over extended periods of time (ca. minutes) (13).

Surprisingly, the unliganded HIV-1 Env on the surface of native virions was found to be dynamic,  
sampling at least three distinct conformations, observed as low-, intermediate- and high-FRET  
states (9) (Fig 1). The low-FRET state was the most populated state and thus must reflect the  
closed ground state conformation of the prefusion Env, an interpretation that was confirmed  
studying ground state-stabilizing mutations. The two higher-FRET states were stabilized to  
varying extents by soluble CD4 (sCD4) and the coreceptor-mimicking antibody 17b. When  
introduced together, sCD4 and 17b stabilized the intermediate-FRET state that thus likely  
represents the coreceptor-stabilized state. Different responses to sCD4 for distinct HIV-1  
isolates didn't allow a precise assignment of the high-FRET state. Importantly, because all three  
FRET states were observed in the absence of ligands, conformations that are stabilized by CD4  
and coreceptor, are therefore intrinsically accessible to the unliganded HIV-1 Env.

We also compared the conformational sampling of Env for the laboratory-adapted  
normalization-sensitive HIV-1 isolate NL4-3 with the neutralization-resistant clinically isolated  
JR-FL. In contrast to NL4-3 that opened relatively frequently, the neutralization-resistant JR-FL  
Env rarely opened and as a consequence fewer molecules had access to the coreceptor-  
stabilized state. Given that JR-FL evolved under pressure from the host immune system to  
conceal functional centers, and thus escape neutralization by antibodies, this provided the first  
dynamics-based rationale for differential neutralization sensitivity and resistance among HIV-1  
isolates.

Hidden Markov modeling of the smFRET trajectories from both strains revealed the relative  
frequencies of the observed transitions, and confirmed that HIV-1 Env is intrinsically capable of  
sampling three conformations. The CD4- and coreceptor-stabilized states are less energetically  
stable, thereby providing a mechanism by which vulnerable functional states are protected from  
antibodies. Consistent with this model, antibody 17b required extended incubation times to  
stabilize the activated state in the absence of sCD4. This analysis also indicated that the high-  
FRET state is a necessary intermediate during the activation of HIV-1 Env by CD4 and the
coreceptor-mimicking antibody 17b. This analysis thus directly visualized the sequence of molecular events, underlying the two-step activation of HIV-1 Env by CD4 and coreceptor.

The establishment of an smFRET assay for the conformational state of native HIV-1 Env molecules on the surface of virions allowed us to visualize the conformational consequences of broadly neutralizing antibodies and small-molecule inhibitors. Surprisingly, multiple broadly neutralizing antibodies, despite engaging the Env trimer in very different ways, stabilized the ground-state conformation (9) (Figure 2A, B). The ground-state stabilization of Env by these broadly neutralizing antibodies indicates that they recognize the closed conformation of HIV-Env. Our data are consistent with observations by Guttman and colleagues who used hydrogen-deuterium exchange to arrive at a similar conclusion (14). Moreover, the especially strong ground-state stabilization by antibodies such as PGT145 (9) and PGT122 (8) (Figure 2A, B) also raises the possibility that in addition to binding the closed conformation, broadly neutralizing antibodies may inhibit transition out of the ground state, thereby preventing activation of Env. A potent small molecule HIV-1 entry inhibitor, BMS-626529 (15), similarly stabilized the closed ground state. This suggests that ground state stabilization may be a general means of inhibiting HIV-1 entry.

A possible rationale for ground-state stabilization as an antiviral strategy is provided by our observation that the unliganded HIV-1 Env has intrinsic access to the activated conformational states stabilized by CD4 and the coreceptor mimicking antibody 17b. This could indicate that access to the open conformations is required for function because binding of CD4 and coreceptor may depend on capture of pre-existing conformations. Data from us, Guttman and colleagues, and others strongly suggest a conformational capture mechanism during binding of antibodies such as 17b (9, 14). Whether CD4 binding occurs in the same manner is subject of current research.

The observed strong ground-state stabilization of HIV-1 Env by PGT122 may in retrospect explain why this antibody was able to reduce conformational heterogeneity and allow the crystallization of a soluble trimer (8, 10) (Figure 2C). Moreover, our smFRET data identify this structure as the ground-state conformation of the Env trimer (8). Since broadly neutralizing antibodies specifically recognize this conformation, any immunogen intended to elicit these potent antibodies must present this conformation. Therefore, the vaccine research field is currently engineering Env mutants that lock the trimer in that ground-state conformation. These locked trimer scaffolds will not transition to the receptor-stabilized states, and thus not present...
highly immunogenic open conformations to the immune system that only distract the immune response and do not result in protective immunity.

These considerations explain why recent advances in the understanding of the structure and dynamics of the HIV-1 Env trimer gives hope that new effective vaccine scaffolds can be generated (8, 9). However, many questions remain to be addressed. The biggest hurdle that requires solving is how a vaccine based on one HIV-1 strain can elicit protection against heterologous challenge by a different HIV-1 strain. Also, while recent structures of the Env trimer represent critical advances (8, 10, 11, 16) they are all based on the use of engineered soluble trimers and it remains an open question to what extent these artificially stabilized trimers faithfully reflect all features of the wild-type HIV-1 Env trimer (17). Many additional aspects of how the HIV-1 Env fusion machine promotes viral entry remain to be understood. While we could demonstrate that gp120 resembles an allosteric immune evasion machine that has intrinsic access to all conformational states required for function, the underlying fusion machine gp41 is expected to undergo irreversible conformational changes following triggering by the coreceptor, which ultimately lead to membrane fusion. Last but not least, researchers studying other viral glycoproteins may ask themselves if their viral glycoproteins may similarly present a dynamic equilibrium of various conformations that may better explain observed phenotypes. It is our hope that smFRET technologies that allow the visualization of the conformational state of viral glycoproteins on the surface of native virions may play a pivotal role in these future studies.

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FIG 1. The HIV-1 Env is conformationally dynamic and has inherent access to conformational states that are stabilized by soluble CD4 and the coreceptor-surrogate antibody 17b. (A) HIV-1 virions containing a single dually labeled Env molecule among native trimers were immobilized on the surface of passivated quartz microscope slides and imaged via TIRF microscopy. (Top left) Representative fluorescence (Cy3B, donor, green; Cy5(4S)-COT, acceptor, red) and (bottom left) FRET (blue) trajectories obtained from a single HIV-1 NL4-3 Env on the surface of an intact virion. Idealization of FRET trajectories (red) was achieved by fitting each trace to a three-state Markov model. (Top right) FRET trajectories from individual unliganded HIV-1NL4-3 Env proteins were compiled into a population FRET histogram and fit to the sum of three Gaussian distributions (red) with means 0.1, 0.3, and 0.6 (black). (B, C) Fluorescence and FRET trajectories, and FRET histograms in the presence of soluble CD4 (B) or soluble CD4 and coreceptor-mimicking antibody 17b. Adapted from Munro et al., Science. 2014 Nov 7;346(6210):759-63, with permission from AAAS (9).
FIG. 2. Broadly neutralizing antibodies recognize the closed ground-state conformation. (A, B) An experiment performed as in FIG. 1A for the unliganded dually labeled HIV JR-FL Env (A) and in complex with the broadly PGT122 (B) that reduces the occupancy of the high-FRET state, and stabilizes the ground state. Adapted from Pancera et al. Nature. 2014 Oct 23;514(7523):455-61 (8). (C) Structure of the closed ground-state conformation of the soluble HIV-1 Env trimer with the gp120 subunit in orange, gp41 in red and glycan shield in green. Figure provided courtesy of Marie Pancera, Jonathan Stuckey and Peter D. Kwong.