Novel Strategy for Stabilizing HIV Surface Proteins for AIDS Vaccine Development

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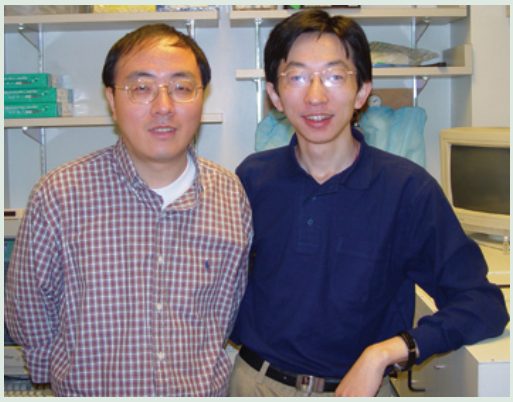
Despite recent advances in retroviral drug therapy, the need for a vaccine that can slow or stop the spread of the human immunodeficiency virus (HIV), the causal agent of AIDS, remains urgent. The surface of the virus consists of three copies of glycoproteins (proteins and carbohydrate) in a trimeric configuration. This glycoprotein complex mediates entry of the virus into immune system cells. Vaccine candidates that mimic the HIV glycoproteins are expected to elicit antibodies able to block this viral-entry process and thus neutralize the virus. Scientists from the Weill Medical College of Cornell University in New York City, Duke University in Durham, North Carolina, and the University of Pennsylvania in Philadelphia have suggested a potential strategy for the production of HIV envelope proteins in a stably folded form that might serve as vaccine candidates.



Authors (from left): Min Lu and Jie Liu

The current combination of drug therapies has not yet succeeded in eradicating the human immunodeficiency virus (HIV), the causal agent of AIDS. Even worse, new drug-resistant variants of HIV are emerging at an alarming rate. A vaccine that would block HIV's entry into host cells would thus most efficiently prevent the spread of HIV infection worldwide.

When either the human (HIV) or simian (SIV) immunodeficiency virus infect the human body, the viral membrane fuses with the membrane of a host cell, and then inserts the viral RNA (genetic information in the form of a single strand) inside the host cell. This RNA is used to create clones of the virus inside the host cell, and these new viruses then spread outside the host cell to invade other cells.

The virus harbors two types of glycoproteins (proteins and carbohydrate) on its membrane, called glycoprotein 120 (gp120) and gp41, that are assembled in the form of a trimer. When the virus attaches to a host cell, these glycoproteins bind by a lock-and-key mechanism to other proteins, called receptors, on the surface of the host cell.

When gp120 and gp41 bind to the host cell's surface receptors, the glycoproteins undergo a conformational change, creating a transient species called the pre-hairpin intermediate, in which gp41 is present simultaneously in both the viral and cellular membranes. The pre-hairpin intermediate ultimately transforms into a structure made of a trimer of hairpins (three hairpin-like structures assembled together) called the fusion-active state of gp41.

An anti-HIV vaccine would elicit antibodies from the host immune system that can neutralize HIV. Since gp120 is readily dissociated or shed from HIV because of its non-covalent association with the external portion, or ectodomain, of gp41, a major challenge in vaccine research has been to preserve completely the two types of glycoproteins together in vaccine preparations. To date, such efforts have relied on attempts to stabilize the glycoprotein complex by introducing disulfide bonds between the gp41 ectodomains or between the gp120 and gp41 subunits.

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We have shown that a laboratory-created variant of SIVmac251, called CPmac, exhibits a remarkably stable association between the gp120 and gp41 subunits. In contrast to previously described HIV isolates, gp120 and gp41of this mutant remain associated during destruction of HIV by ionic detergents and can be isolated by monoclonal antibodies targeted to either gp120 or gp41. This unique property results from five amino acid substitutions in the gp41 ectodomain.

To understand why the gp120-gp41 structure was stable in CPmac, we sought to delineate the role of five CPmac mutations in the folding, thermodynamics, and conformation of the gp41 ectodomain. Crystallographic studies show that the CPmac mutant sequences fit to the trimer-of-hairpins structure of gp41. Thermal unfolding studies show that the gp41 trimer-of-hairpins structure is thermodynamically coupled to the stability of the gp120/gp41 complex.

Our results show that the gp120/gp41 complex could be stabilized by introducing mutations that destabilize the hairpin structure. If this idea proves correct, our results may lead to a novel strategy for stabilizing the HIV envelope glycoprotein complex and using it in structural and immunogenicity (induction of immune response) studies.

Crystal structure of the CPmac gp41 core. This structure is very similar to the trimer-of-hairpins structure present in the ectodomain of the glycoprotein 41 (gp41) of the parent simian immunodeficiency virus SIVmac251. (Left) Lateral view of superposition of the backbone traces for the wild-type (green) and CPmac (red) gp41 cores. The amino-terminal helices point toward the bottom of the page and carboxyl-terminal helices point toward the top. (Right) Axial view looking down the three-fold axis of the trimers.