X-Ray Analysis of a New Potent Anti-HIV Drug Actinohivin Overcoming Multi-Drug Resistance

The effectiveness of several inhibitor medicines currently being used to treat the HIV/AIDS pandemic is rapidly diminishing because of the appearance of resistant mutants through mutations of the target proteins. To overcome this multi-drug resistance problem, a new lectin actinohivin has been found to exhibit a potent anti-HIV activity. In order to clarify the specific interactions of actinohivin with the branched mannose moieties of the high-mannose type glycans (HMTG) bound to gp120 of HIV, the structure has been successfully solved by X-ray crystallography using synchrotron radiation at the Photon Factory of KEK. It has been revealed that actinohivin is composed of three similar structural modules associated with a pseudo three-fold symmetry. In each module, there is a valley with a pocket for carbohydrate-binding which may be able to accept two branched units of Manα(1-2)Man of an HMTG. This feature further suggests that the high specificity of actinohivin to gp120 might be ascribed to the ability to accept three HMTGs on gp120s.

Every year, about 3 million people are infected with HIV, and over 2 million people die with AIDS. Since HIV is a retro virus, its envelope protein gp120 participating in infection to human cells rapidly mutates. Therefore, there is little prospect of developing a vaccine at present. Although over 20 kinds of inhibitors targeting the enzymes essential for the life-cycle of HIV after it enters the cell are currently used as medicines for treating HIV/AIDS, their effectiveness has rapidly diminished with the appearance of resistant mutants through mutations of the target proteins. Based on a new idea of targeting HMTGs which are bound to gp120 (see Fig. (1)) in order to overcome the multi-drug resistance problem, we have found a lectin actinohivin (AH) which exhibits potent anti-HIV activity. In order to clarify the specific interactions of AH with branched mannose moieties of HMTG, the crystal structure of AH has been successfully solved by X-ray crystallography [1].

The X-ray data were taken at 100 K with synchrotron radiation (λ= 1.07 Å) at BL-5A, and the crystal structure was determined at 1.19 Å resolution. The two proteins found in the asymmetric unit have a similar structure, so the root-mean-square deviation between the corresponding C_α atoms is only 0.17 Å when superimposed on each other. Therefore, one of the two AHs is shown in [Fig. 2(a)]. The structure appears to be composed of three modules which are similar to each other, as speculated from the tandem repeats in the sequence. However, the folding is not so simple. Although the three modules contain a β-sheet with four anti-parallel strands and a short 3_{10} helix (π), except module 2, which has an additional β-strand, the secondary-structure topologies are slightly different between the modules. As seen in [Fig. 2(b)], the *N*-terminal β-strand (β_0) starts in module 2. The subsequent three β-strands (β_1, β_2 and β_3), a long loop (L) and a π-helix form module 1, which is followed by the second module (β_4, β_5, β_6, β_7, L and β_2) and the third module (β_8, β_9, β_{10}, β_{11}, L and β_3). The last β_{12}-strand completes the first module 1 so as to close the cyclic assembly. Only module 2 is structurally stabilized by forming a larger β-sheet with five β-strands.

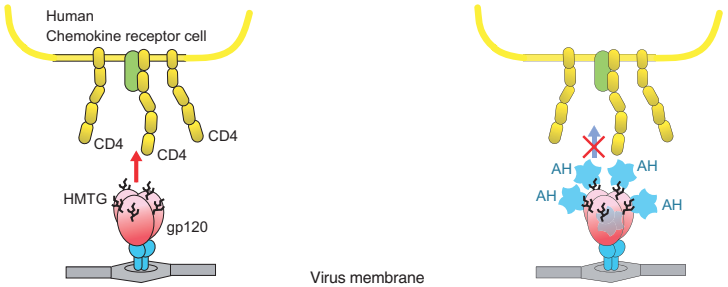

Figure 1
Schematic diagram of AH interference for gp120 binding to chemokine receptor of human CD^{4+} for the initiation of HIV entry.

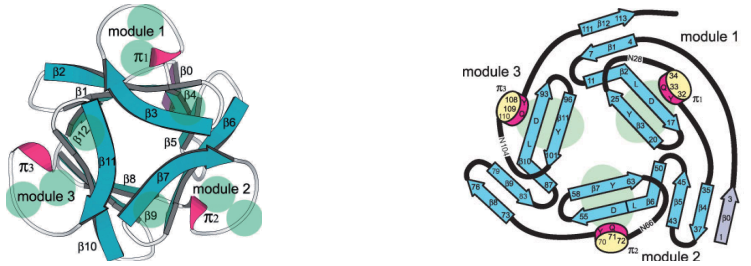

Figure 2
Structural features of AH. (a) Tertiary structure of AH. (b) Secondary structure topology. Ellipsoids colored in light green indicate the three Man-binding pockets composed of the LD-QXW motifs. Arrows, disks and values show β-strands, π-helices and residue numbers, respectively.

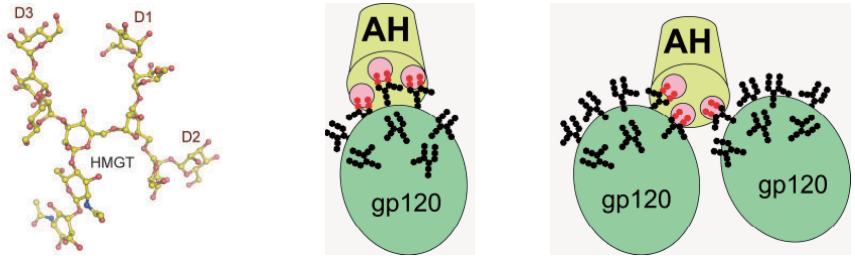

Figure 3
Three branched mannose chains of HMTG (a), AH bound to three HMTGs on a gp120 (b) and AH bridged between two gp120s (c).

The three modules are associated with a pseudo three-fold symmetry, in which the three β-sheets form a triangular barrel. Inside the barrel, hydrophobic residues form a stable core. On the outer surface, a long loop with a π-helix of each module runs from the top to the bottom of the barrel. This loop and the preceding β-strands containing the carbohydrate-binding motifs LD-QXW form a valley with a pocket for carbohydrate-binding, except W which participates in the central-core formation. The size of a valley is approximately 10 Å in length, 15 Å in width and 3 Å in depth, which creates enough space to accept two units of Manα(1-2)Man of an HMTG (see Fig. 3(a)). The binding assay with various fragments of HMTG suggests that the end of the D1 branch (Man4) accommodates in a pocket, and that the end of D2 or D3 (Man7 or Man9) contacts with a valley. Therefore, it is possible to speculate that each carbohydrate-binding pocket of the three modules of AH accepts the D1 and D2/D3 branches of Man8 and Man9; the combination of D1 and D3 may be preferred because both are closely located in their conformations. The three pockets are located to form an almost regular triangle at a distance of 17 Å between the aspartate residues in the three conserved motifs on AH. This separation might allow simultaneous acceptance of three HMTGs on a gp120 [Fig. 3(b)] and of those between the two neighboring gp120s, as shown in [Fig. 3(c)]. It is expected that further structural and functional studies will reveal the specific aspects of AH which lead to a safe microbicide to help prevent HIV transmission.

REFERENCE

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