5A,NW12A/2010G088

Specificity and efficiency in activity of anti-HIV actinohivin for sugar binding

Masaru TSUNODA[1*], Kaoru SUZUKI[2], Atsushi TAKAHASHI[1], Jiandong JIANG[3], Md. Mominul Hoque[1], Haruo TANAKA[1] and Akio TAKENAKA[1,3]

[1]Faculty of Pharmacy, [2]Faculty of Science and Engineering, [3]Graduate School of Science and Engineering, Iwaki Meisei University, 5-5-1 Chuodai iino, Iwaki, Fukushima 970-8551, Japan

Introduction

HIV/AIDS is a major health concern, a global pandemic which remains a relatively uncontrolled infectious disease. Over 20 kinds of inhibitors targeting the HIV enzymes (e.g. reverse transcriptase, integrase and protease) are currently used as medicines to disturb the HIV lifecycle after HIV entry into cells. These antiretroviral drugs have recently been evaluated further for their dual effects as treatments and in preventing HIV infections. In addition, some proteins which are able to bind the surface glycoprotein of HIV are now expected to prevent HIV entry into cells.

This effect on entry inhibition is also applicable to help suppress the spread of infection. Structurally, trimeric gp120 protruded from the HIV surface binds to human CD^{4+} cell to initiate entry. Each gp120 is highly glycosylated to cover the surface with highmannose type glycans (HMTG). As candidates for suppressing gp120-binding to susceptible cells, several carbohydrate-binding proteins (lectins) have been isolated and characterized. We succeeded in discovering a new lectin, actinohivin (hereafter designated AH) from an actinomycete, *Longispora albida*. This lectin possesses a potent, specific anti-HIV activity to inhibits the entry of various HIV-1 and HIV-2 strains into susceptible cells, as well as T-cell-tropic and macrophage-tropic syncytium formation. Therefore, we have at first determined the crystal structure of the *apo* form[1] and then that in complex with Man-α(1,2)-Man (hereafter MB) of HMTG by X-ray analyses.

Experimental

Crystallization

AH for crystallization was prepared several times. Acetonitrile-treated, lyophilized AH was dissolved in water and its concentration was adjusted to 20mg/ml. An MB solution (in water) was adjusted to the same concentration. The two solutions were mixed at equal volume to prepare a protein solution for crystallization. Crystallization screening of AH in complex with MB was carried out by the hanging-drop vapor-diffusion method at 298 K. In each well, a droplet of protein solution (2.0μl) mixed with the same volume of the reservoir solution was equilibrated to the reservoir solution (700μl). Commercially available crystallization kits were applied for the reservoir solution at the initial trial. From several conditions under which crystalline precipitates appeared, a suitable one was further optimized.

X-ray data collection

As crystals were obtained from a solution containing 50% (v/v) MPD, it was regarded that they were already cryo-protected. A crystal suitable for X-ray experiment was picked up and mounted into a Cryo-Loo. X-ray experiments were performed at 100 K using the synchrotron radiation with wavelength of 1.00 Å in Photon Factory. Diffraction images were recorded on an ADSC 210r CCD detector. In total 180 frames of the patterns were taken at 1º oscillation steps with 1 second exposure per frame. Raw data images were indexed and intensities around Bragg spots were integrated to a resolution of 1.90 Å, and then scaled and merged using the computer program Mosflm of the CCP4 suite. Intensities were further converted to structure-factor amplitudes with TRUNCATE from the CCP4 suite.

Results and Discussion

The numbers of observed reflections were 68064 in a resolution range 27.84~1.90 Å and an R_{merge} value was reasonably low at 11.2% for 10409 unique reflections with the completeness of 99.9%. The unit cell is composed according to the space group $P22_12_1$ with the cell dimensions of a = 27.8, b = 66.8 and c = 67.1 Å. These habits are quite different from those of the previous crystal ($P2_13$, a=56.2 Å). The previous crystal has a 3-fold symmetry, around the axis AH is disordered by rotating 120°. However the present crystal has no such a 3-fold symmetry. Therefore it is expected there is no such disorder of AH molecule. A calculated Matthews coefficient (V_M) and the solvent content (2.49 $Å^3$/Da and 50.60%, respectively) suggest that the asymmetric unit contains one AH molecule. This crystal could reveal not only the detailed interaction geometries, but also other effects on both structures of AH and the bound MB.

Reference

[1] H. Tanaka, et al. *Proc. Natl. Acad. Sci. USA*, 106, 15633-15638. (2009)

*masaru@iwakimu.ac.jp