BL-5A, NE-3A, NW-12A/2011G124, 125

Crystal structures of beta-glucan recognition protein from *Plodia interpunctella*

Mayumi KANAGAWA[1], Tadashi SATOH[1], Akemi IKEDA[1], Yoshiyuki ADACHI[2], Naohito OHNO[2], Yoshiki YAMAGUCHI[1*]

[1]RIKEN Advanced Science Institute, Wako, Saitama 351-0198, Japan, [2]Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

Introduction

Beta-glucan recognition protein (beta-GRP), a pattern recognition receptor of an innate immune system, evokes Toll pathway by binding to beta-glucan in pathogens. Beta-GRP binds to triple helical beta-glucan but not to denatured beta-glucan or shorter beta-1,3-linked glucose oligomers. However, details of the structural basis of the biological activity of beta-glucan have not been elucidated. Here we report how the triplex beta-glucan is recognized by beta-GRP [1].

Experimental Procedure

The $(His)_6$-MBP-TRX-fused *Plodia* beta-GRP N-terminal domain was purified with a Ni-Sepharose column and then digested with TEV protease. The MBP-TRX tag was removed by chromatography through a Ni Sepharose column. The protein was further purified by size exclusion chromatography. Beta-GRP-N crystals were obtained by the sitting drop vapor diffusion method with ligand laminarihexaose or without ligand and data sets were collected at PF BL5A, PF-AR NW12A and NE3A beamlines. The crystal structures were solved by molecular replacement method using a program Molrep. The refined structures of beta-GRP-N with ligand and without ligand have a crystallographic *R*-factor of 22.2% (R_{free} = 26.9%) in the 20.00-2.20 Å resolution range and 20.7% (R_{free} = 23.8%) in the 20.00-1.58 Å, respectively.

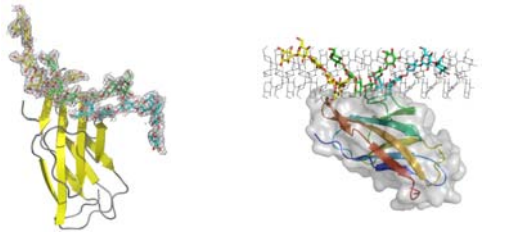

Fig. 1 Crystal structure of beta-GRP-N with ligand (left) and a structural model for binding between with beta-GRP-N and triplex beta-glucan (right).

Results and Discussion

The *Plodia* beta-GRP-N has an immunoglobulin-like beta-sandwich fold composed of two antiparallel beta-sheets containing three or five beta-strands (Fig. 1). There is little structural difference between liganded and unliganded forms of beta-GRP-Ns. Surprisingly, the laminarihexaoses are arranged in a right handed helical structure and fits well with beta-1,3-glucan triple helix structure derived from x-ray-fiber diffraction. Ligand binding of beta-GRP-N is attained through convex beta-sheet and a characteristic long loop. Six glucose residues from three laminarihexaose chains are involved in binding to beta-GRP-N, and eight amino acid residues show extensive polar and non-polar interactions. Our crystallographic data provide a structural basis for the unique recognition by such receptor of the triple helical structure of beta-1,3-glucan.

Reference

[1] M. Kanagawa et al., *J. Biol. Chem.*, **286**(33):29158-29165, (2011).

*yyoshiki@riken.jp