Crystal Structure of PILRα bound with *O*-Glycan and its Attached Peptide

Herpes simplex virus-1 (HSV-1) is the prototype of the Herpesviridae family and causes mucocutaneous lesions as well as lethal encephalitis. Recently, an entry receptor for one of the essential HSV-1 surface proteins, glycoprotein B, has been identified to be human paired immunoglobulin(Ig)-like type 2 receptor a (PILRα). We have revealed that a sialylated *O*-linked sugar attached peptide of HSV-gB (SnT-T(GPA)PAP) is an essential unit for PILRα-HSV-gB recognition and determined the free and the ligand bound structures, showing that PILRα recognizes both a sialic acid of the *O*-glycan and HSV-gB peptide sequence, and notably, exhibited large conformational rearrangements.

HSV-1 is well known for its extreme difficulty of complete elimination, because it can be latent in ganglia after infection and the reduction of immune responses can potentially induce the recurrence of herpes. To date, some reports showed that several viral envelope proteins are prerequisite for entry and infection. Among them, glycoprotein D (gD) is known to recognize herpes virus entry mediator (HVEM) and nectin-1. On the other hand, recently it was shown that human paired immunoglobulin-like type 2 receptor α (PILRα) associates with gB of HSV-1 and mediates membrane fusion during HSV-1 infection [1] (Fig. 1).

To gain structural insights for HSV-gB-PILRα recognition relevant to HSV-1 infection, we prepared the recombinant human PILRα protein comprising the critical V-set Ig-like domain (residues 13-131, hereafter designated as PILRα) and successfully crystallized the protein [2]. We determined the crystal structure of free PILRα at 1.4-Å resolution [3]. To calculate the experimental phases, iodide-anion derivative crystals were made by soaking in cryoprotectant solution including 1 M KI for 30 seconds and 1.8-Å SAD data were collected at beamline BL-17A. The PILRα ectodomain harbors a V-set Ig-like β-sandwich fold [Fig. 2(a)]. A DALI search identified sialoadhesin/Siglec(sialic-acid-binding-immunoglobulin-like-lectin)-1 [4] as the closest structure of PILRα (r.m.s.d. = 1.6 Å for 97 Cα atoms (PDB ID: 1URL)). The PILRα structure exhibits a similar structure and topology to the reported structures of members (Siglec-1, -5 and -7) of the Siglec family [5, 6], which consists of 14 Siglecs in humans. Furthermore, we successfully crystallized and determined the structure of the complex of PILRα with a sialylated *O*-linked sugar (sialyl T antigen, sTn) attached to a peptide of HSV-gB protein (residues 50-56), NH_2-GPAT(sTn)PAP-CO_2H, as described above [7]. The overall structure of the complex state was similar to the free form and the omit map of electron densities for the HSV-gB peptide was clearly identified [Fig. 2(b)]. However, PILRα unexpectedly induced large structural rearrangements to confer the simultaneous binding sites for both sugar and peptide regions of this *O*-glycosylated HSV-gB peptide [Fig. 2(b)].

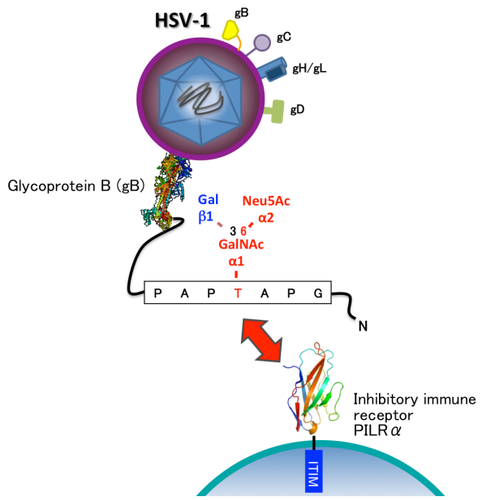

Figure 1: Schematic depiction of the interaction between gB from HSV-1 and PILRα. The *O*-linked GalNAc-(Gal)-Neu5Ac and attached N-terminal loop region of gB used in this study are highlighted.

Our structural analysis revealed the molecular details of protein-*O*-glycosylated peptide recognition, which is not well understood, with another example of the complex structure of P-selection (the polypeptide alpha-*N*-acetylgalactosaminyltransferase (pp-GalNAc-T10) complexed with GalNAc-*O*-Ser was crystallized but its structure unfortunately demonstrated no electron density for the serine residue) [8]. The PILRα residues that are contacting with the sialylated *O*-linked sugar as well as the Pro-rich peptide are essential in forming the unique compact conformation of the HSV-1 gB sTn peptide. They are highly conserved in human and

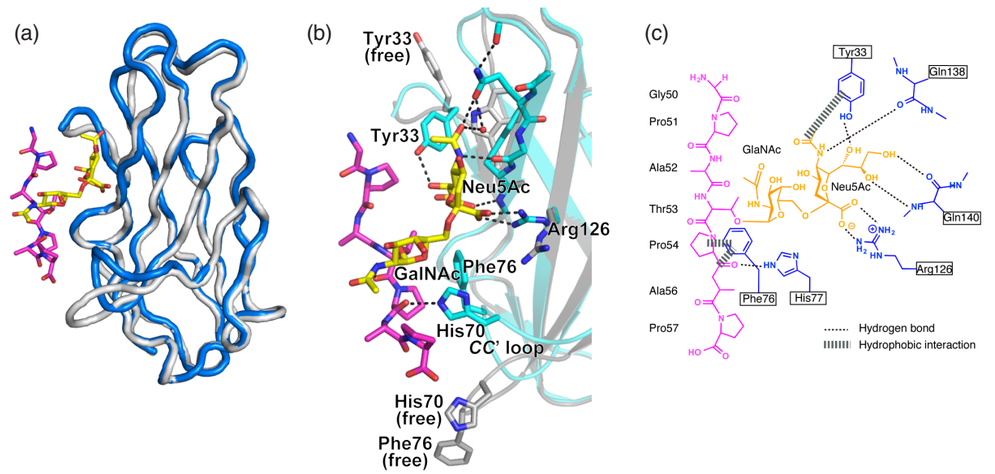

Figure 2: Interaction between SnT-T(GPA)PAP from HSV-1 and PILRα. (a) Superposition of the PILRα–sTn peptide complex (dark blue) with the free form of PILRα (gray) is shown in the tube model. The sTn-T(GPA)PAP peptide is shown as a stick model. The glycopeptides are colored yellow (sugar) and magenta (peptide), respectively. (b) Detailed view of the interaction between PILRα (cyan, stick and cartoon). The free form of PILRα (gray) is also presented. The glycopeptides are colored as (a). (c) Schematic representation of the simultaneous recognition by PILRα of the *O*-glycan and the attached peptide of the sTn peptide. Sialic acid (yellow), GalNAc (yellow), the peptide region (magenta), and PILRα residues (blue) are shown.

mouse PILRs, suggesting that this mode of sialylated O-glycosylated peptide recognition is maintained in species even though some distinct specificities exist. The way the PILRα contacts the sugar peptide is radically different from the previous descriptions of Siglecs in complex with sialylated sugar molecules. Tyr33 and Trp139 recognizing the glycerol group of a sialic acid are not well structurally conserved in the Siglec family members, possibly ensuring the adaptability of the recognition toward distinct sugar structures. Furthermore, the *CC'* loop of PILRα was significantly changed to fit both sugar-peptide connection and peptide regions, but those of other Siglec family members were basically not affected by the sugar binding. PILRα thus has a wider range of binding interface to simultaneously recognize both the *O*-linked sugar and its peptide [Fig. 2(c)].

The complex formation accompanied a wide range of conformational changes, which are possibly one of the largest ones among Ig-like domains, emphasizing that the ligand bound structure is essential for designing anti-HSV-1 entry inhibitor drugs. Importantly, the ligand specificity of PILRα seemed to be determined by the Thr53-Pro54-Ala55-Pro56, because the N-terminal GPA peptide region was not largely involved in the PILRα complex formation.

HSV-1 is difficult to eliminate completely, and dampened immune responses induce herpes recurrence [9]. HSV-1 seems to have evolved to utilize PILRα as an entry receptor for gB, as well as to suppress broad immune responses. The gB-PILRα interaction is crucial for HSV-1 to infect PILR-expressing cells, such as monocytes. Importantly, the sTn peptide definitely inhibited the entry of HSV-1 and thus can serve an initial frame for a potential therapeutic compound which may contribute to the rational design of antiviral drugs.

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