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Structure of the saccharide-binding domain of the human natural killer cell inhibitory receptor p75/AIRM1

Nazzareno Dimasi,[a]* Alessandro Moretta,[b,c] Lorenzo Moretta,[a,c] Roberto Biassoni[a] and Roy A. Mariuzza[d]

[a]Istituto Giannina Gaslini, Largo Gerolamo Gaslini 5, 16147 Genova, Italy, [b]Dipartimento di Medicina Sperimentale, Sezione di Istologia, Università di Genova, Via Marsano 10, 16132 Genova, Italy, [c]Dipartimento di Medicina Sperimentale, Sezione Patalogia Gernerale, Università di Genova, Via Alberti 2, 16132 Genova, Italy, and [d]Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, Maryland 20850, USA

Correspondence e-mail: ndimasi@email.it

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The high-resolution crystal structure of the functional N-terminal domain from the extracellular region of the human natural killer cell inhibitory receptor p75/AIRM1 or Siglec-7 has been determined at 1.45 Å resolution; it was obtained from a crystal belonging to a primitive monoclinic space group, with unit-cell parameters $a = 32.65$, $b = 49.72$, $c = 39.79$ Å, $\alpha = \gamma = 90$, $\beta = 113^\circ$. The structure reported here belongs to a different space group than the previously described Siglec-7 structure and was obtained using a bacterial expression system. The structure unveils the fine structural requirements adopted by a natural killer cell inhibitory receptor of the Siglec family in target-cell recognition and binding.

1. Introduction

Siglecs, expressed by specific cell types, are a structurally related protein family that bind carbohydrates, particularly sialic acid oligosaccharides (Crocker, 2002). Based on differences in the amino-acid sequence, they can be divided into two subgroups. The first subgroup, embracing Siglec-1, Siglec-2 and MAG (myelin-associated glycoprotein), share about 30% amino-acid sequence identity in the extracellular region, but display different cytoplasmic tails. The second subgroup, including the CD33-related Siglec, share 50–80% sequence identity in the extracellular region and have two highly conserved tyrosine-based motifs in their cytoplasmic tails that are involved in inhibitory functions (Crocker & Varki, 2001). p75/AIRM1, also termed Siglec-7, is a type I transmembrane glycoprotein belonging to the CD33-related Siglecs and displays an amino-terminal V-domain and two C2-set extracellular Ig-like domains. It is the major Siglec identified in natural killer (NK) cells, but is also expressed on normal and leukaemic myeloid cells. Interestingly, it is not only involved in the inhibition of NK-mediated cytolysis (Falco *et al.*, 1999), but is also able to inhibit the proliferation of both acute (AML) and chronic (CML) myeloid leukaemic cells (Mingari *et al.*, 2001). In view of its potential clinical interest, we report the high-resolution crystal structure of the extracellular IgV-like domain of Siglec-7 (referred to herein as the p75/AIRM1 structure) determined using a previous Siglec-7 crystal structure with a different space group as a model.

2. Materials and methods

Cloning, protein expression, *in vitro* folding, purification and crystallization have been described previously (Dimasi *et al.*, 2003). The structure was solved by molecular replacement in *AMoRe* (Navaza, 1994), using the Siglec-7 structure (PDB code 1o7s) as a search model (Alphey *et al.*, 2003), in space group $P2_1$. A single solution was found with an R factor of 0.48 and a correlation coefficient of 0.29. The model phases were used as an input for *warpNtrace* (Perrakis *et al.*, 1997), which built 98 of 131 residues. This model was refined using iterative rounds of *CNS* (Brünger *et al.*, 1998) and manual model building in *O* (Jones *et al.*, 1991). Subsequent rounds of refinement were performed using *SHELX*97 (Sheldrick & Schneider, 1997), which included anisotropic B factors. The final model ($R_{\rm cryst}$ = 17.1% and $R_{\rm free}$ = 21.8%) lacks two N-terminal residues, the C–C' loop (residues 69–73) and two C-terminal residues. Refinement statistics are summarized in Table 1.

3. Results and discussion

The investigation of the roles of carbohydrate-binding proteins that mediate processes central to immune regulation by interacting with carbohydrate groups expressed on the cell surface is a new and interesting research area. p75/AIRM1, also known as Siglec-7, is an inhibitory receptor expressed by human natural killer cells, the function of which is to inhibit their cytolytic activity. We have begun dissecting the function of p75/AIRM1 both *in vitro* and *in vivo*, with a final goal of identifying its cellular or viral counter-ligand. To this end, we have proceeded to characterize the biochemical and biophysical binding parameters for potential sialic acid oligosaccharide ligands (N. Dimasi *et al.*, in preparation). In parallel, we have crystallized (Dimasi *et al.*, 2003) and determined the X-ray crystal struc-

Table 1
p75/AIRM1 (Siglec-7) IgV-like domain X-ray data-collection and refinement statistics.

Values for the highest resolution shell (1.51–1.45 Å) are given in parentheses.

Data collection	
Source	F1 beamline, CHESS
Wavelength (Å)	0.934
Temperature (K)	100
Resolution (Å)	1.45
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 32.65, b = 49.72, c = 39.79, $\alpha = \gamma$ = 90, β = 113
Mosaicity (°)	0.59
No. observations	238401
Unique reflections	20819
Data completeness (%)	89.3 (68.6)
$\langle I/\sigma(I)\rangle$	15.3 (2.5)
$R_{\rm merge}$† (%)	6.0 (38.8)
Refinement	
Resolution range (Å)	15–1.45
$R_{\rm cryst}$‡ (%)	17.1
$R_{\rm free}$§ (%)	21.8
No. water molecules	67
R.m.s. deviations from ideality	
Bond lengths (Å)	0.010
Bond angles (°)	1.64
Ramachandran plot statistics¶	
Most favoured (%)	95.8
Allowed (%)	4.2
Disallowed (%)	0.0

† $R_{\rm merge} = \sum_h \sum_i |I_{hi} - \langle I_h\rangle| / \sum_h \sum_i I_{hi}$, where F_c is the calculated structure factor. ‡ $R_{\rm cryst} = \sum ||F_o| - |F_c|| / \sum |F_o|$, where F_c is the calculated structure factor. § $R_{\rm free}$ is as $R_{\rm cryst}$, but calculated for a randomly selected 4.0% of reflections not included in the refinement. ¶ As calculated by *PROCHECK* (Laskowski *et al.*, 1993).

ture of the p75/AIRM1 saccharide-binding domain at 1.45 Å resolution (Table 1; Fig. 1*a*). The p75/AIRM1 structure described here was obtained in space group $P2_1$, which differs from the previously determined Siglec-7 crystal structure ($P4_12_12$) used as a search model in the molecular replacement (Alphey *et al.*, 2003). Key features of the Siglec-7 structure as reported previously (Alphey *et al.*, 2003) are summarized below. Following this brief description, differences between the p75/AIRM1 and Siglec-7 structures are highlighted.

The Siglec-7 and p75/AIRM1 structures show a classical IgV-like domain organization characterized by a β-sandwich formed by β-sheets *ABED* and *A′G′ FCC′* (Fig. 1*a*). A feature of the p75/AIRM1 structure is the presence of an intra-sheet disulfide bond between Cys46 and Cys106 (Fig. 1*a*) that is common to all sialoadhesin family members (May *et al.*, 1998). This disulfide bond replaces the inter-sheet disulfide bridge commonly observed in other Ig-like domains. In p75/AIRM1, the Cys in strand *F* observed in other Ig-like domains is replaced by Phe123 (Fig. 1*a*), which packs against the disulfide bridge. The absence of the inter-sheet disulfide bridge in p75/AIRM1 results in an opening and extension of the two β-sheets and may expose residues in order to facilitate ligand recognition or confer conformational plasticity on the binding site, which is needed to accommodate variability within the potential oligosaccharide ligands (N. Dimasi *et al.*, in preparation; Yamaji *et al.*, 2002; Nicoll *et al.*, 2003).

As for Siglec-1 (May *et al.*, 1998), the putative carbohydrate-binding site in p75/AIRM1 can be located between strands *A* and *G* (Fig. 1*b*). Of particular note is that the partial opening and widening of the p75/AIRM1 β-sandwich caused by the absence of the inter-sheet disulfide bridge, as discussed previously, provides a very large flat surface that perfectly matches the surface area in the 3′-sialyllactose–Siglec-1 complex structure (Fig. 1*b*). In addition to a conserved arginine at position 124 in p75/AIRM1 (Figs. 1*a* and 1*b*), which forms a salt bridge with the carboxylate group of sialic acid in the 3′-sialyllactose–Siglec-1 complex (May *et al.*, 1998), the putative binding site of p75/AIRM1 contains a number of hydrophobic and basic residues that may contribute to the binding specificity (Fig. 1*b*).

The Siglec-7 structure involves residues 18–144, whereas the p75/AIRM1 structure involves residues 18–150, *i.e.* six more residues at the C-terminal end. The most important aspect of the structure described here when compared with the Siglec-7 structure is that the p75/AIRM1 structure was obtained using a bacterial expression system, whereas the Siglec-7 structure was obtained using CHO mammalian cells. As a result, the crystal structure of Siglec-7 reveals a well defined Asn105-linked glycan. Conversely, due to the bacterial expression system, the p75/AIRM1 does not contain any glycan chain. To determine whether the non-glycosylated form differs from the glycosylated form and to gain insight into possible main-chain distortions arising from the glycan chain, 112 amino-acid residues forming the core of the p75/AIRM1 structure were superimposed on the structure used for molecular replacement (Siglec-7; PDB code 1o7s), obtaining an r.m.s. deviation of 0.5 Å. This value of the r.m.s. deviation indicates that the sugar chain does not significantly alter the overall structural arrangement of the protein and, in turn, supports the bacterial expression system as an efficient way to express p75/AIRM1 and other closely related members of the Siglec family in contrast to the time-consuming and more expensive mammalian expression system. Furthermore, we can use the high-resolution structure described here as a 'molecular probe' in order to locate this domain in our ongoing attempt to solve the full extracellular region of p75/AIRM1. This future work is aimed at the elucidation of the precise structural requirements for sialic acid recognition and binding by this natural killer inhibitory receptor.

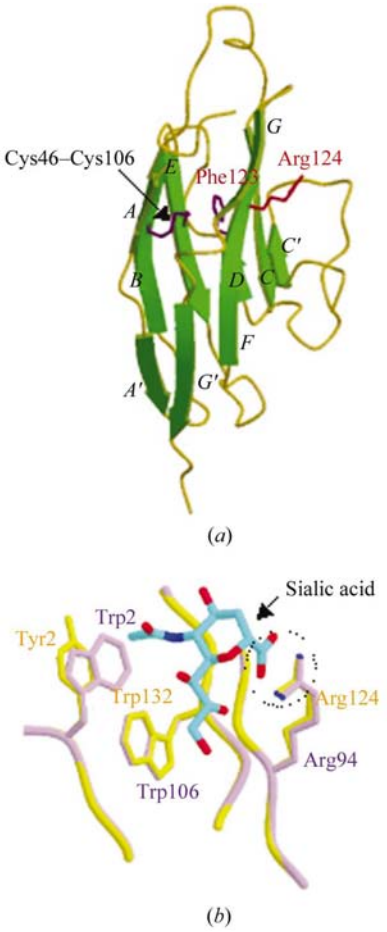

Figure 1
(*a*) N-terminal IgV-like domain of p75/AIRM1 (PDB code 1nko). β-Strands are labelled according to the standard immunoglobulin nomenclature. The intra-sheet disulfide bond between Cys46 and Cys106, and the Phe123 that replaces the Cys in standard Ig-like folds are shown in magenta. The position of Arg124 that is responsible for the primary binding to the N-terminal sialic acid in sialoadhesins is shown in magenta. (*b*) Superposition of p75/AIRM1 (in gold) and the Siglec-1–3′-sialyllactose complex (in violet) at the ligand-binding site (PDB codes 1nko and 1qfo, respectively). The terminal sialic acid in the 3′-sialyllactose complex structure is shown in ball-and-stick representation. The amino-acid side chains that contribute to the binding to the terminal sialic acid in the Siglec-1–3′-sialyllactose complex and the homologues in p75/AIRM1 are shown. The figure highlights the key position of Arg94 in Siglec-1 and Arg124 in p75/AIRM1, which forms a salt bridge with the carboxylate group of the sialic acid; site-directed mutagenesis studies (Vinson *et al.*, 1996) have shown that mutating this arginine residue completely abolishes the binding of Siglec-1 to sialic acid-containing oligosaccharides.

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addenda and errata

Structure of the saccharide-binding domain of the human natural killer cell inhibitory receptor p75/AIRM1. Erratum

Nazzareno Dimasi,[a]* Helen Attrill,[b,c] Daan M. F. van Aalten,[c] Alessandro Moretta,[d,e] Lorenzo Moretta,[a,e] Roberto Biassoni[a] and Roy A. Mariuzza[f]

[a]Istituto Giannina Gaslini, Largo Gerolamo Gaslini 5, 16147 Genova, Italy, [b]Division of Cell Biology and Immunology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, [c]Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, [d]Dipartimento di Medicina Sperimentale, Sezione di Istologia, Via Marsano 10, Università di Genova,Italy, [e]Dipartimento di Medicina Sperimentale, Sezione Patalogia Gernerale, Via Alberti 2, Università di Genova,Italy, and [f]Center for Advanced Research in Biotechnology, W. M. Keck Laboratory for Structural Biology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, Maryland 2085, USA. Correspondence e-mail: ndimasi@email.it

In the paper by Dimasi *et al.* [(2004), *Acta Cryst.* D**60**, 401–403] two authors were not included. The correct list of authors is given above. It should also be noted that Nazzareno Dimasi and Helen Attrill contributed equally to the work described in the paper, and the following acknowledgent also applies to the paper.

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