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Structural studies on ligand binding ability of Siglec-2 using molecular modeling techniques

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Abstract

Siglecs are attractive therapeutic target of the major homologous subfamily of I-type lectins. The primary role of Siglecs may actually lie in the recognition and phagocytosis of bacterial pathogens that express sialic acids, maintenance of myelin organization, and inhibition of neurite outgrowth, cell-cell interactions between neurons and glial cells etc. Siglec-2, a member of the Siglec family expressed on the surface of maturing B cells and B cell lymphomas and regulates signal transduction. In this work, 3-D structure of human Siglec-2 was predicted using molecular modeling techniques. The structure of the complex in solution of Siglec-2 with ligand, 6'-Sialyl-N-acetyl lactose (6'-SialylLacNAc) was predicted using a novel docking technique. The structural analysis of the complex and calculation of theoretical dissociation constant value will help to ascertain functional roles of such sugar binding protein.

Keywords: Modeling, sialic acid, Siglec-2, structure.

Introduction

The Siglecs are a specialized subgroup of the Ig super family that can recognize sialylated glycoconjugates (Crocker et al., 1998). Sialic acid (Neu5Ac) is an acidic, nine-carbon monosaccharide occurring glycocalyx on the cell surface. Multi-cellular organisms use the sialic acid conjugates for non-specific electrostatic repulsion between cell types and to mediate cell adhesion, protein–protein interactions, and protein trafficking via sialic acid recognizing receptors. (Blixt et al., 2003; Kelm et al., 1994; Powell and Varki, 1994; Brinkman-Van der Linden and Varki, 2000; Cornish et al., 1998; Nicoll et al., 1999; Varki, 1997; Karlsson, 1998; Crocker et al., 1998; Crocker and Varki, 2001). Siglecs are type 1 membrane proteins, recognizes sialylated glycoconjugates by an N-terminal sialic acid-binding V-set Ig domain which followed by a transmembrane domain, and a cytoplasmic tail and variable number of C2-set Ig-like domains (Angata et al., 2001). Siglecs can be divided into two subgroups: Sialoadhesin (Siglec-1), Siglec-2 (CD22), MAG (Siglec-4) and

Siglec-15 constitute one subgroup, share ~25–30% sequence identity in the extracellular region, and have divergent cytoplasmic tails and the second subgroup consists of the CD33-related Siglecs. They share 50–80% sequence similarity and have in their cytoplasmic tails two highly conserved tyrosine-based motifs. Expression of each human Siglec in a cell type-specific fashion, mainly in the hematopoietic and immune systems of humans, suggesting involvement in discrete functions ranging from regulation of neuronal cell growth and maintenance of myelination in the nervous system (MAG) (Li et al., 1994; Montag et al., 1994) and control of myeloid cell interactions (sialoadhesin) (Crocker et al., 1997) and CD33 (Freeman et al., 1995) to activation of B cells (CD22 [Cyster and Goodnow, 1997].

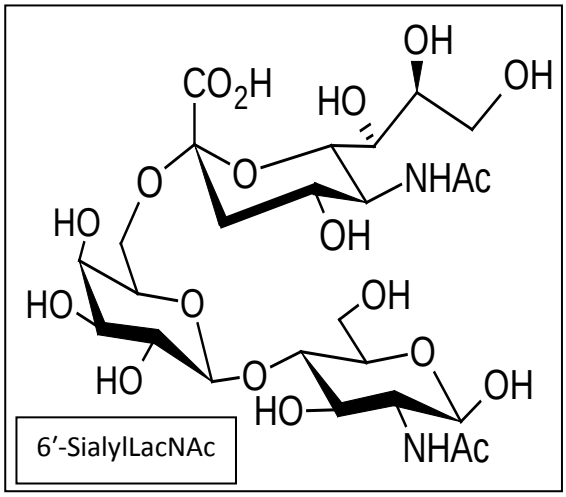

Figure 1. Glyco-chain structure of the ligand used in this study.

In the present study, I have predicted the 3-D structure of human Siglec-2 (hSiglec-2) along with the specific ligand, 6'-SialylLacNAc (Fig. 1). The structural analysis of the predicted complex was done. The theoretical dissociation constant value was also calculated for the complex which helped me to compare the relative binding affinity.

Materials and Methods

The starting scaffold for modeling was the x-ray crystallographically determined structure of *Mus Musculus* (PDB ID: 1QFP). The initial structure of hSiglec-2 was obtained using the LOOPP (Teodorescu et al., 2004; Meller and Elber, 2001; Tobi and Elber, 2000) server due to less sequence homology with mSiglec-1 and the structure was refined using our in-house software package of ANALYN and MODELYN (Mandal, 1998). Initial structure of the Siglec-ligand complex was obtained by the superposition of the modeled hSiglec-2 structure with the experimental structure of *Mus Musculus* (PDB ID: 1QFO) followed by repeated energy minimization and dynamics simulations. The structure was refined using DISCOVER module of InsightII 2005 of Accelrys (San Diego, CA). Structural optimization was done using cff91 force-field and energy minimization (100 steps each of steepest descent and conjugate gradient methods) followed by dynamics simulations. A typical dynamics run consisted of 100000 steps of one femto-second after 1000 steps of equilibration with a conformational sampling of 1 in 100 steps at 300K. At the end of the dynamics simulation, lowest potential energy conformation with was picked for the next cycle of refinement using the module ANALYSIS of InsightII. This combination of dynamics and minimization were repeated until satisfactory conformational parameters were obtained.

In order to investigate the influence of water on the ligand binding, water molecules were added as a sphere of radius 18Å having its center at an atom roughly at the center of the ligand molecule so as to surround it completely using the Assembly/Soak option of InsightII. In the aqueous environment, structure optimization of the ligand was done using energy minimization and molecular dynamics simulation in presence and absence

of the protein molecule. From the values of the free energies of complex formation of ligand in water and water-protein environments the absolute binding energy was calculated using the relation $\Delta G_{bind} = \alpha \Delta \langle V^{el}_{l\text{-}s} \rangle + \beta \Delta \langle V^{vdw}_{l\text{-}s} \rangle$ where ΔG_{bind} is the absolute binding energy, Δ stands for differences in the electrical ($V^{el}_{l\text{-}s}$) and van der Waals ($V^{vdw}_{l\text{-}s}$) components of the free energies of the ligand solvent (l-s) systems i.e., in pure water and protein containing water environments following the linear interaction energy approximation method of (Aqvist et al., 1994) The weight factors of the electrical and van der Waals contributions were taken respectively as 0.5 (α) and 0.16 (β) as proposed by Aqvist et al., and used by earlier workers (Aqvist and Mowbray, 1995; Hulte´n et al., 1997). Dissociation constant K_d was calculated by taking the inverse of K_a (association constant). K_a was calculated using the thermodynamic relation $\Delta G_{bind} = -RT\ln K_a$ where R is the ideal gas constant and T is the absolute temperature.

MODELYN was run on both on IBM-compatible PC in the windows environment and FUEL workstation of Silicon Graphics, Inc. in the IRIX environment. Altrix 350 server of Silicon Graphics, Inc. in the IRIX environment and FUEL workstation were used to run InsightII. The electrostatic potential surface of the protein was determined by MOLMOL (Koradi et al., 1996). For checking the structural parameters PROCHECK (Laskowski et al., 1993) was used. FUEL in the UNIX operating system was used to run both MOLMOL and PROCHECK. The binding affinity of the Siglec-ligand complex was obtained using the DOCKING module of InsightII.

Structure of the ligand was generated using the BUILDER module of InsightII followed by optimization with repeated energy minimization and molecular dynamics.

Results

General structural characteristics of the predicted model was determined by measuring all the bond distances and bond angles and calculating the deviation of these parameters from the standard values for appropriate types of bonds and angles. The quality of backbone conformations were determined by calculating the phi and psi dihedral angles and drawing Ramachandran's plots for the structure. Table 1 presents the RMSD (root mean square deviation) of bond lengths and bond angles of the predicted structure along with the percentages of backbone Phi-Psi angles in different areas of Ramachandran's plots obtained after the prediction of 3D structures.

RMSD from the respective standard values of the bond lengths around 0.02 Å and those of bond angles around 3 degrees indicate good general structural parameters of the modeled structure. The good quality of the backbone conformations of the modeled structure indicated by the values of above 95% Phi-Psi pairs in the core and allowed areas of Ramachandran's plot.

PROCHECK was used for side chain planarity of the planar groups in phenylalanine, tyrosine, tryptophan, histidine, arginine, glutamine, asparagines, glutamic acid, and aspartic acid and deviations from planarity were identified by measuring RMS(root mean square) distances of planar atoms from the best-fitted plane, residues having RMS distances >0.03Å for rings and 0.02Å for other groups were marked as outliers (Laskowski et al., 1993) (Table 2).

Table 1. General and backbone structural parameters of the modeled structure of the target sequence as well as the x-ray structures of the Siglecs.

Siglecs	Accession No	% of AA Identity (positive score)	RMS deviation		% of Phi-Psi pairs in the area			
			Bond (Å)	Angle (°)	Core	Allowed	Generously allowed	Dis-allowed
mSiglec-1	1QFP	100	0.018	2.33	77.2	19.8	2.0	1.0
mSiglec-1	1QFO	100	0.016	2.51	83.0	16.0	0.0	1.0
hSiglec-2	AAB06448	16	0.023	2.85	65.7	28.4	5.9	0.0

Table 2. General and backbone structural parameters of the modeled structure of the target sequence in comparison with the x-ray structures of the Siglecs.

Siglecs	Accession No	All atom clashcore (per 1000 atom)	Rotamer outliers (%)	Planarity outliers (%)
mSiglec-1	1QFP	3.16	3.42	0.0
mSiglec-1	1QFO	3.26	4.81	0.0
hSiglec-2	AAB06448	3.26	7.62	0.0

Table 3. Empirical free energies, their difference in water and water-protein environments and corresponding ΔG and K_d values for the complex formation between hSiglec-2 and the specific ligand in the aqueous solution. Abbreviations used in this table: VdW, van der Waals, Elect, electrical.

Complex	Free energy in kcals/mol			Difference		ΔG_{bind} in kcals/mol	K_d
	Vdw	Elect	Total	Vdw	Elect		
Siglec-2-6′-SialylLacNAc in solution	-73.45	-208.06	-281.51	+6.53	-10.78	-4.35	0.724 mM
6′- SialylLacNAc *	-79.98	-197.28	-277.26				
* Value corresponding to the interaction energy in presence of water molecules only.							

Table 4. Hydrogen-bond network within the binding site of hSiglec-2 in complex with 6′-SialylLacNAc. Distances are measured between hydrogen and acceptor or donor atom.

Ligand–protein hydrogen-bonds		
Atoms of 6′-SialylLacNAc	Atoms of hSiglec-2	Distance(Å)
Neu5Ac		
O1A	Lys-105:NZ	1.83
O1B	Arg-98:NH1	2.45
O10	Lys-105:N	2.48
O4	Thr-103:O	1.98
Intra-molecular hydrogen-bonds		
Atoms of 6′-SialylLacNAc	Atoms of 6′-SialylLacNAc	Distance(Å)
Neu5Ac O8	Neu5Ac O1B	2.19
Nag O4	Nag O6	2.10
Nag O7	Nag O1	1.78

Protein geometry of the modeled structure was checked by calculating clashcores and rotamer outliers using MOLPROBITY (Davis et al., 2004) (Table 2). Siglec-2 (CD22) is a B cell-specific glycoprotein of the Ig super family, highly expressed on the surface of maturing B cells and B cell lymphomas (Haas et al., 2006; Collins et al., 2006). Its extracellular domain contains seven Ig domains, of which the outermost N-terminal domain recognizes sialic acid containing glycan ligands, specifically α(2,6)-linked sialic acid through which CD22 can induce cell adhesion if the ligand is expressed on target cells (Collins et al., 2006). α(2,6) Sia is a common N-linked terminal carbohydrate which is expressed on several glycoproteins in the serum and also on the surface of several cell types, among them lymphocytes (Ghosh et al., 2006). In addition to regulating signal transduction through its cytoplasmic domain, CD22 regulates B cell development and function through ligand-generated signals (Haas et al., 2006). I have modeled the structure of hSiglec-2 applying threading method of 3-D structure prediction using LOOPP server as described in the materials and methods section. Due to less sequence similarity (only 16% AA identity) with the mSiglec-1, homology modeling is not applicable for the 3-D structure prediction of hSiglec-2. As studied earlier (Blixt et al., 2003) α(2,6) Sia-linked ligand 6′-SialylLacNAc (NeuAcα2,6Galβ1,4GlcNAc) has been docked into the binding site of the modeled structure to study the nature of interaction of the protein – ligand complex. At first ligand molecule was constructed using 'BUILDER' module of InsightII followed by optimization with repeated energy minimization and dynamics simulations. After that the ligand molecule was superposed taking the sialic acid part of the ligand with the equivalent part of the x-ray structure containing 3′-Sialyllactose (1QFO). Then the modeled protein is superposed with the 3′-Sialyllactose bound protein (1QFO) with respect to the structurally conserved regions followed by transfer of the superposed 6′-SialylLacNAc molecule to the binding site.

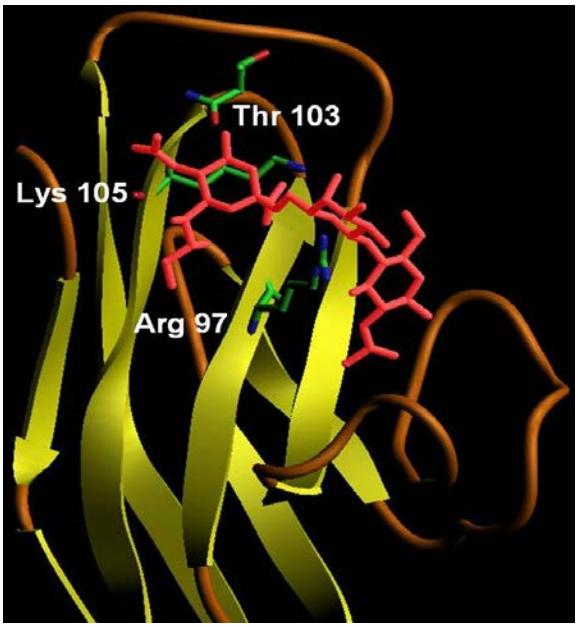

Figure 2. Mode of ligand binding in hSiglec-2: Ligand binding environment is shown in the secondary structure environment of the modeled lectin. Beta sheets are shown in yellow with an arrow indicating the C-terminus and random coils as thin cylinder coloured in maroon. The residues of the protein involved in hydrogen bonding with the ligand are shown in stick representation, coloured as atoms (C=Green, O=Red and N=Indigo) and the ligand 6′-SialylLacNAc is in red colour.

Optimization of the structure of the resulting complex was done by repeated molecular dynamics and energy minimization in presence of water as described in the materials and methods section. Values of ΔG_{bind} were calculated by the linear interaction energy approximation as described in materials and methods for the complex and presented in Table 3. It may be noted that the calculated ΔG_{bind} value for the complex of 6′-SialylLacNAc with hSiglec-2 is negative indicating that the complex formation of 6′-SialylLacNAc with the protein

in the aqueous medium is thermodynamically favorable. Values of ΔG_{bind} for 6'-SialylLacNAc -protein complex corresponds respectively to dissociation contestants (K_d) of 0.724 mM. The value is comparable with the experimental findings (Blixt et al., 2003). The essential interaction between Arg-97 and the sialic acid carboxylate group is conserved in the structure as reported in previous studies (Zaccai et al., 2003; May et al., 1998; Bukrinsky et al., 2004). Side chain of Lys-105 and Thr-103 (Fig. 2 & Table 4) are also involved in direct hydrogen-bonding with the ligand. The bound conformation of the ligand is stabilized by the three intra-molecular hydrogen-bonding.

Discussion

I have modeled the 3-D structure of human Siglec-2. The predicted structure was refined to obtain best backbone and side chain conformations by executing repeated molecular dynamics and energy minimization and picking the most reliable structure. Although, the structural models do not cover the entire sequence of these biochemical lectins, which participate in many crucial phenomena of the mammalian life process, my predictions were limited only to the extent of the experimental structures available for proteins homologous to the Siglec-2. None-the-less, the structure encompassed the important segments known to participate in their biological activities.

I have also predicted the structure of the complex of the modeled human Siglec-2 with the specific ligand, 6'-SialylLacNAc known so far from experimental studies. The nature of interactions of the ligand with the Siglec-2 was examined in details in order to under the origin of their specificity at the atomic levels. The involvement of the crucial amino acids, identified by experimental techniques, was confirmed from the modeled structure by exploring the involvement of evolutionary conserved amino acids. The participation of the various loop structures of the Siglec-2 in binding to the specific ligand was explored to understand their conformational implications. The chemical environment leading the stability of the bound ligand was analyzed in atomic details in presence of water molecules to simulate closely the aqueous environment. Binding constant was predicted for the modeled complex and compared with the experimental values. Thus, my structural studies using predicted model of human Siglec-2 and the complex with specific ligand, 6'-SialylLacNAc have contributed significantly in understanding the interactions involving sialic acid containing bioactive molecules which are implicated in many important biochemical phenomena.

Conflict of interest

Author declares that there is no conflict of interest.

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Abbreviations

Glc = Glucose; Gal = Galactose; GlcNAc = N-acetyl glucose; LacNAc = N-acetyl lactose; NeuAc/ Neu5Ac = N-acetyl neuraminic acid (sialic acid); hSiglec = human Siglec; mSiglec = mouse Siglec; sia = sialic acid.

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