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Comparison of the Conformational Behavior of Sialyllactose Complexed with the two Viral Attachment Proteins Influenza A Hemagglutinin and the Murine Polyomavirus[$]

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Abstract

The attachment of a virus to the host cell surface is influenced by enthalpic and entropic factors. A detailed evaluation of all possible energetic interactions including the effects of solvent molecules seems to be a promising way to gain deeper insights in the overall process of binding. Here we performed intensive molecular dynamics studies to compare the conformational space available for the unbound sialyllactose in aqueous solution and when complexed with influenza A hemagglutinin and the murine polyoma virus. In general the conformational freedom of sialyllactose is considerably reduced compared to the free state. Remarkably, two different conformations of the Siaα(2-3)Galβ glycosidic linkages are preferred (which are both populated in the free state) when complexed with either protein.

Keywords: Protein-Carbohydrate interactions, MD simulations, Flexibility, Sialyllactose, Influenza A virus hemagglutinin, Murine polyoma virus

Introduction

Recognition of and stable binding to an appropriate receptor on the surface of the host cell by a virion is the first step of viral infection. Receptor recognition has to be selective and specific because virions which are bound to non-receptor structures cannot initiate infection. Gangliosides are sialic acid (N-acetyl-neuraminic acid) containing glycosphingolipids that are ubiquitous components of mammalian plasma membranes. Sialyllactose (SL) (Neu5Acα2-3Galβ1-4Glc1-1Ceramid) (Figure 1) is the sugar head group of the GM3 ganglioside. Despite this ubiquity, such oligosaccharides appear to be an essential receptor component of many animal viruses from different virus families, such as influenza A and C viruses, Newcastle disease, cardioviruses and murine and primate polyoma viruses. For such viruses enzymatic removal of sialic acids from cell surfaces leads to loss of virion binding, demonstrating the essential role of sialic acid for this initial step.

The determination of two high resolution crystal structures of viral attachment proteins - influenza A hemagglutinin (HA) [1] (Figure 2) and the murine polyoma virus [2] (Figure 3) - has provided opportunities for a detailed analysis

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Table 1. *Glycosidic linkages: mean values of the trajectories for Φ,Ψ in degrees and their standard deviations in the free and the bound state*

	free state		hemagglutinin		polyoma	
	mean	std.dev.	mean	std.dev.	mean	std.dev.
Φ (Siaα(2-3)Galβ)	94	22	-60	9	-164	11
Ψ (Siaα(2-3)Galβ)	22	14	12	11	-20	10
Φ (Galβ(1-4)Glc)	41	17	42	9	39	14
Ψ (Galβ(1-4)Glc)	-11	17	3	12	-13	15

and comparison of the structural features and the dynamics of the protein-sialyllactose complexes.

The loss of rotational, translational and conformational freedom that accompany the formation of a ligand-receptor complex are entropic in origin and have to be compensated by the largely attractive enthalpic components of the energy of binding. In cases where the bound conformation of a flexible molecule is close to its lowest energy conformation the resulting penalty for this contribution will be small and only the loss in internal rotational entropy has to be regarded [3,4].

It has been experimentally proven that oligosaccharides generally exhibit several conformations in equilibrium in solution. However, since usually only one unique conformation is significant for a specific oligosaccharide-macromolecule interaction, it is important to explore in detail the conformational space which is accessible by the carbohydrate. Several NMR, molecular mechanics and molecular dynamics studies of isolated oligosaccharides containing sialic acids in different solvents showed [5-7] that several of the possible conformations are populated.

Aiming towards a better understanding of the binding process of a carbohydrate to a protein surface we performed intensive molecular dynamics studies to compare the conformational space available for the ligand in aqueous solution in comparison to the conformational space when complexed with the virus.

Methods and materials

The 3D-structure of HA (entry 1HHG) and murine polyoma virus VP1 (entry 1SID) both complexed with SL were ex-

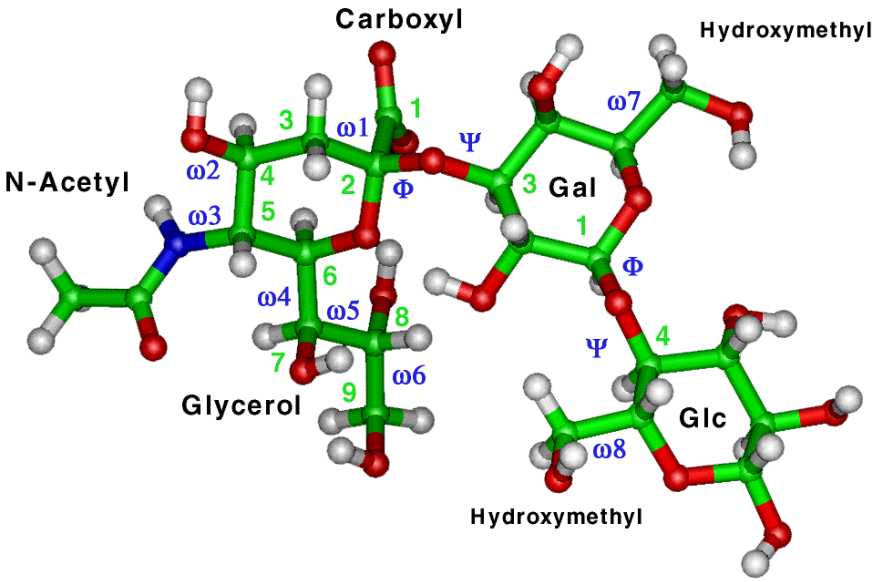

Figure 1. *3D structure and numbering of sialyllactose (green: carbon, red: oxygen, blue: nitrogen, white: hydrogen).*
Definition of torsional angles:
Φ(Siaα(2-3)Galβ) = C1-C2-O-C3;
Ψ (Siaα(2-3)Galβ) = C2-O-C3-H;
Φ (Galβ(1-4)Glc) = H-C1-O-C4;
Ψ (Galβ(1-4)Glc) = C1-O-C4-H;
ω1 = O1-C1-C2-C3;
ω2 = C3-C4-O-H;
ω3 = C4-C5-N-H;
ω4 = C5-C6-C7-C8;
ω5 = C6-C7-C8-C9;
ω6 = C7-C8-C9-O;
ω7(Gal)= C4-C5-C6-O;
ω8(Glc) = C4-C5-C6-O

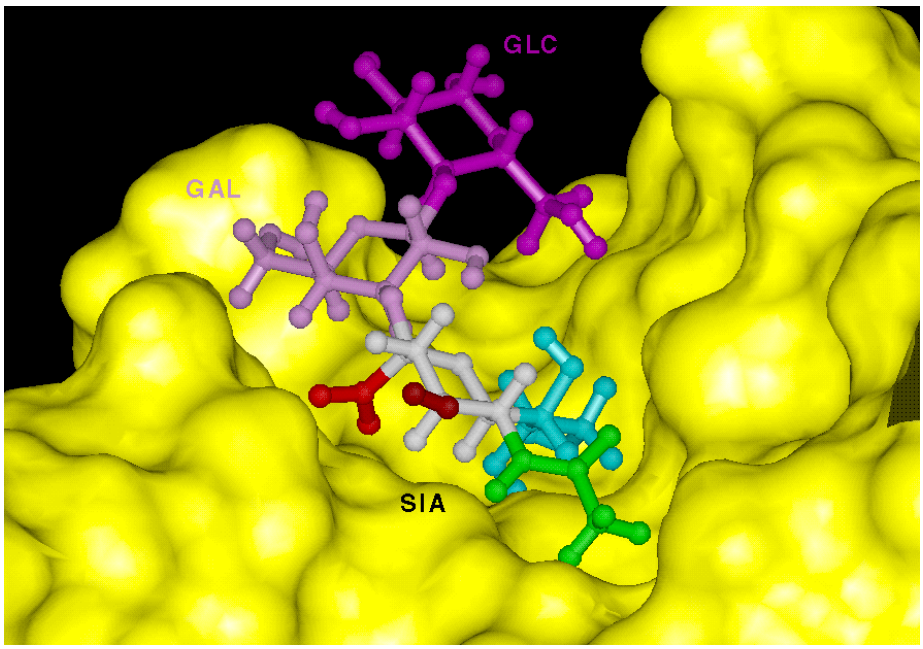

Figure 2. *Complex of sialyllactose and influenza virus A hemagglutinin as determined by X-ray crystallography (PDB-entry 1HGG) [1]. The figure shows the water-accessible surface of the protein colored in yellow. The sialic acid moiety of sialyllactose (SIA) is colored by subunit (ring: grey, glycerol side chain: cyan, 4-OH group: brown, carboxyl group: red, N-acetyl side chain: green)*

tracted from the Brookhaven Protein Database. The handling and visualization of the 3D molecular structure, the construction of missing H-atoms and the singling out of the receptor binding site was accomplished with the INSIGHTII (BIOSYM Technologies, 9685 Scranton Road, San Diego, CA 92121-2777) molecular modeling software.

Computational methods such as molecular mechanics, molecular dynamics or Monte Carlo simulations require an appropriate force field. The force field applied for study of protein-sugar complexes must correctly describe the potential energy surfaces of both, proteins and carbohydrates. For this study CVFF was selected. It is a well-established force field, devoted to describe the conformational behavior of proteins. Recently it has been reported that good agreement with experimental results for model oligosaccharides can be achieved using CVFF [7-9].

In order to reduce the required CPU time a volume of 20 Å around the SL was extracted from the X-ray structure. Only the residues forming the active site (all residues within a sphere of 10 Å around SL) and SL itself were completely relaxed during simulations. The positions of all other atoms of the protein were fixed but their non-covalent interactions with all other atoms were evaluated. A dielectric constant of 4 and a double cutoff of 18 and 20 Å for the non-covalent interactions was applied. No explicit water molecules were added to model the solvation effects. The potential types of

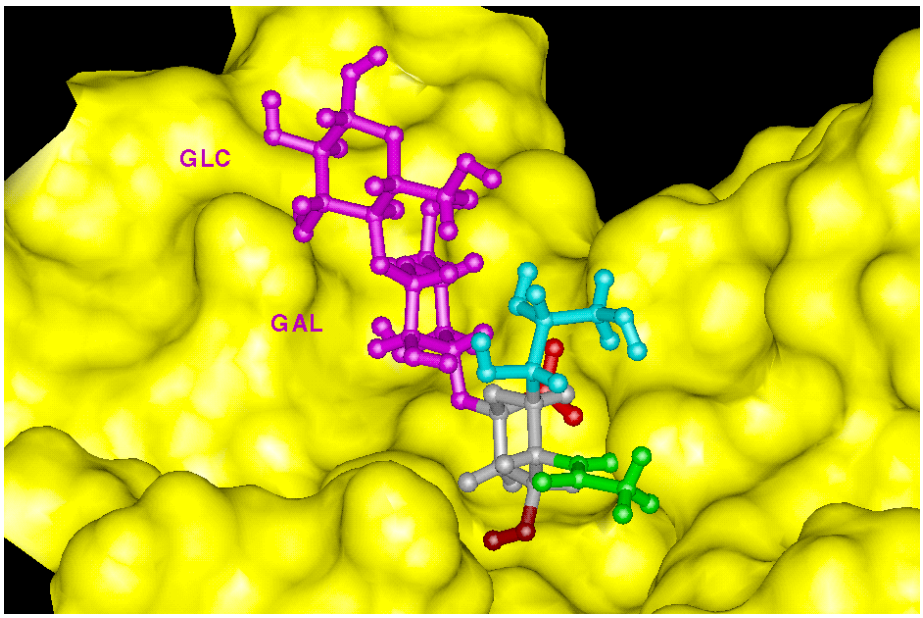

Figure 3. *Complex of sialyllactose and murine polyoma virus VP1 protein as determined by X-ray crystallography (PDB-entry 1SID) [2]. The figure shows the water-accessible surface of the protein colored in yellow. The sialic acid moiety of sialyllactose (SIA) is colored by subunit (ring: grey, glycerol side chain: cyan, 4-OH group: brown, carboxyl group: red, N-acetyl side chain: green).*

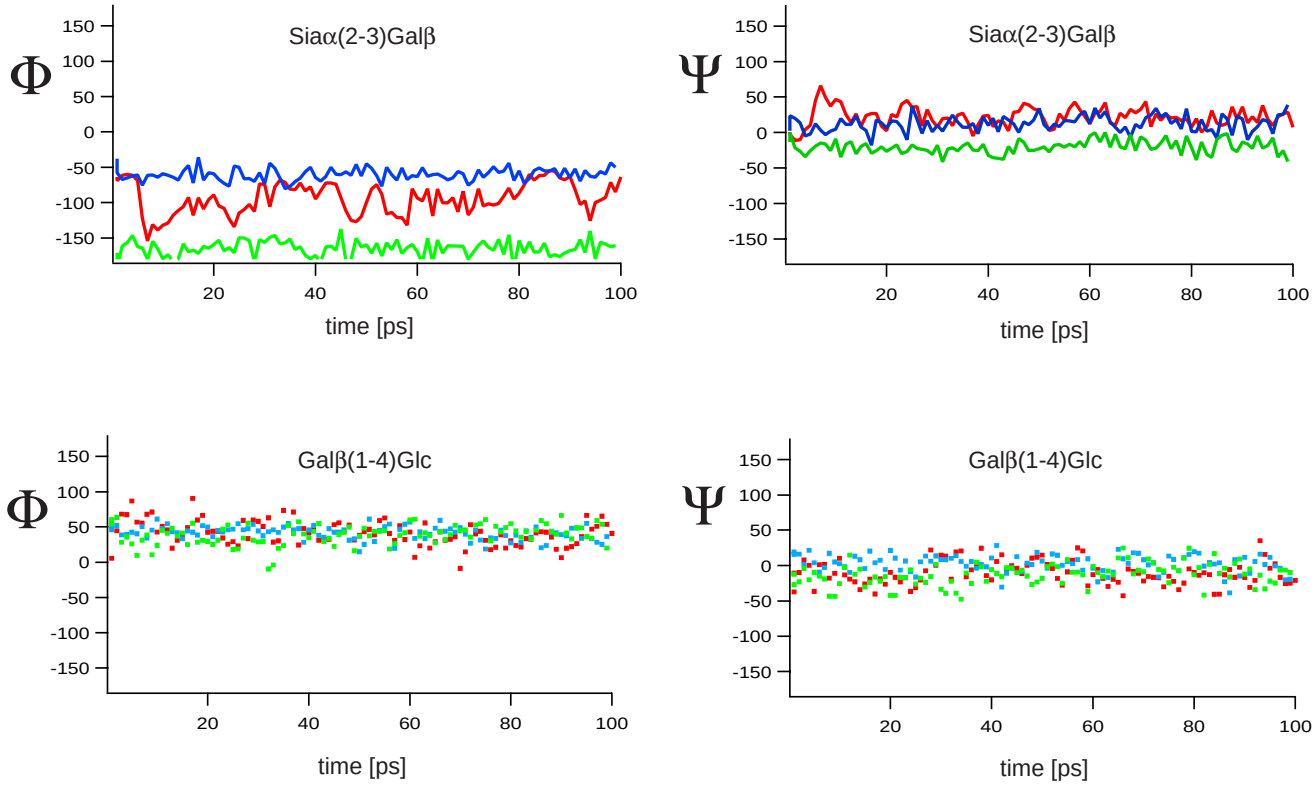

Figure 4. *Comparison of the trajectories of the glycosidic torsional angles Φ/Ψ of sialyllactose in the solvated free state (red), bound to influenza A virus hemagglutinin (blue) and bound to murine polyoma virus VP1 (green) at a simulation temperature of 300 K. The torsional angles (for definition see Figure 1) are given in degrees.*

atoms were assigned using the automatic assignment module of INSIGHTII. Charges were added using the automatic charge assignment procedure of INSIGHT II for the protein as well as for the sugar part.

The MD simulation protocol included an initial step of minimization (conjugate gradient, 1000 steps), an equilibration time of 10 ps and a production time of 100 ps. The simulations were performed at a temperature of 300 K. An integration step of 1 fs was used throughout all simulations. The temperature and the total energy were monitored for each simulation and kept stable during the production time. The evaluation of the result was accomplished with the ANALYSIS module of INSIGHTII.

Results and Discussion

It is well known that the major conformational flexibility in oligosaccharides arises from the rotational freedom of the torsional angles of the glycosidic linkages. Figure 4 gives a comparison of the Φ/Ψ torsional angles Siaα(2-3)Galβ glycosidic linkages in the free and in the bound state. A controversial discussion about the flexibility of this linkage can be found in the literature. Some authors claimed that this linkage adopts only a single conformation [10]. Others reported experimental data indicating that especially this linkage exhibits larger flexibility [5-7]. Frequently occurring transitions between two or three conformations have been found by MD simulations using different force fields and simulation protocols. In accordance with the reported data we also found that the torsion angle Φ is rather flexible and exhibits several conformations in the unbound state. When complexed with HA and VP1 only a single conformation is populated. Remarkably, two different conformations in the complex with HA (Φ /Ψ = -60°/30°) and VP1 (Φ/Ψ = -170°/-30°) are populated. By NMR-experiments it was found that both conformations are populated in the free state [8].

Figure 4 displays the trajectories of the Φ, Ψ torsion angels for the Galβ1-4Glc linkage also. Two possible conformations (Φ/Ψ = 55°/-5° and -15°/-45°) for this linkage have been described in literature [5-7] . The barrier between the two minima is very low, so that it is more correct to speak of fluctuations within an extended energy valley rather than transitions between distinct conformers. Table 1 reports the mean values of the trajectories for Φ, Ψ and their standard deviations in the free state and when bound to one of the two proteins. Although the mean values are rather similar for all three cases, the mobility of both torsion angles is considerably reduced in the bound state.

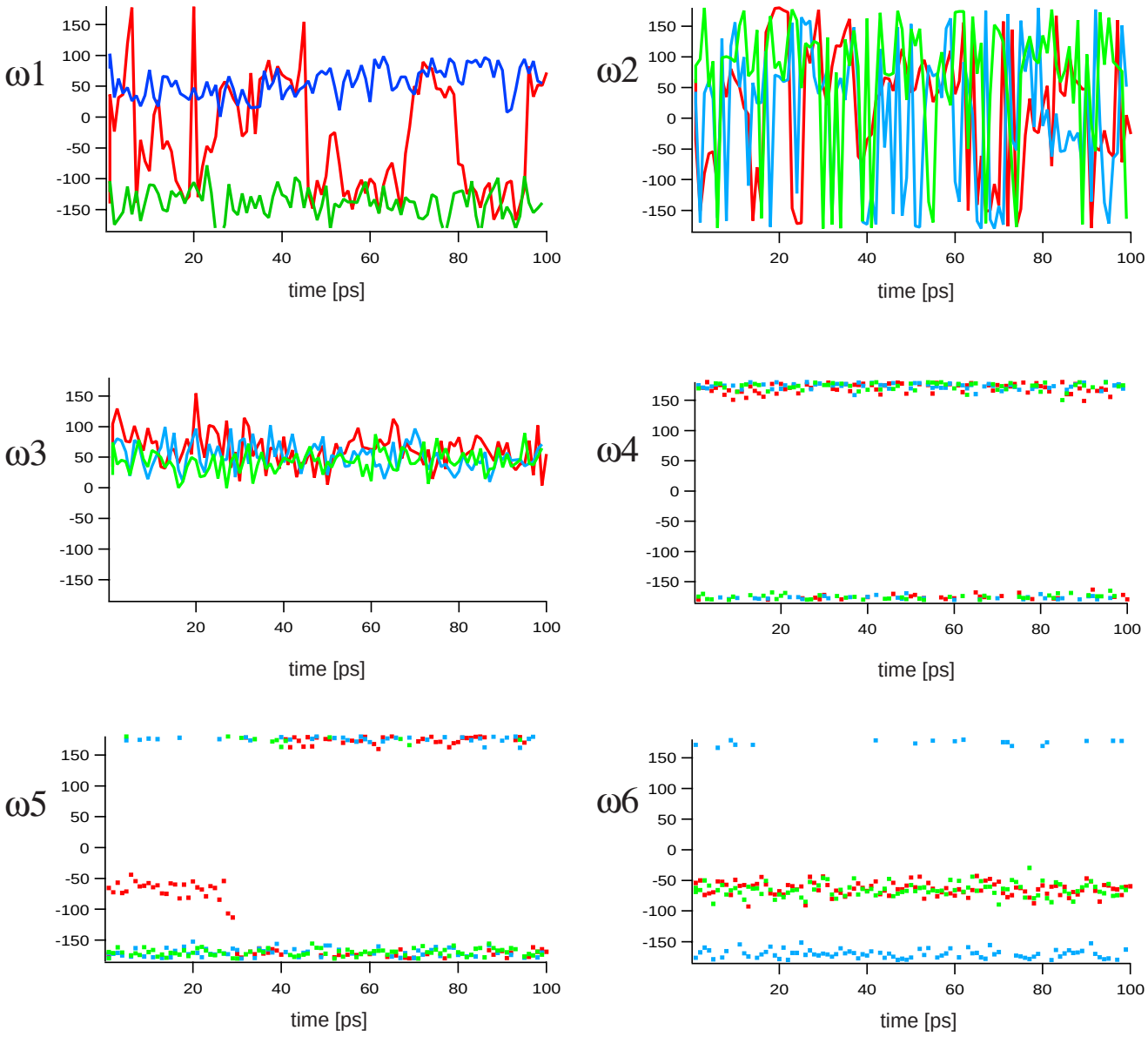

Figure 5. *Comparison of the trajectories of the ω torsional angles of sialyllactose in the solvated free state (red), bound to influenza A virus hemagglutinin (blue) and bound to murine polyoma virus VP1 (green) at a simulation temperature of 300 K. The torsional angles (for definition see Figure 1) are given in degrees.*

The carboxyl group of sialic acid adopts two orientations in solution showing frequent jumps between them (Figure 5; ω1). This degree of rotational freedom is completely frozen in both complexes. Only one orientation is populated and no jump to the other orientation occurs during the simulation.

The 4-hydroxyl group exhibits considerable conformational freedom also in the the bound state (Figure 5; ω2). Although frequent jumps occur between the possible orientations of the 4-hydroxyl group their population is quite different for the three simulations. The histogram of the ω2 angle (Figure 6) shows maxima for the free state at -60°, +60° and 180°. The orientation +60° is slightly preferred due to a weak intramolecular H-bond with the oxygen atom of the N-acetyl side chain. The frequency plot of the ω2 angle for the free state looks very similar to the one of the bound state for the HA complex but looks quite different for the VP1 complex. Therefore it can be concluded that the 4-hydroxyl group shows only weak interaction with the protein in the HA complex whereas the interaction with the VP1 protein is significant stronger. We confirmed these findings by analysing the interaction energy on a residue level (Figure 7). In the VP1 case the intermolecular interaction energy for the 4-hydroxyl group is sixfold higher than in the HA case.

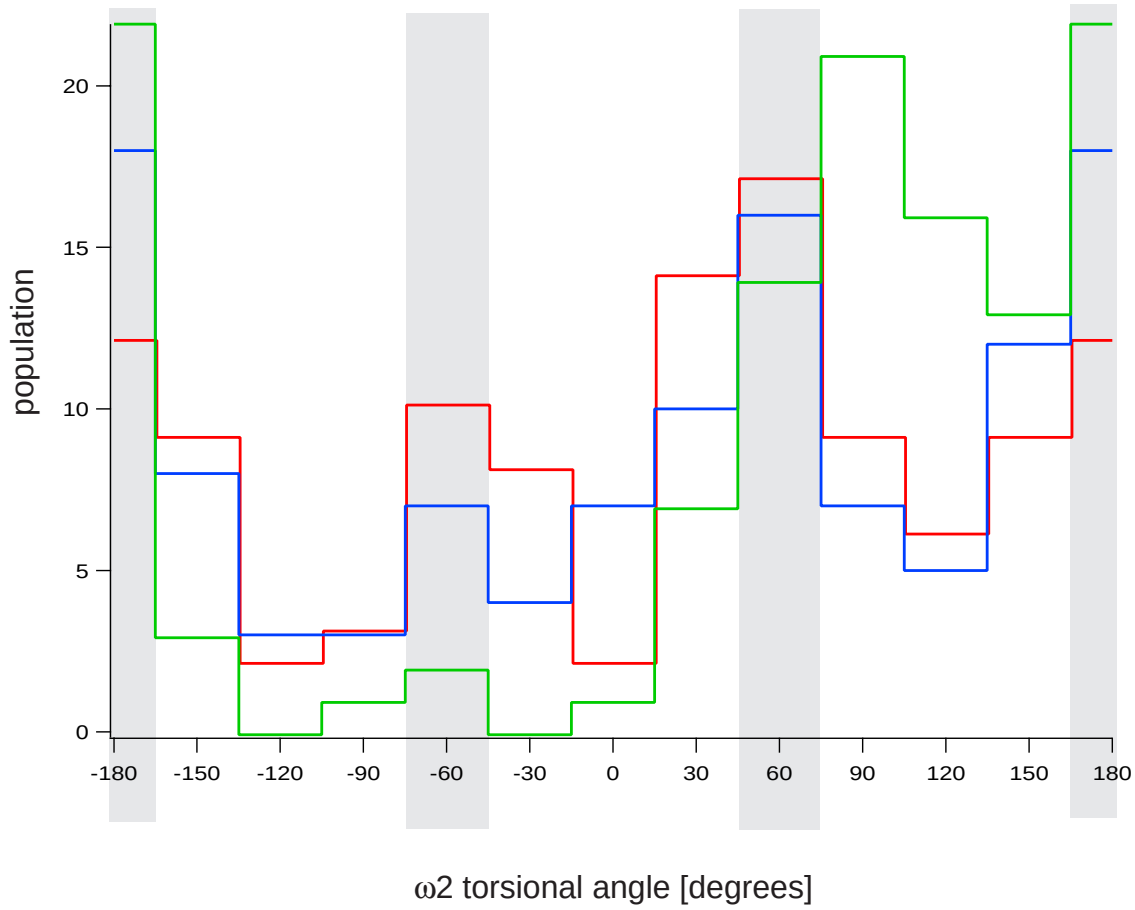

Figure 6. *Histogram of the ω2 torsional angle of the 4-hydroxyl group in the solvated free state (red), bound to influenza A virus hemagglutinin (blue) and bound to murine polyoma virus VP1 (green) at a simulation temperature of 300 K. For the definition of the ω2 torsional angle see Figure1. The theoretical preferred orientations (-60°, 60°, 180°) are highlighted in grey.*

The glycerol side chain shows only one conformational change in the free state. Thus MD simulations can reproduce the relative rigidity of the glycerol side chain which has been found experimentally [5,7]. In the bound state no jumps to other conformations occur but two different conformations of the C7-C8-C9-O9 torsion angles are populated in the HA (-60°) and VP1 (180°) complex (Figure 5; ω4, ω5, ω6).

The mobility of the N-acetyl side is reduced in the bound state also (Figure 5; ω3) (Std.dev.: 27° (free state), 22° (HA), 18° (VP1)).

Summary and Conclusion

The attachment process of a virus to the host cell surface is influenced by enthalpic and entropic factors [4]. A detailed evaluation of all possible energetic interactions including the effects of solvent molecules seems to be a promising way to gain deeper insights in the overall process of binding. Here we compared the reduction of the conformational freedom of unbound sialyllactose and when complexed with influenza A hemagglutinin and the murine polyoma virus. In general the conformational freedom of the complexed SL is considerably reduced compared to the free state. Remarkebly two different conformations of the Siaα(2-3)Galβ glycosidic linkages exist (which are both populated in the free state) when complexed with either protein.

Molecular dynamics (MD) simulations provide insights into the dynamic behavior of protein-carbohydrate complexes i.e. yields information which is not available by experimental methods. The described approach does not include the evaluation of solvent effects. Additional MD Simulations including explicit solvent molecules are required.

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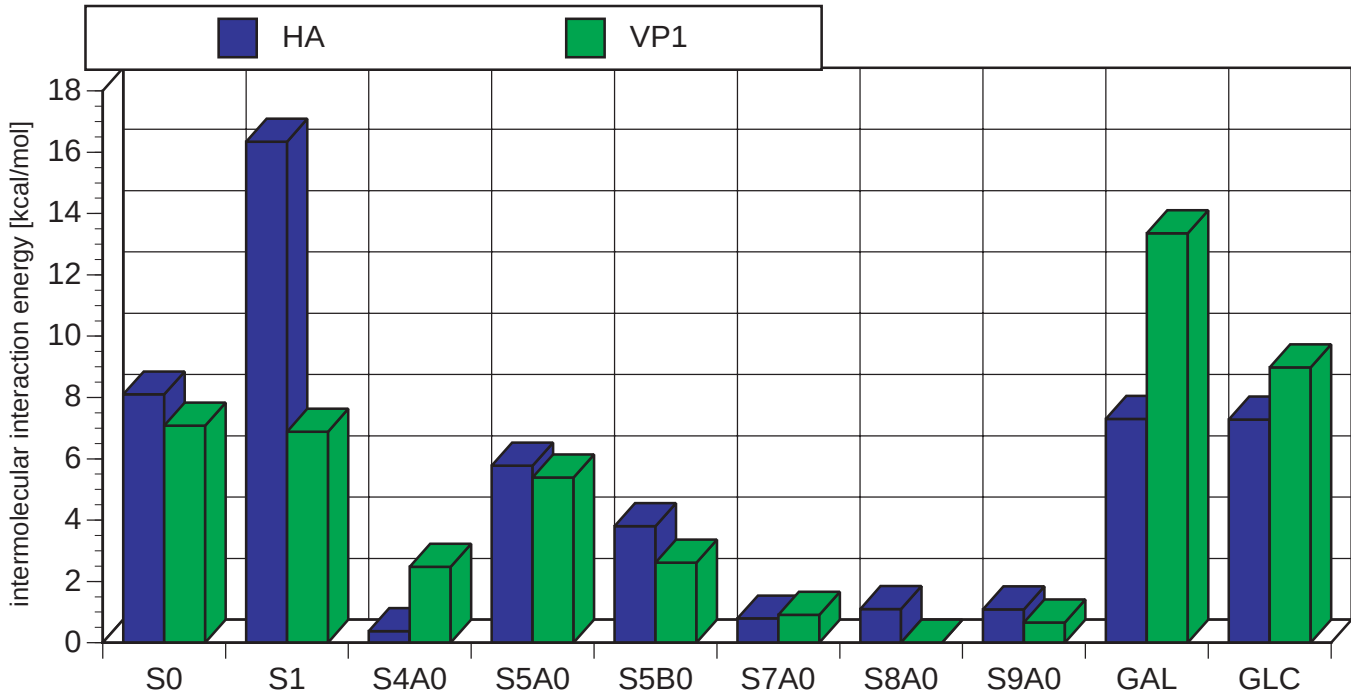

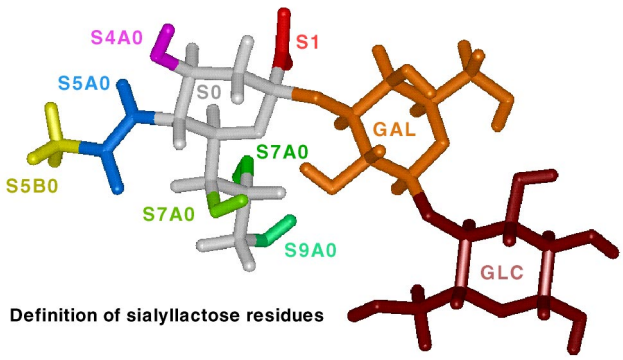

Figure 7. *Analysis of the intermolecular interaction energy between residues of sialyllactose and virus protein. Ten snapshots were stored in regular intervals during the molecular dynamics simulation and minimized using the conjugate gradient algorithm until a convergence of 0.001 kcal/mol had been reached. For all ten optimized structures the total interaction energies (van der Waals plus Coulomb interactions at a dielectric constant of 4) of each residue of sialyllactose with various residues of HA and VP1 were calculated using the "enclose residue-residue interaction" option of the DISCOVER program. All residues of the protein within a sphere of 5 Å were enclosed in the calculation.*

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