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Sialyldisaccharide conformations: a molecular dynamics perspective

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Abstract Sialyldisaccharides are significant terminal components of glycoconjugates and their negative charge and conformation are extensively utilized in molecular recognition processes. The conformation and flexibility of four biologically important sialyldisaccharides [Neu5Acα(2-3)Gal, Neu5Acα(2-6)Gal, Neu5Acα(2-8)Neu5Ac and Neu5Acα(2-9)Neu5Ac] are studied using Molecular Dynamics simulations of 20 ns duration to deduce the conformational preferences of the sialyldisaccharides and the interactions which stabilize the conformations. This study clearly describes the possible conformational models of sialyldisaccharides deduced from 20 ns Molecular Dynamics simulations and our results confirm the role of water in the structural stabilization of sialyldisaccharides. An extensive analysis on the sialyldisaccharide structures available in PDB also confirms the conformational regions found by experiments are detected in MD simulations of 20 ns duration. The three dimensional structural coordinates for all the MD derived sialyldisaccharide conformations are deposited in the 3DSDSCAR database and these conformational models will be useful for glycobiologists and biotechnologists to understand the biological functions of sialic acid containing glycoconjugates.

Keywords Sialic acid · Sialyldisaccharide · Conformation · MD simulations

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Introduction

Sialic acids are important structural motifs frequently found in glycoconjugates and appear as terminal sugars in oligosaccharide chains of glycoproteins and glycolipids. Because of their terminal position, size and negative charge, sialic acids are involved in numerous significant biological events mainly including phenomena related to cellular and molecular recognition and structural stabilization [1–10]. Glycoconjugates containing sialic acids are dominant receptors for many pathogenic agents that include viruses, bacteria and parasites [11–15]. Sialic acids present themselves in increasingly diverse forms and this elegant structural diversity arises from the nature of these nine carbon monosaccharides and their linkages to other sugars. The most commonly occurring form of sialic acid is Neu5Ac (*N*-Acetylneuraminic acid). The linkage diversity of sialic acids occurs in four main configurations, α(2-3), α(2-6), α(2-8) and α(2-9). When bound with other sugars, Neu5Ac forms α(2-3) and α(2-6) linkages whereas with other sialic acids, it forms α(2-8) and α(2-9) linkages. In gangliosides, sialic acids occur internally or externally and the functions of a ganglioside are preferentially determined by the number of sialic acids and the position of occurrence of sialic acids. Polysialic acids are important class of homopolymers of sialic acid which feature either of α(2-8) or α(2-9) or both type of linkages between the sialic acid residues. The existence of various linkages of sialic acid with other sugars is exploited by most of the invading membrane binding-pathogens which exhibit remarkable specificity towards the linkage and conformation of sialic acid at the receptor oligosaccharide chain. Terminal sialyldisaccharides Neu5Acα(2-3)Gal and Neu5Acα(2-6)Gal are recognized with different specificities by different toxins. Most of the avian influenza viruses preferentially recognize sialic acids with α(2-3) linkages whereas human

influenza viruses recognize sialic acids with α(2-6) linkages. Swine flu viruses can recognize both α-(2-3) and α-(2-6) linked sialic acids with different specificities. α(2-8) linkage also plays crucial roles in pathogenic infection [16–25]. The structure and conformation of polysialic acids are important in initiating the neuronal processes and in pathogenic recognition processes [26].

A large amount of data is available about the linkage specificities of various pathogens however the atomistic level details of receptor specificities is poorly understood. A clear examination of sialic acid receptor specificities at atomistic level is the basis for the development of entry inhibitors that could be used in prevention and therapy of pathogenic infection. It is apparent that the development of clear and well defined models to study the conformations and dynamics of sialyldisaccharides is an important step in understanding the biological nature of processes by which the disaccharides interact with their environments. To model the sialyldisaccharides, it is necessary to explore and characterize their conformational landscape as a function of glycosidic and exocyclic torsions which dictates the three dimensional structure of carbohydrates. The functional importance of sialyldisaccharides has been understood to an amenable extent through experimental methods however their conformational features are not given emphasis because of the difficulties associated with elucidating the three dimensional structure of carbohydrates by NMR and X-ray crystallography. Use of experimental methods to study the carbohydrate conformation is limited by the presence of multiple conformers adopted by the oligosaccharides in solution and the arduous efforts needed for crystallizing the sugars. These limitations of the experimental methods necessitate the use of theoretical methods like Molecular Dynamics simulations (MD) because the MD approach provides the atomistic details over a length and time scale which is inaccessible to experimental methods. MD simulations performed in aqueous environment for a considerable duration can explore the conformational space of saccharides in solution which are relevant to the biological conformation [27–35]. In the present study, we have carried out aqueous MD simulations of 20 ns duration on four different biologically important sialyldisaccharides [Neu5Acα(2-3)Gal, Neu5Acα(2-6)Gal, Neu5Acα(2-8)Neu5Ac and Neu5Acα(2-9)Neu5Ac] to understand their solution structure and dynamics.

As to the time duration of the simulation, we understand from our previous simulations that MD simulations of 10 ns duration can explore all the possible conformations of a given saccharide. The conformation of Neu5Acα(2-3)Gal has been investigated using 1 ns MD simulation by Suresh and Veluraja [36] and the results of the simulation suggested that the disaccharide can exist in two distinct conformational regions. However a 10 ns MD simulation performed on the same disaccharide by Veluraja and Margulis [37] resulted in three conformational regions, a new conformational region along with the two regions proposed earlier by Suresh and Veluraja. It is also found that, even when the initial conformations are given in high energy regions, the disaccharide conformation likely falls back to the biologically relevant conformation during a 10 ns simulation [37]. We have carried out MD simulations of 20 ns duration for the same disaccharide in order to validate the conformational convergence of earlier simulation.

Materials and methods

The initial models of the sialyldisaccharides Neu5Acα(2-6)Gal, Neu5Acα(2-8)Neu5Ac and Neu5Acα(2-9)Neu5Ac are generated using the standard geometry [38, 39]. The input parameters for the MD simulations are prepared using generalized amber force field from AMBER9 [40]. Significant work in the development of carbohydrate force field has been made by GLYCAM group and for now, many variants of force field parameters are available for carbohydrate simulation. The differences are unlikely the major concerns while attempting to simulate only carbohydrates using a single force field. Even though both gaff and GLYCAM span over similar conformational regions for a 10 ns MD simulation of Neu5Acα(2-3)Gal (data not shown), we have used generalized amber force field because of its thorough parameterization and its advantage in studying protein-carbohydrate interactions. The disaccharide is placed at the centre of the box and TIP3P water molecules from the AMBER solvent library are added into it. Necessary sodium ions are added to neutralize the system as sialic acid carries a negative charge. Periodic boundary conditions are used to treat the boundary of the system to minimize the edge effects. Particle Mesh Ewald is used to treat electrostatic interactions. The PME grid sizes of Neu5Acα(2-3)Gal, Neu5Acα(2-6)Gal, Neu5Acα(2-8)Neu5Ac and Neu5Acα(2-9)Neu5Ac are $37 \times 22 \times 37$, $37 \times 21 \times 41$, $40 \times 45 \times 53$ and $34 \times 21 \times 60$ respectively. The resulting system is minimized for 1,000 steps, equilibrated for 1 ns and then simulated for 20 ns duration using the molecular dynamics software NAMD–NAnoscale Molecular Dynamics [41] with the time step of one femto second. During the simulation, the temperature, pressure and the number of particles are kept constant. The temperature is maintained at 300 K throughout the simulation. The information about the ensemble is recorded over every pico second and a total of 20,000 structures are collected for a 20 ns MD simulation. The simulation trajectories are analysed by using the visualization software VMD (visual molecular dynamics) [42].

Results

The model structures of sialyldisaccharides indicating the glycosidic torsional angles along with the exocyclic torsional angles that involve the conformational flexibility are given in Fig. 1. The definitions of dihedral angles around the glycosidic linkages of simulated four sialyldisaccharides are marked.

During the entire simulation time, the pyranose ring of sialic acid and galactose had maintained their respective $^2C_5$ and $^4C_1$ chair conformations with a deviation of 6° in ring dihedral angles.

Conformational features of Neu5Acα(2-3)Gal

The first report on the existence of two conformational regions [(−70°,−10°) and (−155°,−20°)] for Neu5Acα(2-3)Gal was made by Veluraja and Rao [43] using Hard Sphere energy calculation. Molecular dynamics calculations of duration 1 ns carried out by Suresh and Veluraja [36] resulted that the disaccharide can exist in conformational regions around (−66°,10°) and (−164°,−59°) and water mediated hydrogen bonding schemes are proposed to explain the stability at the preferred conformational regions. Later, Veluraja and Margulis performed MD

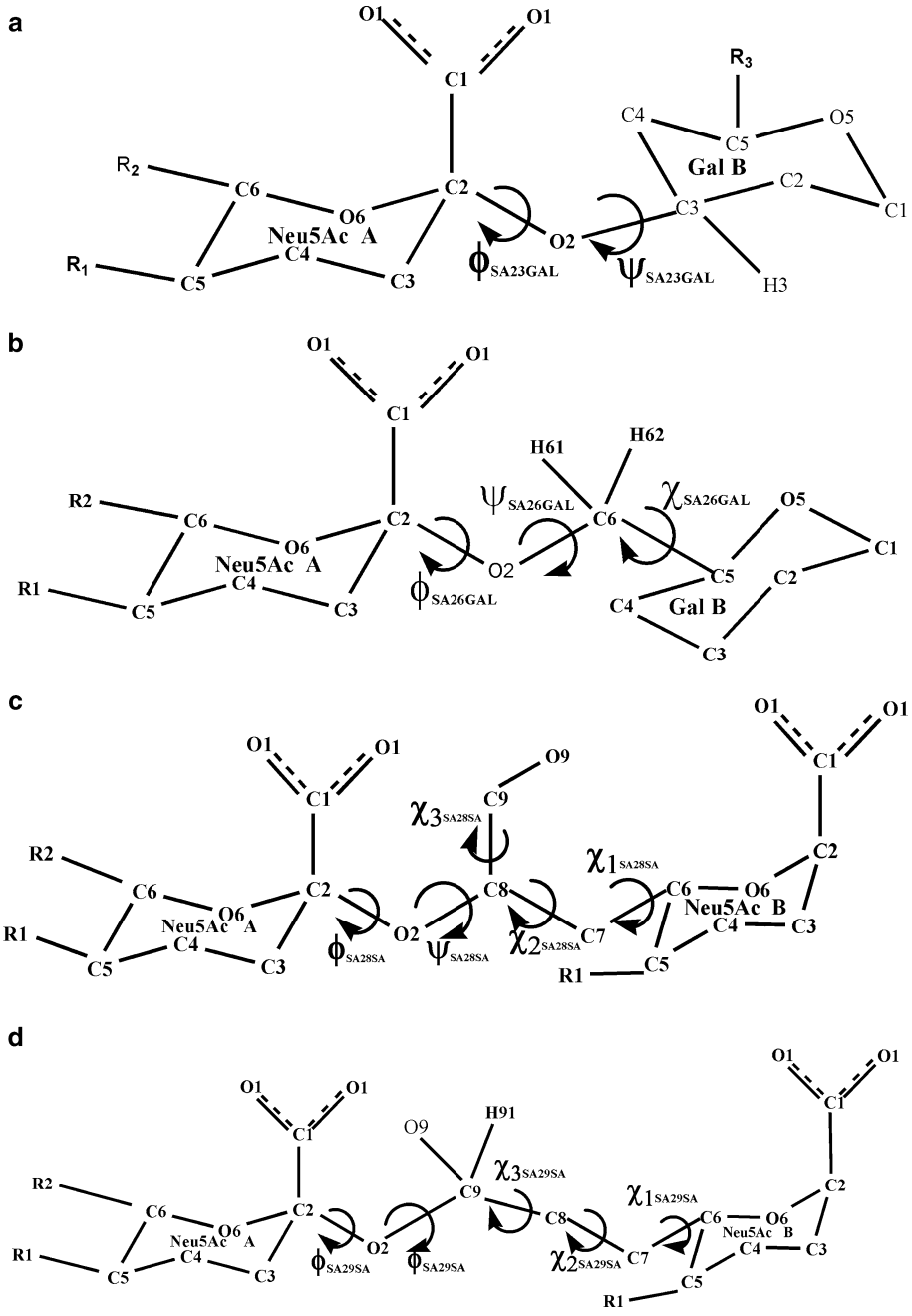

Fig. 1 Sialyldisaccharide models. **a** Neu5Acα(2-3)Gal. $\Phi_{SA23GAL}$ = C1–C2–O2–C3, $\Psi_{SA23GAL}$ = C2–O2–C3–H3. R_1 acetamido group, R_2 glycerol side chain, R_3 CH_2OH. **b** Neu5Acα(2-6)Gal. $\Phi_{SA26GAL}$ = C1–C2–O2–C6, $\Psi_{SA26GAL}$ = C2–O2–C6–H61 and $\chi_{SA26GAL}$ = O2–C6–C5–O5 (H61 is the hydrogen which makes an angle of H61–C6–C5–O5 = 120° when $\chi_{SA26GAL}$ = 0°). R_1 acetamido group, R_2 glycerol side chain. **c** Neu5Acα(2-8)Neu5Ac. Φ_{SA28SA} = C1–C2–O2–C8, Ψ_{SA28SA} = C2–O2–C8–H8, $\chi 1_{SA28SA}$ = C5–C6–C7–C8, $\chi 2_{SA28SA}$ = C6–C7–C8–C9, R_1 acetamido group, R_2 glycerol side chain. **d** Neu5Acα(2-9)Neu5Ac. Φ_{SA29SA} = C1–C2–O2–C9, Ψ_{SA29SA} = C2–O2–C9–H91, $\chi_{1SA29SA}$ = C5–C6–C7–C8, $\chi_{2SA29SA}$ = C6–C7–C8–C9, $\chi_{3SA29SA}$ = C7–C8–C9–O2, H91 is that hydrogen which makes an angle of 120° when $\chi_{3SA29SA}$ = 0°. R_1 acetamido group, R_2 glycerol side chain

simulations of 10 ns duration and predicted three conformational regions for Neu5Acα(2-3)Gal [37]. In the present work, the conformational preferences of Neu5Acα(2-3)Gal are investigated using 20 ns MD simulations and resulted in reprising the same three conformational regions that are found by earlier 10 ns simulations by Veluraja and Margulis. The glycosidic torsional angle values ($\Phi_{SA23GAL}$, $\Psi_{SA23GAL}$) of the three distinct conformational regions are around (−100°,−50°), (−70°,0°) and (−150°,−30°) and these three conformational structures are stabilized either by direct or water mediated hydrogen bonding between the sugar residues. The distribution of glycosidic torsions over every 10 ns duration (1–10, 2–11, 3–12, 4–13, 5–14, 6–15, 7–16, 8–17, 9–18, 10–19 and 11–20 ns) in the respective dihedral plots (Fig. 2a–k) are similar which indicates that MD simulations of 10 ns duration are sufficient to attain convergence while simulating a disaccharide. The conformational map for the 20 ns duration is given in Fig. 2l. Similar conformational convergence has been observed for 20 ns MD simulations carried out for the other three sialyldisaccharides under the present study. In the previous study [36], the relative energies for the different conformational regions were calculated by systematically sampling 100 structures in every conformation. In the present work, the relative energies of the conformations are calculated by conformational averaging which includes

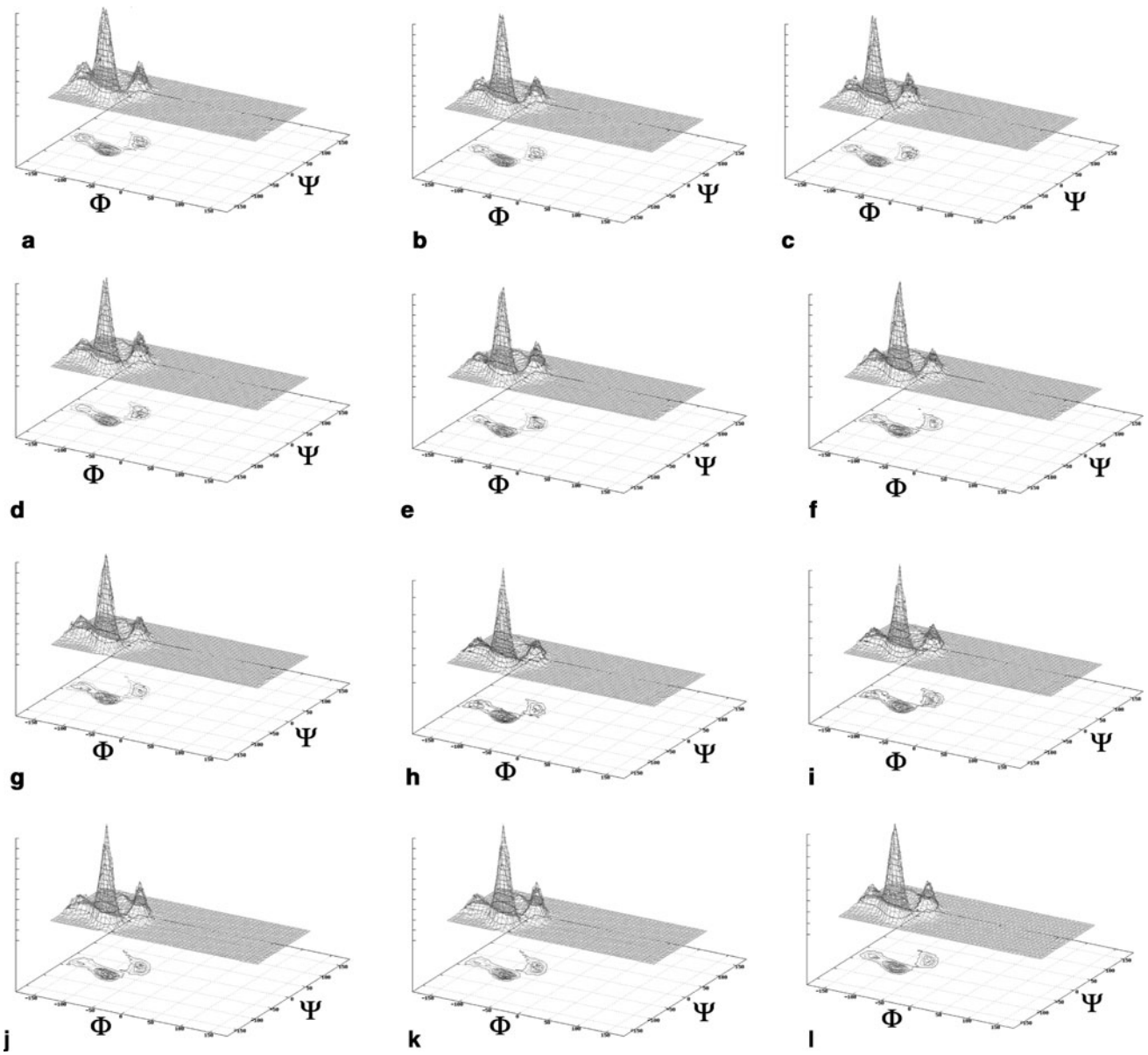

Fig. 2 The conformational convergence of 10 ns MD simulations for the disaccharide Neu5Acα(2-3)Gal

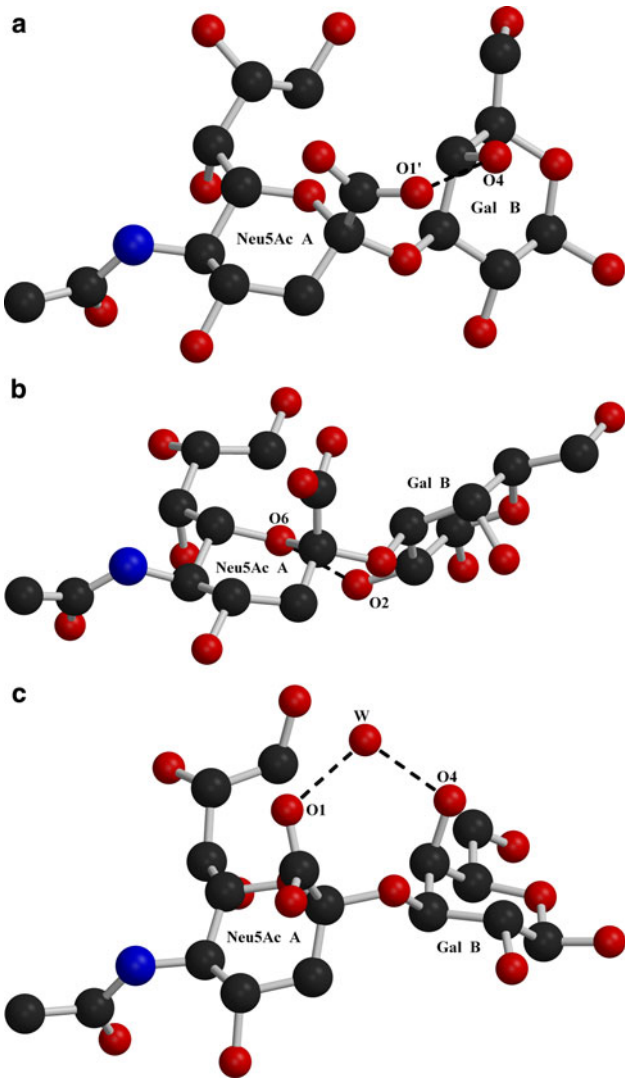

Fig. 3 Three different conformational models for Neu5Acα(2-3)Gal

averaging over thousands of structures for a 20 ns simulation. This increase in number of structures reduces the noise in the energy calculation. The interactions, water occupancy in water mediated hydrogen bonds and the relative energy values are given in the Table 1. The structural models corresponding to the three conformations are given in Fig. 3. The models are rendered using MOLSCRIPT [44].

Conformational features of Neu5Acα(2-6)Gal

Neu5Acα(2-6)Gal occurs mostly as terminal moiety of the carbohydrate chains protruding from the cell surface. The conformation of Neu5Acα(2-6)Gal is dictated by the glycosidic torsions ($\Phi_{SA26GAL}$, $\Psi_{SA26GAL}$) along with the exocyclic torsion ($\chi_{SA26GAL}$) extending from the ring of the galactose residue towards the glycosidic linkage. Molecular Dynamics simulations carried out for 1 ns duration by Suresh and Veluraja [36] revealed that Neu5Acα(2-6)Gal can exist in two conformational regions similar to that of the earlier report using Hard Sphere calculations by Veluraja and Rao [43]. Since 1 ns MD simulation is too short for this disaccharide to sweep the entire conformational space, a 20 ns MD simulation is carried out in order to bring out the conformational features of Neu5Acα(2-6)Gal. The glycosidic conformational space for Neu5Acα(2-6)Gal computed from the 20 ns simulation trajectories is shown in Fig. 4. The map clearly indicates that the glycosidic torsions ($\Phi_{SA26GAL}$, $\Psi_{SA26GAL}$) are clustered around two distinct regions and the torsional angle values corresponding to these two regions are (−70°,70°) and (−150°,70°). The exocyclic torsion ($\chi_{SA26GAL}$) can take two values, +60° and −60°. A detailed conformational analysis reveals that the disaccharide can adopt four different conformations based on the glycosidic and exocyclic torsions as follows, (−70°,70°,60°), (−150°,70°,60°), (−150°,70°,−60°) and (−70°,70°,−60°). Of the four conformations, (−70°,70°,60°) and (−150°,70°,60°) are the minimum energy conformations of Neu5Acα(2-6)Gal and the energy of the latter is higher than the global minimum energy conformer (former) by 0.1 kcal/mol which is marginal. Existence of these two conformers is equally probable in the aqueous solution. When the exocyclic torsion $\chi_{SA26GAL}$ shifts to −60° in these two conformations (−70°,−50°) and (−150°,−50°), the energy increases about 3.5 and 10.5 kcal/mol respectively. The occurrence of conformer with highest energy in solution is less probable. The hydrogen bonding schemes which stabilize the first three different conformations are given in Table 1 along with the relative energy values and water occupancy. The three different conformational models are given in Fig. 5.

Conformational features of Neu5Acα(2-8)Neu5Ac

The conformation of Neu5Acα(2-8)Neu5Ac is dictated by glycosidic torsions (Φ_{SA28SA}, Ψ_{SA28SA}) and also by the exocyclic torsions $\chi 1_{SA28SA}$ and $\chi 2_{SA28SA}$. Two conformational regions were identified for Neu5Acα(2-8)Neu5Ac by Veluraja and Rao using Hard Sphere calculations and later similar regions were reported by Suresh and Veluraja using MD simulations of 1 ns duration [36, 43]. Reports from other research groups also suggest the similar conformational patterns for the same disialoside [45]. To explore the entire conformational space of Neu5Acα(2-8)Neu5Ac, a 20 ns MD simulation is carried out and the glycosidic torsional map obtained from the 20,000 simulation structures of the disaccharide is given in Fig. 6. As evident from the dihedral map, Neu5Acα(2-8)Neu5Ac favours four different glycosidic conformations and the respective glycosidic dihedral angle values (Φ_{SA28SA}, Ψ_{SA28SA}) are (−130°,50°), (−150°,−30°), (−70°,30°) and

Table 1 Conformational models for sialyldisaccharides along with hydrogen bonding pattern and relative energy values

Molecule	Conformation	Interaction stabilizing the structure		Occupancy of water (%)	Relative energy (Kcal/mol)
		Direct hydrogen bond	Water mediated hydrogen bond		
Neu5Acα(2-3)Gal	$\Phi_{SA23GAL}$, $\Psi_{SA23GAL}$				
Conformer I	−100,−50	O1/O1′(A)–O4 (B)	–	–	0.00 ± 0.11
Conformer II	−70,0	O6 (A)–O2 (B)	–	–	0.98 ± 0.23
Conformer III	−150,−30		O1/O1′(A)–W–O4(B)	92	2.38 ± 0.29
Neu5Acα(2-6)Gal	$\Phi_{SA26GAL}$, $\Psi_{SA26GAL}$, $\chi_{SA26GAL}$				
Conformer I	−70,70,60	–	O7 (A)–W–O5(B)	31	0.00 ± 0.06
Conformer II	−150,70,60	–	O1/O1′ (A)–W–O5(B)	70	0.10 ± 0.10
Conformer III	−70,70,−60	O2(A)–O4 (B)	O1/O1′ (A)–W–O5(B)	32	3.51 ± 0.02
			O7 (A)–W–O4 (B)		
Conformer IV	−150,70,−60	O2(A)–O4 (B)	O1/O1′(A)–W–O4 (B)	51	10.5 ± 0.43
Neu5Acα(2-8) Neu5Ac	Φ_{SA28SA}, Ψ_{SA28SA}				
Conformer I	−130,50	O6(A)–O9 (B)	O9 (A)–W–O1/O1′ (B)	23	
			O1/ O1′ (A)–W–N5(B)	90	0.00 ± 0.87
			O1/O1′ (A)–W–O1/O1′ (B)	35	
Conformer II	−150,−30	O8 (A)–N5 (B)	O1/O1′ (A)–W–O7 (B)	63	1.50 ± 0.33
Conformer III	−70,30	O6(A)–O9 (B)	O9 (A)–W–O1/O1′ (B)	32	
			O7 (A)–W–O9 (B)	14	1.60 ± 0.61
			O1/O1′ (A)–W–N5(B)	62	
Conformer IV	−100,−50	O9(A)–N5(B)	O9(A)–W–O1/O1′ (B)	15	6.52 ± 0.54
		O1/O1′ (A)–O7(B)	O1/O1′ (A)–W–O10(B)	62	
Neu5Acα(2-9) Neu5Ac	Φ_{SA29SA}, Ψ_{SA29SA}, $\chi_{2SA29SA}$				
Conformer I	−150,70,60	O2(A)–O7(B)	O1/O1′ (A)–W–O8 (B)	68	0.00 ± 0.15
			O4(A)–W–O2 (B)	74	
Conformer II	−150,70,−80	–	O1/ O1′(A)–W–O8(B)	60	0.25 ± 0.28
			O1/O1′ (A)–W–O1/O1′ (B)	32	
Conformer III	−70,50,180	–	O1/O1′ (A)–W–O8 (B)	80	3.74 ± 0.34
Conformer IV	−70,50,−80	–	O4 (A)–W–O10(B)	2	5.96 ± 0.41
			O1/O1′ (A)–W–O1/O1′ (B)	98	
Conformer V	−70,90,180	O2(A)–O7 (B)	O6/O7 (A)–W–O7/O10(B)	34	6.29 ± 0.39
Conformer VI	−150,70,180	O2(A)–O8 (B)	O1/O1′ (A)–W–O8 (B)	68	6.45 ± 0.34
Conformer VII	−70,90,−80	–	O4/O7/ O10 (A)–W–O10(B)	62	6.89 ± 0.39
			O1/O1′ (A)–W–O1/O1′(B)	14	
Conformer VIII	−70,50,60	O2(A)–O7(B)	O7(A)–W–O1/O1′(B)	68	12.85 ± 0.34
			O10(A)–W–O1/O1′(B)	5	

W indicates water; (A) and (B) denote the first and second residues respectively. In all the disaccharides, Neu5Ac is the first residue

(−100°,−50°). The exocyclic torsion $\chi 1_{SA28SA}$ prefers 180° and $\chi 2_{SA28SA}$ prefers 60°. Except the conformation at (−100°,−50°), the relative energies of the rest of the conformers are marginal, viz 0.0, 1.5 and 1.6 kcal/mol for the conformers at (−130°,50°), (−150°,−30°), (−70°,30°) respectively. These three conformers are stabilized by water mediated hydrogen bonding between the carboxylic acid group of one residue and glycerol side chain of the other as given in Table 1. The occupancy of water in mater mediated hydrogen bonds are also given in Table 1. The corresponding structural models are given in Fig. 7.

Conformational features of Neu5Acα(2-9)Neu5Ac

The pronounced flexibility of Neu5Acα(2-9)Neu5Ac is due to the fact that it has three exocyclic torsions involved in dictating the conformational preferences apart from the glycosidic torsional angles. The exocyclic torsions are

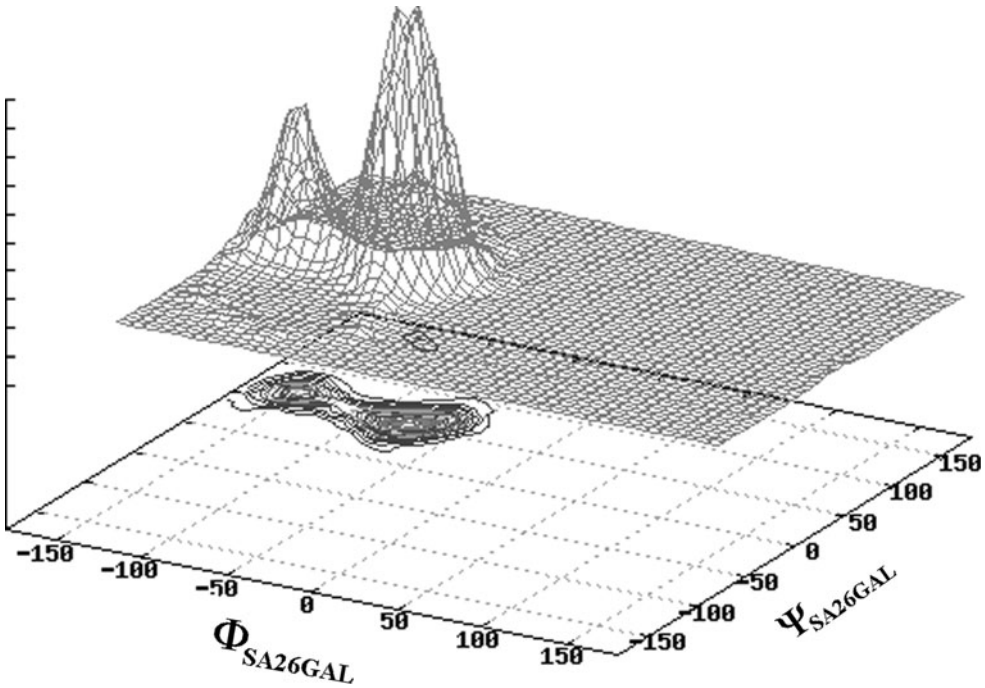

Fig. 4 Distribution of glycosidic torsions around glycosidic linkage of Neu5Acα(2-6)Gal in the 20,000 structures arrived through MD simulation

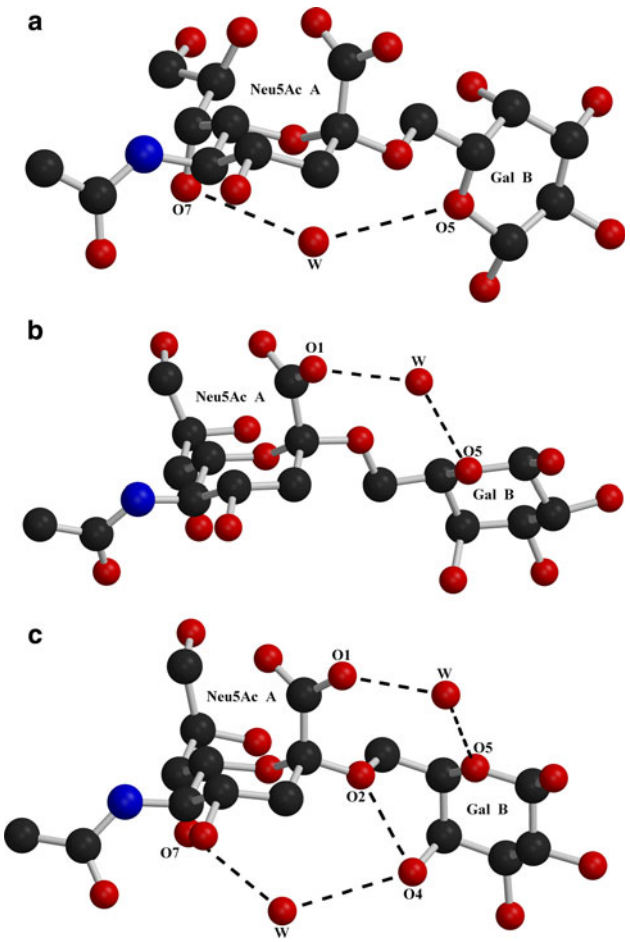

Fig. 5 Three different conformational models for Neu5Acα(2-6)Gal

marked as $\chi_{1SA29SA}$, $\chi_{2SA29SA}$ and $\chi_{3SA29SA}$ in Fig. 1d. MD simulations of 2 ns duration carried out by Suresh and Veluraja [36] revealed two conformational regions for Neu5Acα(2-9)Neu5Ac. The conformational map of Neu5Acα(2-9)Neu5Ac obtained from the present 20 ns MD simulation in terms of glycosidic torsions is given in Fig. 8. It is observed that the glycosidic torsions are centered on two distinct regions (−70°,70°) and (−150°,70°). The map also indicated the presence of conformations at (50°,50°) and (−100°,−50°), both having negligible population. Throughout the 20 ns simulation, the exocyclic torsions $\chi_{1SA29SA}$ and $\chi_{3SA29SA}$ preferred single conformation and the torsional angle values are 180° and 60°, respectively. The another exocyclic torsion $\chi_{2SA29SA}$ is flexible and can take three values, 60°, 180° and −80°, however it cannot take 60° when the glycosidic torsion is at (−70°,90°). A careful analysis of the simulation trajectories brings out eight different conformers with torsional values (Φ_{SA29SA}, Ψ_{SA29SA}, $\chi_{2SA29SA}$) for these disaccharides as given below: (−150°,70°,60°), (−150°,70°,−80°), (−70°,50°,180°), (−70°,50°,−80°), (−70°,90°,180°), (−150°,70°,180°), (−70°,90°,−80°) and (−70°,50°,60°). The first three conformers show a slight difference in the energy viz, 0.00, 0.25 and 3.74 kcal/mol, respectively. The next four conformers have ≈6 kcal/mol more than the minimum energy conformers. The hydrogen bonding schemes, water occupancy and relative energy values corresponding to all the conformational states are given in Table 1. Structural models for the first three minimum energy conformers are given in Fig. 9.

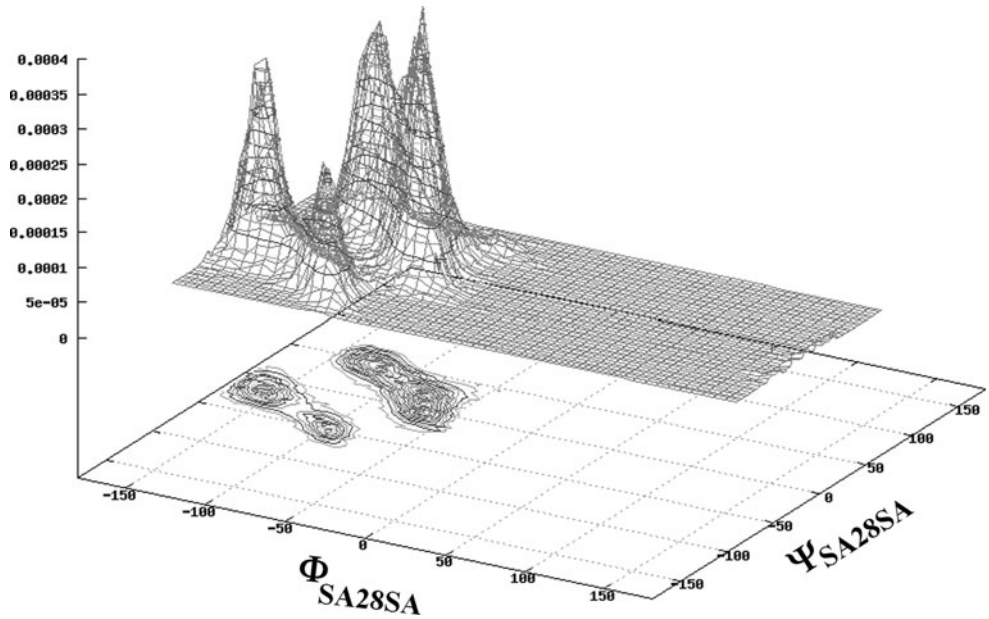

Fig. 6 Distribution of glycosidic torsions around glycosidic linkage of Neu5Acα(2-8) Neu5Ac in the 20,000 structures arrived through MD simulation

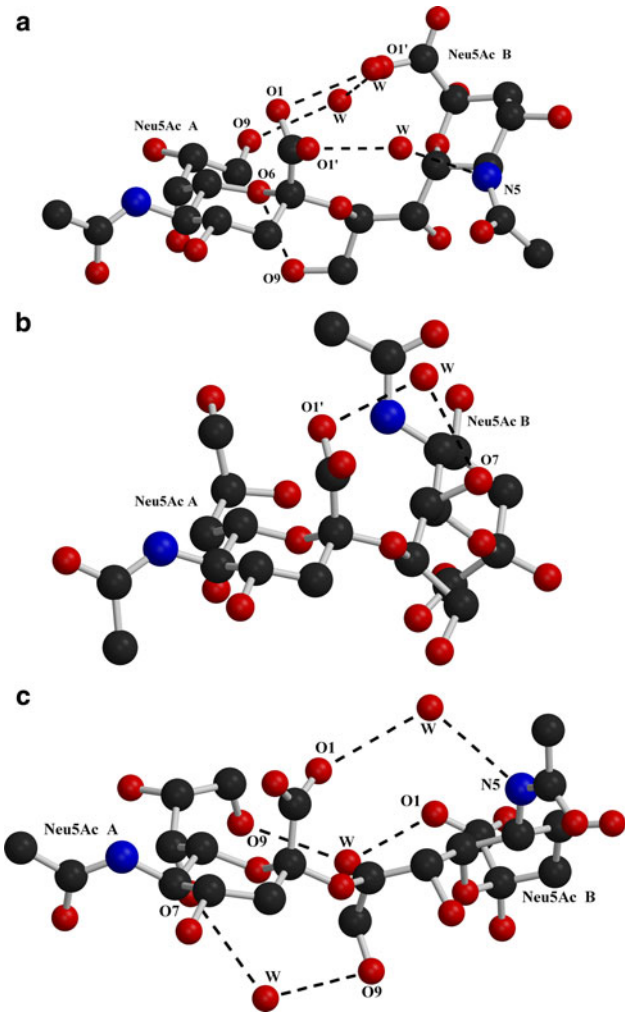

Fig. 7 Three different conformational models for Neu5Acα(2-8)Neu5Ac

Discussion

Molecular modelling of sialyldisaccharides is an important step towards understanding the basis of protein–carbohydrate interactions. The abundance of sialic acid in the cell walls makes it a key molecule in numerous cell surface biological events. As the terminal glycans of glycoproteins and gangliosides, and the versatility of structure and conformation, sialyldisaccharides can influence the course of molecular recognition processes. We have arrived at the atomistic level description of the conformational features of four biologically important sialyldisaccharides by performing 20 ns Molecular Dynamics simulations. The glycosidic linkage between the terminal sialic acid and the penultimate Galactose or Neu5Ac residue does not occur in a single conformation and hence the flexibility at the glycosidic linkage should be taken into account when molecular recognition events are studied for understanding the substrate specificity. Apart from addressing the flexibility at the glycosidic linkage, we have analysed the possible conformational flexibility due to the glycerol side chain of Neu5Ac involved in glycosidic linkage (exocyclic torsions). Owing to the fact that presence of water molecules affects the conformations of oligosaccharides considerably, we have simulated the sialyldisaccharides in aqueous environment and studied the role of water in stabilizing the structure and providing conformational similarity to the sialyldisaccharides. The general trend of water occupancy in water mediated hydrogen bonding interactions which stabilizes the disaccharide conformation is more than 50 %. The conformational flexibility of the sialyldisaccharides depends not only upon the glycosidic torsions but also upon the exocyclic torsions. After

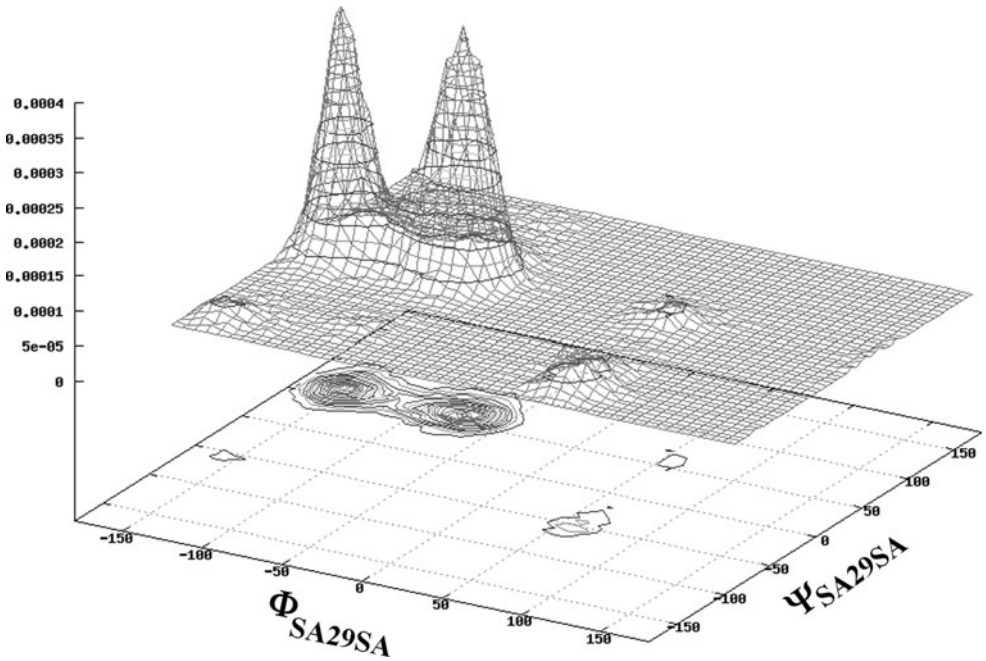

Fig. 8 Distribution of glycosidic torsions around glycosidic linkage of Neu5Acα(2-9)Neu5Ac in the 20,000 structures arrived through MD simulation

thorough analysis, it can be stated that each of the four sialyldisaccharides prefer three conformational models even though the number of exocyclic torsions can vary viz Neu5Acα(2-3)Gal: 0; Neu5Acα(2-6)Gal:1; Neu5Acα(2-8)Neu5Ac:2; Neu5Acα(2-9)Neu5Ac:3. The restriction in multiplicity of conformational states can be attributed to the direct/water mediated hydrogen bonding between the inter residues of the sialyldisaccharides. This also suggests that water plays a significant role in biomolecular interactions involving sialyldisaccharides. An analysis of experimentally derived structures of sialyldisaccharides that exist in Protein Data Bank is carried out in order to validate the accuracy of theoretical predictions about the sialyldisaccharide conformations. Using the keyword "SIA GAL" in ligand search yielded 82 PDB structures out of which 55 complexes possess Neu5Acα(2-3)Gal and 24 complexes possess Neu5Acα(2-6)Gal. The glycosidic torsional angles are measured for the ligands Neu5Acα(2-3)Gal and Neu5Acα(2-6)Gal in the complex structures and the corresponding dihedral maps are given in Fig. 10. In the conformational map of Neu5Acα(2-3)Gal (Fig. 10a), two experimental conformations are observed instead of the three proposed theoretical conformations. Restriction of one conformational region in the complexed structure is due to the interactions of either carboxylic acid group of sialic acid or the O-4 of Galactose with the active site residues. The conformational map of Neu5Acα(2-6)Gal (Fig. 10b) shows the distribution of dihedral angles in the theoretically proposed conformational region however a few dihedral angles fall in a region apart from cluster of points (wide spread). An analysis of the PDB structures corresponding to the points which fall away from the clustered regions shows that the conformational shifts can be accounted for the formation of hydrogen bonds that bridges the sugar residues (SIA and GAL) through amino acid side chains (*1PTO*) or for the distortion in sugar ring geometry during refinement (*3H32*).

In the case of recognition of sialyloligosaccharides by lectins which is a biologically significant phenomenon, sialic acids at the terminal chains act as either substrates or inhibitors. Lectins show remarkable specificity towards sialic acids. This specificity arises from the three dimensional structure and conformation of terminal disaccharide of the interacting sugar chain which invariably contain sialic acid. The binding forces are dominantly contributed by the network of hydrogen bonds and the structuring of water molecules in the binding site. The functional groups of sialic acid along with the hydroxyl groups are actively involved in forming hydrogen bonds with the lectins and this fact is evident from the crystal structures of sialyloligosaccharides complexed with lectins [46, 47]. The three dimensional structure of polysialic acids is influenced by many factors including inter-residue interactions and lactonization. It has been reported that the negative charge of the carboxylic acid at C-1 position of Neu5Ac is crucial in determining the conformation of polysialic acids and polysialic acids can form helical structure when the chain consists of more than ten monomers whereas at shorter lengths it occurs as linear chain [26, 48–52]. A three dimensional structural study of α(2-8) linked polysialic

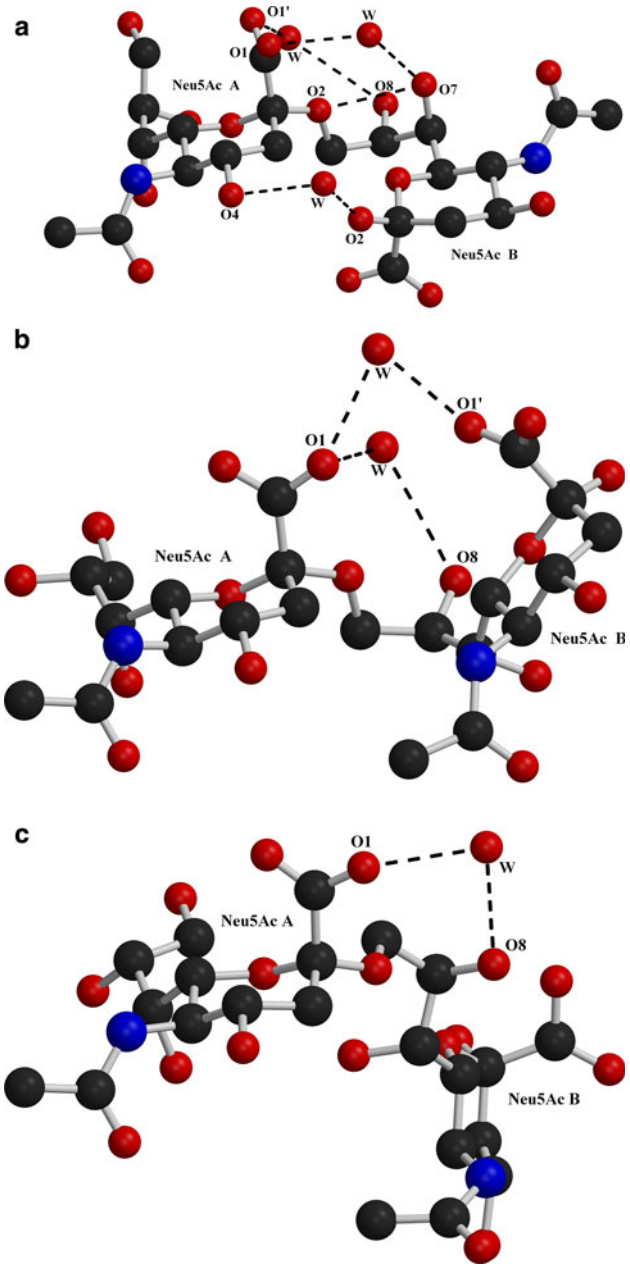

Fig. 9 Three different conformational models for Neu5Acα(2-9)Neu5Ac

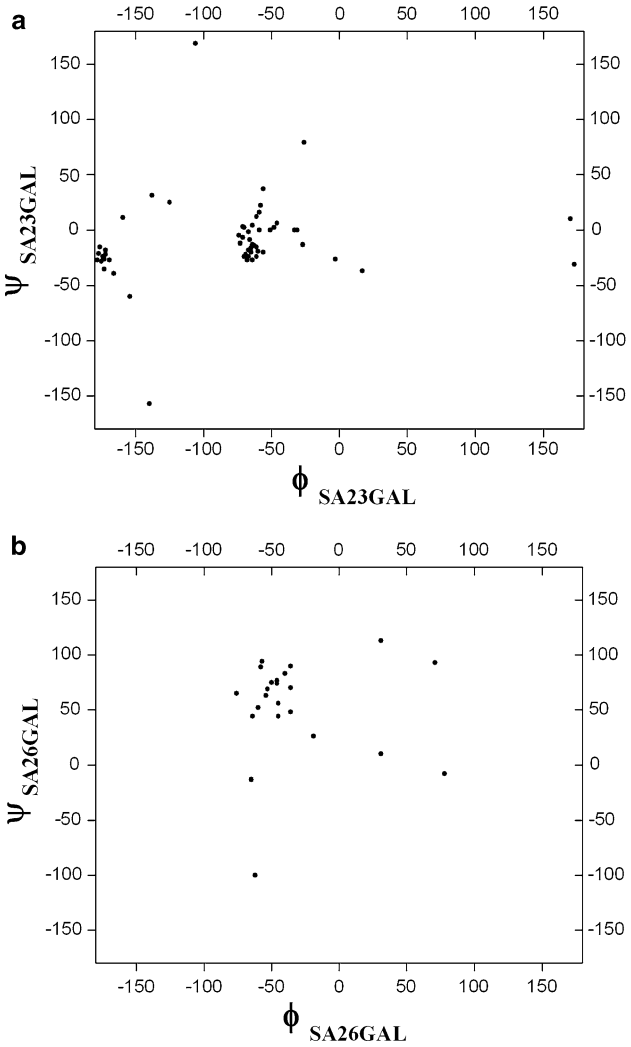

Fig. 10 Observed torsional angle distribution for experimentally derived structures of **a** Neu5Acα(2-3)Gal, **b** Neu5Acα(2-6)Gal

acid by Baumann et al., clearly shows that the polymer can adopt three helical structures with different n values, where n is the number of Sialic acid molecules per turn of the helix [53]. However the interactions stabilizing the helical structure are not discussed. From our conformational analysis of α(2-8) linked disialoside, we understand that the hydrogen bonding interactions between the carboxylic acid group of one Sialic acid with the glycerol side chain of the successive Sialic acid is the reason for the helical structure and stability of the polysialic acid. This result coincides well with the proposed helical structure of Baumann et al.

The flexibility at the glycerol side chain of Neu5Ac can be attributed to the formation of helix. The acetamido group conformation has almost no effect on the overall conformation of the sialyldisaccharides as well as on polysialic acid. To the best of our knowledge, this is the first report on the conformation of sialyldisaccharides by using longer MD simulations. Understanding the structure/function relationship of sialyldisaccharides will aid to our understanding of the protein-carbohydrate interactions and the role of conformation and dynamics of oligosaccharide chains that are a part of glycoproteins. Also the structural studies on disaccharides have given viable results which can be applied in designing sialyldisaccharide analogue inhibitors that could replace the natural receptors and thus either inhibit or induce the biological functions as desired. As oligosaccharide synthesis is gaining momentum with the advent of experimental techniques, it can be believed that the oligosaccharides and sialyldisaccharides can be

synthesized and used in carbohydrate therapeutics and diagnostics within a foreseeable time. The conformational models predicted by using the 20 ns MD simulation are deposited at the 3DSDSCAR Database (http://3dsdscar.org) which is a dedicated three dimensional structural database for sialic acid-containing carbohydrates. The structural coordinates of the conformational models can be viewed and downloaded from the database. These conformational models will be useful for the glycobiologists and structural biologists to understand the atomic level interactions underlying the conformational preferences of protein-carbohydrate interactions.

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