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Conformational Free Energy Surface of α-*N*-Acetylneuraminic Acid: An Interplay Between Hydrogen Bonding and Solvation

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The conformational free energy surface of α-*N*-acetylneuraminic acid (Neu5Ac, sialic acid) in the space of ring-puckering coordinates was calculated using the metadynamics method. Free energy surfaces in vacuum and with an explicit solvent were calculated in GLYCAM 06 force field. In vacuum three structures are almost equivalently populated, namely, the $^{2}C_{5}$ chair and the $B_{3,6}/^{2}S_{6}$ and $^{O}S_{3}$ boat/skew-boat conformations. The $B_{3,6}/^{2}S_{6}$ structure is stabilized by an ionic hydrogen bond between the amide N−H bond and the carboxylic group. However, this structure is unfavorable in a water environment in which the experimentally observed $^{2}C_{5}$ chair conformation is predicted to be more stable than the other structures. These results indicate that environment significantly influences conformation of Neu5Ac and that Neu5Ac-processing enzymes might modify a conformation of their substrates solely by a changing polarity of the environment. The structure of Neu5Ac bound in influenza neuraminidase ($^{4}S_{2}/B_{2,5}$) belongs to conformations preferred in a water environment. The free energy penalty of this conformational change was calculated (relative to $^{2}C_{5}$) as 10.2 ± 2.0 and 17.3 ± 2.0 kJ/mol for $^{4,O}B/^{O}S_{3}$ and $^{4}S_{2}$, respectively. This result indicates that mimicking of the enzyme-bound conformation is likely to be a viable strategy for the design of neuraminidase inhibitors.

Introduction

Experimentally determined structures of carbohydrate-processing enzymes, in complexes with their substrates and products, demonstrate that pyranoses are not always bound in structures corresponding to solution equilibrium; rather they are often bound in less favorable conformations that facilitate catalysis.[1−4] Therefore, the equilibrium and kinetics of conformational transitions in saccharides are very important for understanding the mechanisms of carbohydrate-processing enzymes and for designing their inhibitors.

Sialic acids frequently occur as terminal groups of glycoproteins and glycolipids, including cell-surface glycoconjugates.[5−7] Recognition of sialic acid residue plays an important role in a wide range of biological processes. Sialic acid recognizing proteins from pathogenic organisms are therefore targets for the design of therapeutic agents.[8,9] For example, inhibitors of the influenza virus neuraminidase (acetylneuraminyl hydrolase, EC. 3.2.1.18) have been shown to be effective antiviral agents in humans. The most frequently occurring sialic acid is *N*-acetylneuraminic acid (Neu5Ac, 5-*N*-acetyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid, Figure 1).

It was found that ring conformations of sialic acid in solution and bound in complex with influenza neuraminidase differ; however, this conformational behavior is not fully understood.[10−14] In water solution Neu5Ac exists predominantly in the $^{2}C_{5}$ chair conformation,[15−20] and its anomeric equilibrium is shifted toward β-anomer, contrary to its α-linkage typically found in glycoconjugates. Experimental structures of Neu5Ac−neuraminidase complex[10−14] showed that this enzyme binds free Neu5Ac in its α-anomer, not the β-anomer. Moreover, Neu5Ac is bound not in the most favorable solution conformation ($^{2}C_{5}$) but rather in a boat conformation ($^{4}S_{2}/B_{2,5}$).

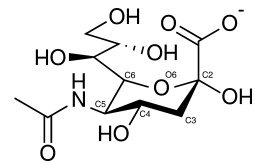

Figure 1. Schematic representation of sialic acid (α-*N*-acetylneuraminic acid, Neu5Ac, 5-*N*-acetyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid) in the $^{2}C_{5}$ conformation with numbered atoms.

An important aspect of the conformational equilibria of Neu5Ac arises from the fact that therapeutically used inhibitors on viral neuraminidase are analogues of the transition state of its reaction.[8,9] The distortion of Neu5Ac ring upon substrate binding is accompanied by a formation of a salt bridge between the negatively charged carboxylic group of Neu5Ac and the side chain of one arginine residue. The reaction then proceeds via a semiplanar oxocarbenium transition state toward formation of an intermediate. It remains unclear whether this intermediate is covalently trapped to the enzyme or it is stabilized only by negative charge of the active site. The intermediate is analogously hydrolyzed in the second step of catalytic cycle. This mechanism model is supported by theoretical and experimental studies[9,21,22] as well as by the fact that semiplanar Neu5Ac analogues mimic the transition state and are therefore potent inhibitors. The fact that the substrate ring is distorted prior to the catalytic step might be explained by lowering the free energy barrier of the first step of catalysis.

Conformational energetics of monosaccharides has been intensively studied in terms of potential energy surface,[23−35] employing different levels of theory including density functional

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theory and ab initio methods. These studies have provided a theoretical basis for development of molecular mechanics force fields such as GLYCAM 04 and 06[36] or carbohydrate-tuned versions of GROMOS[37] or OPLS[38] force fields. However, a potential energy surface of an unsolvated molecule only provides information about the situation in vacuum and at the temperature of 0 K. In order to theoretically study equilibria and kinetics in a water solution and at biological temperatures it is necessary to study the free energy surface of the system.

In principle, it is possible to determine free energy differences by molecular dynamics simulation. Probabilities of different states of the system during a very long simulation can be, in principle, converted into free energy values. However, this approach is not always applicable because studied processes are usually associated with high free energy barriers, and therefore they take place in time scales much longer than those accessible through molecular dynamics simulation. This drawback can be solved by the application of advanced free energy modeling methods including metadynamics.[39−45]

In metadynamics,[39−45] the system is simulated by a standard molecular dynamics simulation to which a special bias potential is progressively added. The bias potential is typically formulated as the sum of Gaussian hills along the trajectory:

$$V_{\mathrm{bias}}(\boldsymbol{q}) = \sum_{t_i<t} \prod_j w_{t_i} \exp\left[\frac{-(q_j(\boldsymbol{x}) - q_j^{t_i})^2}{2\delta {q_j}^2}\right] \qquad (1)$$

where q are collective variables (parameters proposed to represent the progress of the studied change), x are the Cartesian coordinates of the system, and w and δq are the height and width of a Gaussian hill, respectively. This bias potential is history-dependent, i.e., it accumulates during the simulation, it "floods" already explored free energy minima, and thus it facilitates crossing of free energy barriers. Moreover, the bias potential accumulated over the whole simulation approximates the free energy surface of the system. Metadynamics has been applied in numerous fields ranging from geophysics[42] to medicinal chemistry.[43]

Metadynamics requires a set of collective variables (typically one to three). The bias potential then allows efficient exploration of the space of these variables. The resulting free energy surface is also the function of these variables. The repertoire of collective variables tested in metadynamics includes distances,[39−41,43] dihedral angles,[39,43] coordination numbers,[40,41] essential coordinates,[44] and crystallographic descriptors.[42] Biarnés et al. have for the first time introduced metadynamics in the space of ring-puckering coordinates in an effort to study the free energy surface of glucose in vacuum.[45] In light of this study, we used ring-puckering coordinates to study the free energy surface of sialic acid.

Ring-puckering coordinates, introduced in 1975 by Cremer and Pople,[46] provide a general method of dimensionality reduction of ring conformations. The conformation of six-membered rings can be described using three puckering coordinates, defined as

$$q_x = \sqrt{\frac{1}{3}} \sum_{j=1}^{6} z_j \cos\left[\frac{2\pi}{3}(j-1)\right] \qquad (2)$$

$$q_y = \sqrt{\frac{1}{3}} \sum_{j=1}^{6} z_j \sin\left[\frac{2\pi}{3}(j-1)\right] \qquad (3)$$

$$q_z = \sqrt{\frac{1}{6}} \sum_{j=1}^{6} z_j (-1)^{j-1} \qquad (4)$$

where j is index of each ring atom and z_j is its distance from the ring-average plane. In these coordinates energetically favorable conformations are located on a sphere. Chair conformations are located at both poles of the sphere (q_x and q_y are approximately zero), whereas boat and skew-boat conformations are located on the equator (q_z is approximately zero).

In this study we have investigated the conformational properties of α-anomer of Neu5Ac in its ionized form both in vacuum and in water solution. The aim of the simulation in water is to ascertain the free energy differences between conformers of Neu5Ac recognized by sialidases. The aim of the simulation in vacuum is to assess transferability of in vacuo studies (e.g., ab initio) to the situation in water. The second motivation for the simulation in vacuum was to present Neu5Ac as an interesting system for gas-phase experiments. The results of both simulations provide information that may be useful for the characterization of the neuraminidase mechanism and the design of their inhibitors.

Methods

Metadynamics at the molecular mechanics level of theory was performed in a modified version of Grometa 2.0[47,48]—the metadynamics implementation of GROMACS.[49] Sialic acid was modeled using the GLYCAM 06 force field.[36] Details of conversion of GLYCAM topology from AMBER to GROMACS format are presented as Supporting Information. The system in vacuum was equilibrated by 200 ps classical molecular dynamics (MD) simulation at a constant temperature of 300 K. Electrostatic interactions were treated by Coulombic potential with a single cutoff set to 1.6 nm. Standard AMBER 1-4 scaling scheme was used. For simulation in solvent, the solute was solvated by 1392 TIP3P water molecules and 1 sodium counterion. The system was then equilibrated by 200 ps MD at constant pressure and 200 ps in constant volume, both at a temperature of 300 K. Electrostatics was treated using particle-mesh Ewald method with a cutoff set to 1.0 nm. Time step was set to 1 fs, and bonds in the solute were not constrained.

Each metadynamics run comprised 20 ns MD simulation during which 20 000 3D-Gaussian "hills" were added. Width δq of a hill was 0.01 nm in all three directions. Height w of a hill was 0.1 kJ/mol. The metadynamics formulation known as direct metadynamics was used.[41] Free energy surfaces were visualized using MayaVi and PovRay. Confidence intervals of the free energy minima were calculated for the last 5 ns of metadynamics run at 95% probability.

Results

Cyclohexane was selected to test the applied approach as the simplest six-membered ring for which thermodynamics of conformational changes has been experimentally studied. The experimental value of the free energy barrier of conversion from a chair to any boat conformation has been measured as 42 kJ/mol.[50] The generalized AMBER force field (GAFF)[51] was used as molecular mechanics potential. The progress of metadynamics is illustrated in Figure 2. Metadynamics started from one of the chair conformations. After the addition of approximately 3100 hills it flipped to boat conformations. All six boat and six skew-boat conformations were sampled during additions of a further 3500 hills. Then the molecule flipped to the opposite chair conformation. After the addition of another 3100 hills the system started to rapidly interconvert between previously observed conformations. These can be interpreted as a flooding of all free energy minima.

The resulting free energy surface was analyzed to identify free energy minima. Besides both of the chair conformations, which were identified as global minima, the skew-boat confor-

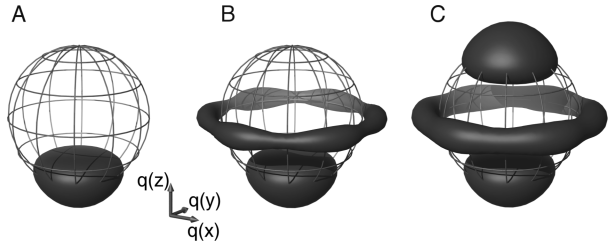

Figure 2. Free energy surface of cyclohexane in vacuum calculated using the GAFF force field. Free energy surfaces after addition of 3000 (A), 6000 (B), and 10 000 (C) hills are presented as isosurfaces at −10 kJ/mol (relative to the unexplored conformations). Diameter of the sphere is 0.05 nm.

mation was identified as the second free energy minimum (27.4 ± 1.1 kJ/mol, relative to the chair conformation). The free energy barrier of conversion from chair to boat/skew-boat was calculated as 48 kJ/mol and was in relatively good agreement with the experimental value (42 kJ/mol).[50] Boat conformations were identified as transition states between two neighboring skew-boats with a free energy barrier of approximately 4 kJ/mol. These results illustrate efficiency of the metadynamics algorithm in modeling the puckering of six-membered rings.

Next, we have investigated the conformational behavior of Neu5Ac using the same procedure. The starting ring conformation for a metadynamics simulation in vacuum was the $^2C_5$ chair conformation. After the addition of approximately 700 hills the system jumped to the $B_{3,6}/^2S_6$ conformation. This conformation is characterized by an ionic hydrogen bond between the amide and carboxylic groups. It stayed in the $B_{3,6}/^2S_6$ conformation during the addition of another approximately 400 hills. The $^OS_3$ conformation was sampled shortly after $^2S_6$. In addition to the $B_{3,6}/^2S_6$, also the $B_{4,O}/^2S_4$ conformation is also characterized by the presence of an ionic hydrogen bond. The inverted chair ($^5C_2$) was sampled for the first time after the addition of approximately 4200 hills from the beginning of the metadynamics run.

As already visible from the development of the metadynamics run, in the resulting free energy surface (Figure 3) there are three free energy minima close in free energy, namely, $^2C_5$, $B_{3,6}/^2S_6$, and $^OS_3$. Their relative free energies are 0.0, −0.5 ± 0.5, and 1.7 ± 0.6 kJ/mol, respectively (relative to $^2C_5$). The free energy barrier between $^2C_5$ and $B_{3,6}/^2S_6$ is approximately 14 kJ/mol. Seven conformations were identified as free energy minima of this system. One skew-boat conformation—$^4S_2$—was not recognized as a free energy minimum because there was no free energy barrier with its neighboring conformation. In general, conformational changes of sialic acid in vacuum are characterized by relatively low free energy values and the existence of two forms with an ionic hydrogen bond.

Metadynamics of solvated sialic acid started from the same initial conformation ($^2C_5$) as in the vacuum simulation. During the metadynamics simulation, the system stayed mostly in the $^2C_5$ conformation for more than 1200 hill additions, which is longer compared to the simulation in vacuum. Then the system jumped into $^{4,O}B/^OS_3$ conformation. The $^2S_6$ conformation, which was sampled shortly after $^{4,O}B/^OS_3$, was not recognized as a separate free energy minimum. The $^5C_2$ was sampled as the last conformational family after the addition of approximately 4500 hills.

The free energy surface in water is depicted in Figure 4. Contrary to the situation in vacuum, where three conformations were within 3 kJ/mol, in water the $^2C_5$ conformation was significantly more stable than other conformations. The system has to overcome the barrier of 27 kJ/mol to get to the $^{4,O}B/^OS_3$

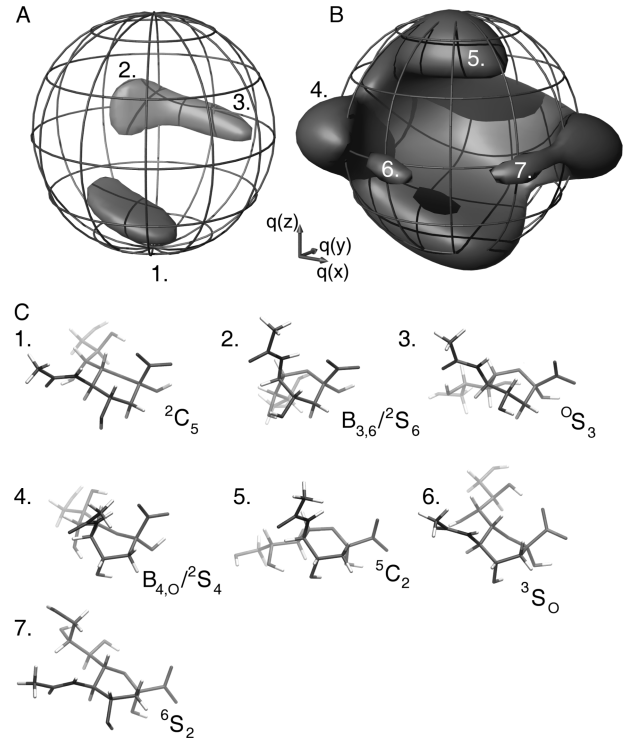

Figure 3. Free energy surface of sialic acid in vacuum calculated using the GLYCAM 06 force field depicted as isosurfaces at 10 (A) and 35 (B) kJ/mol (relative to the free energy minimum—$^2C_5$). Diameter of the sphere is 0.05 nm. Snapshots of metadynamics corresponding to individual free energy minima 1−7 are depicted (C).

conformation, which is 10.2 ± 2.0 kJ/mol higher compared to $^2C_5$. As mentioned earlier, the $^2S_6$ conformation was not recognized as a free energy minimum and its free energy was approximately 30 kJ/mol. Four other boat/skew-boat conformations were identified as free energy minima, with free energy values ranging from 17.3 ± 1.7 ($^4S_2$) to 42.5 ± 1.5 kJ/mol ($^2S_4/B_{4,O}$), relative to $^2C_5$. Free energy barriers between these minima are very low. Free energy of the opposite chair conformation—$^5C_2$—was calculated as 28.1 ± 0.9 kJ/mol, relative to $^2C_5$. Contrary to the free energy surface in vacuum, the ionic hydrogen bond, which is relatively favorable in vacuum, is largely screened by water molecules in solvent. The $^4S_2/B_{2,5}$ conformation, which is present in Neu5Ac bound in neuraminidase, is located in the hemisphere of the conformational space characterized by $^{4,O}B/^OS_3$, $^4S_2$, and $^6S_2$. Free energies of these conformations as determined by metadynamics were between 10 and 20 kJ/mol. We can expect that the free energy of the neuraminidase-bound conformation lies within this range.

The $^2C_5$ conformation was determined as a stable conformation of α-Neu5Ac in vacuum as well as in water. However, in a water environment this conformation is significantly more stable than other conformations. This is in agreement with the fact that Neu5Ac, similarly to most pyranoses, predominantly adopts the chair conformation with the maximum number of equatorial substituents. Schematically drawn free energy surfaces (Figure 5) reveal that not only thermodynamics (free energy differences) but also kinetics (free energy barriers) of conformational changes in Neu5Ac is influenced by the environment. Moreover, a change of environment alters the heights of free energy barriers and can also affect pathways of conformational changes.

Figure 6A illustrates the difference between the conformational behavior of Neu5Ac in water and in vacuum, expressed

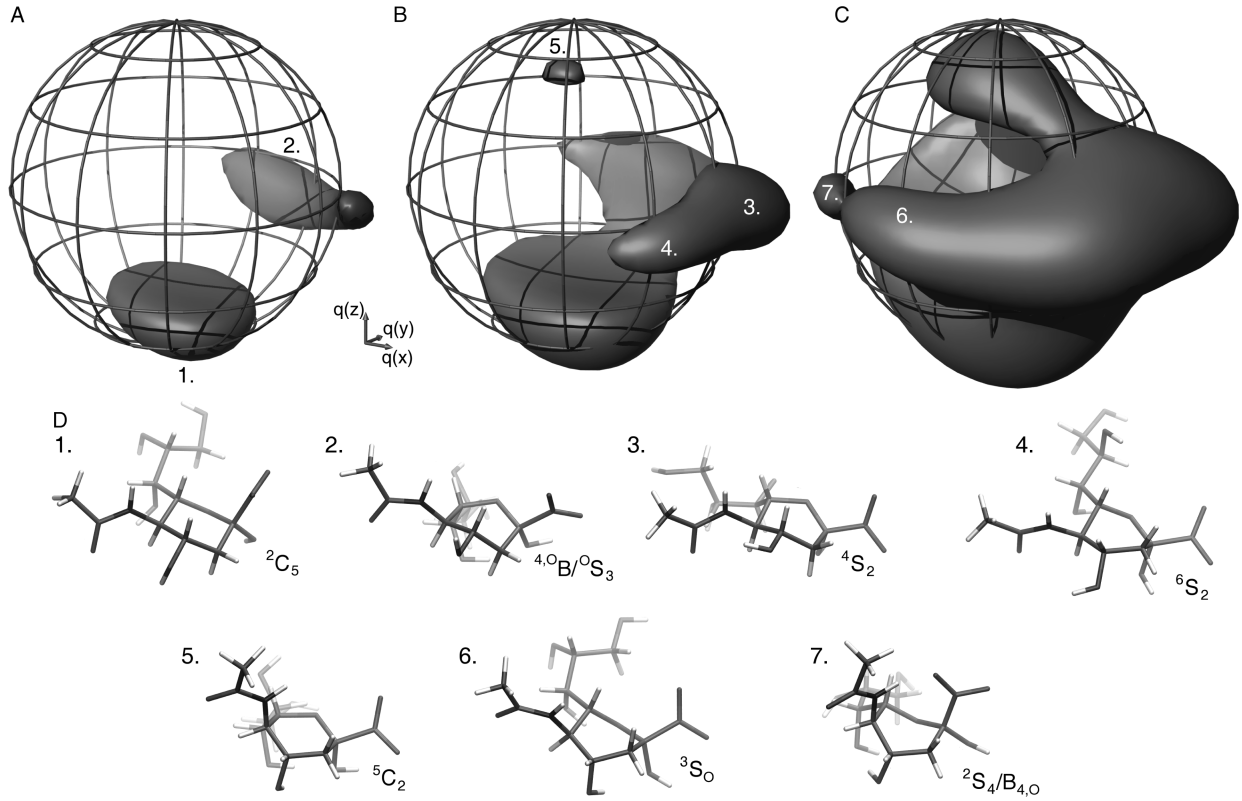

Figure 4. Free energy surface of sialic acid in water calculated using the GLYCAM 06 force field in TIP3P water depicted as isosurfaces at 20 (A), 30 (B), and 45 (C) kJ/mol (relative to the free energy minimum). Diameter of the sphere is 0.05 nm. Snapshots of metadynamics corresponding to individual free energy minima 1−7 are depicted (D).

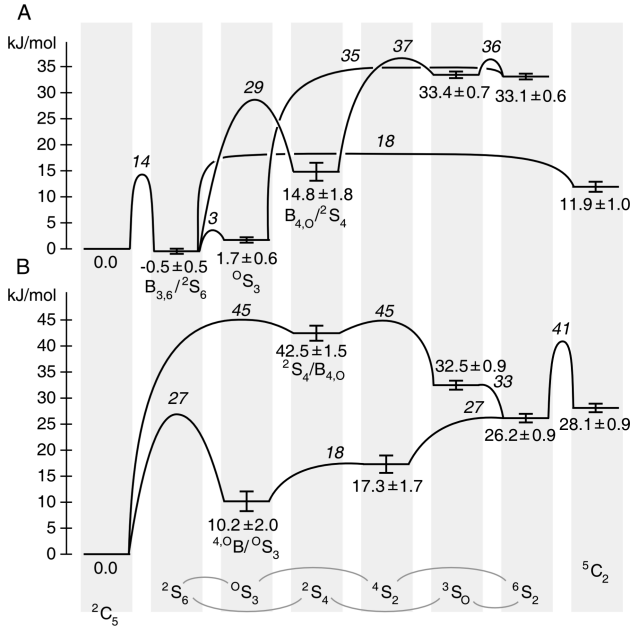

Figure 5. Schematically drawn free energy surfaces of sialic acid in vacuum (A) and in water (B). Free energies of minima and transition states are in kilojoules per mole, relative to $^{2}C_{5}$.

as a difference of free energies in both environments. It can be seen that the sphere in ring-puckering coordinates is divided into two parts of approximately the same size. The region more stable in water includes mainly $^{2}C_{5}$ as well as $^{O}S_{3}$ to $^{6}S_{2}$ conformations. On the other hand, the part more stable in vacuum includes mainly the $B_{3,6}/^{2}S_{6}$ conformation with an ionic hydrogen bond.

Discussion

The metadynamics method has been applied in numerous contexts and has proved to be highly efficient in terms of modeling rare events and in the prediction of free energy differences. As far as we know, the first example of metadynamics in the space of ring-puckering coordinates is the work of Biarnés et al.[45] Their study of the free energy surface of β-D-glucopyranose employed a Car−Parrinello molecular dynamics (CPMD) simulation[52] in vacuum. The free energy surface was determined in the space of two ring-puckering coordinates (q_x and q_y). Contrary to Biarnés et al.,[45] in this study we employed all three coordinates to explore both chair conformations. Instead of ab initio dynamics, we used a molecular mechanics force field which allowed us to run the simulation in an explicitly modeled water environment. Moreover, running a much longer simulation presumably leads to better accuracy in terms of convergence.

On the other hand, the accuracy of a free energy surface calculated using empirical force field depends on the accuracy of the force field. Molecular mechanics modeling of pyranose molecules using general purpose force fields turned out to be very inaccurate due to the complicated conformational behavior of six-membered rings. This led to efforts to develop carbohydrate-tuned versions of the existing biomolecular force fields, including the GLYCAM series. The GLYCAM 06 force field has been developed and evaluated with the Neu5Ac molecule as a part of a training set. Therefore, this force field can accurately model Neu5Ac molecule.

Nevertheless, we decided to evaluate this force field in the modeling of conformations explored during metadynamics run in water by comparison with experimental conformations. The results of metadynamics were compared with experimental data from the Protein Databank (PDB). High-resolution (2.0 Å and better) experimental structures containing α-D-Neu5Ac (either free or as

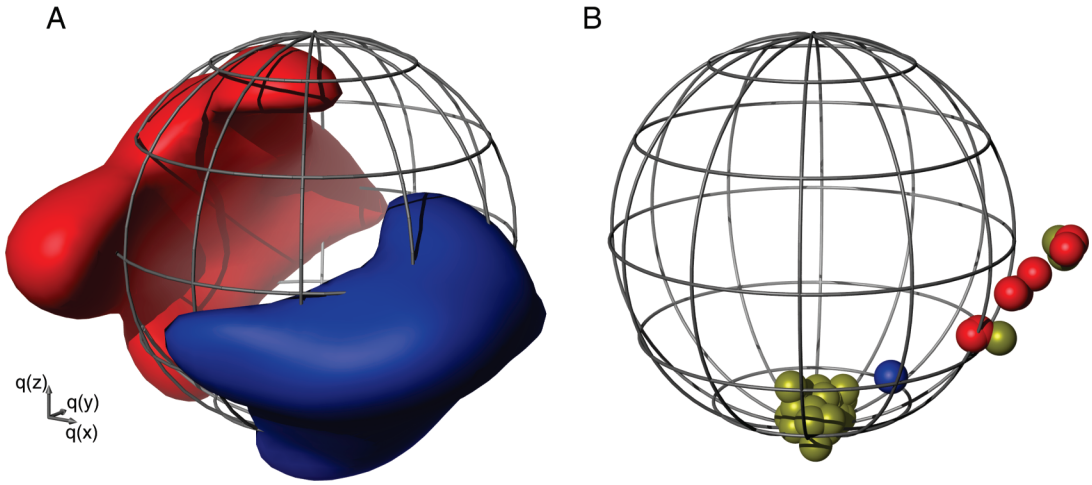

Figure 6. Difference between the free energy surface in vacuum and in water (A, shown as free energy isosurfaces at ±10 kJ/mol). Structures that are more stable in vacuum are in red; those more stable in water are in blue. Free energies of unexplored conformations were set as zero. Conformations of 209 Neu5Ac residues in experimental structures in the Protein Databank were projected to ring-puckering coordinates (B). Neu5Ac residues in the influenza neuraminidase active site are presented as red spheres, others as yellow spheres. A neuraminidase inhibitor mimicking the transition state of the reaction is shown as a blue sphere.

glycosides) were selected using data mining tools at the Web site http://www.glycosciences.de.[53] These residues (72 in total) were then projected to ring-puckering coordinates (Figure 6B and Supporting Information). The majority of residues were in the classical chair conformation. Neu5Ac residues in the active site of influenza neuraminidase were in boat conformation (shown as red spheres). Also, the transition state analogues of the reaction catalyzed by neuraminidase (zanamivir and the active form of oseltamivir) in semiplanar conformation are included (blue spheres). The overall distribution of Neu5Ac conformations in carbohydrate−protein complexes and glycoconjugates is in very good agreement with the calculated free energy surface in water (Figure 6A). Conformations more stable in vacuum were generally not observed in PDB. This result clearly illustrates the accuracy of the force field employed.

The results of our metadynamic simulations clearly show that the free energy surface is considerably different in vacuum and in water. This difference can be explained by the presence of an ionic hydrogen bond, which is screened by water molecules. Similar solvent dependence of carbohydrate conformations have been observed in equilibrium of primary hydroxyl groups in hexopyranosides.[32,54] In the work of Biarnés et al.,[45] the free energy surface of β-D-glucopyranose was determined only in vacuum. The fact that this free energy surface is in good agreement with experimental results in water, and with the observed distribution of glucose conformations in experimental structures of glucosidases, implies that the effect of solvent on the equilibrium of ring conformations of glucose is relatively low, in contrast to Neu5Ac. Also, a recent CPMD study of solvated β-D-glucopyranose[55] indicates relatively limited interactions with a water environment. A metadynamics study of β-D-glucopyranose with and without solvent would further elucidate this issue. Moreover, the difference in dynamics of Neu5Ac in two different environments makes it an interesting object of gas-phase studies, which are gaining popularity in the field of glycochemistry.[56] Similarly to β-D-glucopyranose,[45] solvated Neu5Ac is most stable in the conformation with the maximal number of equatorial substituents, which is $^4C_1$ and $^2C_5$ for glucopyranose and Neu5Ac, respectively. The most favorable pathways of their conversion to boat/skew-boat conformations are analogous in both molecules ($^4C_1$ to $^1S_3$ in glucopyranose and $^2C_5$ to $^4S_2$ in Neu5Ac).

The fact that the conformational behavior of Neu5Ac is strongly influenced by the environment indicates that Neu5Ac-recognizing proteins can stabilize certain conformations simply by the effect of polarities of the environment. In viral neuraminidase the Neu5Ac is bound in $^4S_2/B_{2,5}$ boat conformation. This conformation was modeled using metadynamics as a relatively stable one. Affinity of the enzyme toward Neu5Ac and corresponding glycosides can be divided into two processes. The first is the conformational change of Neu5Ac from $^2C_5$ to a boat conformation. Calculated free energy values of boat and skew-boat conformations ($^{4,O}B/^OS_3$ and $^4S_2$), which are similar to conformations found in viral neuraminidases, range from 10 to 20 kJ/mol, relative to $^2C_5$. In the second step the boat structure is then bound to the binding site. Taking into account the experimental Km of the α-Neu5Ac glycoside cleavage by neuraminidase (55 μmol/L to 1.82 mmol/L),[57] the virtual affinity of neuraminidase toward $^4S_2/B_{2,5}$-form of Neu5Ac is likely to be in the millimolar to micromolar range (dissociation constant 10^{-3} to 10^{-6}). The strength of such an interaction is in the range usual for carbohydrate−protein interactions. Therefore, the conformational change in Neu5Ac does not strikingly disfavor recognition of the substrate by viral neuraminidase. On the other hand, the fact that the substrate is bound in a higher-energy conformation can be exploited by the enzyme to reduce the free energy barrier of the enzymatic reaction. Detailed understanding of the mechanism of neuraminidase-catalyzed hydrolysis is necessary to test this hypothesis.

The therapeutic inhibitor of neuraminidase—Zanamivir (Relenza)—is a representative example of a successful strategy of design for an inhibitor which mimics the transition state of the enzymatic reaction. Another therapeutic neuraminidase inhibitor—oseltamivir (Tamiflu)—also represents an analogue of the transition state of the reaction. It is generally accepted that the reaction proceeds via an oxonium cation transition state, which is more planar than the substrate or product. Conformation of both compounds corresponds to half-chair with a planar system corresponding to C2 atom in Neu5Ac. However, apart from the strategy oriented to transition state mimicking, the free energy difference between solution conformation of Neu5Ac and its conformation in the active site of viral neuraminidase indicates that mimicking of enzyme-bound conformation could be a viable strategy. A potential conformationally locked analogue of Neu5Ac can in principle bind with affinity comparable to that of pure $^4S_2/B_{2,5}$-form but without losing the free energy by its conformational change.

Conclusions

The present results shed some light on the conformational behavior of Neu5Ac. Free energy surfaces of Neu5Ac were described in the space of ring-puckering coordinates and calculated by metadynamics simulation in a GLYCAM 06 force field in vacuum and in explicit solvent, leading to a free energy resolution of more than 2 kJ/mol. The calculated free energy surface in explicit model is in good agreement with available experimental data, namely, conformational distribution in experimental structures. The results show that environment significantly influences the conformational equilibrium of Neu5Ac, which is considerably different in vacuum than in aqueous solution. In vacuum, three structures are almost equivalently populated, namely, $^{2}C_5$ chair and $B_{3,6}/^{2}S_6$ and $^{O}S_3$ boat/skew-boat conformations. The $B_{3,6}/^{2}S_6$ structure is stabilized by an ionic hydrogen bond between the amide N−H bond and the carboxylic group. However, this interaction is unfavorable in water due to a competition with solute−solvent interactions. In water the $^{2}C_5$ chair conformation is significantly more stable than other structures. These results also indicate that the environment of Neu5Ac-processing enzymes might modify the conformation of their substrates as a result of changing the polarity. The structure of Neu5Ac bound in influenza neuraminidase ($^{4}S_2/B_{2,5}$) belongs to conformations more preferred in a water environment. The free energy penalty of conformational change from the solution structure to the enzyme-bound structure was estimated as 10−20 kJ/mol (calculated as the range for $^{4,O}B/^{O}S_3$ and $^{4}S_2$ conformations). The obtained information regarding the conformational behavior of Neu5Ac is relevant for the design of neuraminidase inhibitors, since it indicates that mimicking of the enzyme-bound conformation might be a viable strategy.

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Supporting Information Available: Details on force field conversion and an extension of Figure 6B. This material is available free of charge via the Internet at http://pubs.acs.org.

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