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Molecular dynamics simulation and quantum mechanical calculations on α-D-*N*-acetylneuraminic acid

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ABSTRACT

N-Acetylneuraminic acid is a sugar molecule of biological significance due to its pivotal role in molecular recognition processes. The three dimensional structure and conformation of α-Neu5Ac in biological environments can be clearly observed by molecular dynamics (MD) simulation and quantum mechanical (QM) calculations. A 10 ns MD simulation on α-Neu5Ac yields two conformational models which are stabilized by water mediated hydrogen bond between O-8/O-9 hydroxyl oxygen and carbonyl of carboxylate group. The average life time of the conformers and the residual time of water which mediates the hydrogen bonding interactions are computed. Based on the amphiprotic nature of water, water mediation of each conformer is divided into two different modes, one donor–one acceptor mode and two donor modes. According to the analysis of simulation trajectories, the preferred mode of water mediation for conformers is the one donor–one acceptor mode. The energy and geometry of the MD derived conformational models of α-Neu5Ac are optimized using HF/6-31G* basis set of Gaussian03. QM calculations also resulted that α-Neu5Ac is preferentially stabilized by water mediated hydrogen bonding between O-8 hydroxyl and the carboxylate group where the mediation is one donor–one acceptor type. The optimized geometry of α-Neu5Ac which is in good agreement with the crystal structure of α-D-*N*-acetyl-1-*O*-methylneuraminic acid methyl ester is deposited in the public domain database 3DSDSCAR (http://3dsdscar.org). This optimized structure can be used by biotechnologists, biophysicists and glycobiologists for modelling the sialylglycans and also to design drugs using sialic acid analog inhibitors.

1. Introduction

Right from their discovery in 1957 by Gunner Blix et al., sialic acids always fascinated glycobiologists due to their extensive roles in biological processes.[1–5] Sialic acids are widely distributed in animal tissues mostly as the terminal residue of oligosaccharide chains protruding from the cell surface. The terminal occurrence, existence of more than 50 diversified forms of sialic acids, and its negative charge make their role indispensable for molecular recognition processes. The structural diversity of sialic acids arises from the functional group modifications at different positions of the pyranose ring and the substitution of acetyl, methyl, lactyl, phosphate, or sulfate groups at different hydroxyl positions (C-4, C-7, C-8, and C-9).[6–11] Of all the diverse forms of sialic acids, Neu5Ac (*N*-acetyl neuraminic acid) is the most common sialic acid occurring in nature. Both anomers of Neu5Ac play vital roles in recognition processes such as decoys as well as receptors for pathogens.[12–23] Study of structure and conformation of Neu5Ac is essential to understand the molecular basis of recognition processes involving Neu5Ac.

Experimental and theoretical methods are employed to investigate the conformational flexibility of both the anomers of Neu5Ac. The conformational study of α-Neu5Ac by Sawada et al.[24] had used DFT and considered Neu5Ac as a neutral molecule regardless of its negative charge. Hard sphere energy (HSE) calculations on the conformation of α-Neu5Ac were also reported by Veluraja in 1984.[25] The effect of water on the conformation of α-Neu5Ac was not taken into account because of the limitations associated with the methods and computational power. Despite the biological importance of α-Neu5Ac, no significant attempts have been made to study its conformational aspects. On the other hand, the structure and conformation of β-Neu5Ac had been studied using X-ray by Flippen in 1973,[26] ^{1}H NMR by Brown[27] and ^{13}C NMR by Czarenecki and Thornton.[28] These experimental results were consistent with the ring conformation of β-Neu5Ac, but they were inconsistent with the conformations of glycerol side chain, acetamido group, and the interactions which stabilize the structures. The only theoretical study on the structure and conformation of β-Neu5Ac was made by Veluraja and Rao in the year 1980 using HSE[29] calculations and the proposed models were able to explain the NMR results of Czarniecki and Thornton.[28] In the present work, we have investigated the conformational dynamics of α-D-*N*-acetylneuraminic acid in aqueous solution using molecular dynamics (MD) simulations.

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The conformational models deduced from MD are energy and geometry optimized using quantum mechanical (QM) calculations.

2. Computational details

The molecular structure of sialic acid (α-Neu5Ac) is shown in Figure 1. The dihedral angles denoting the side chain conformational flexibility are marked. The cartesian coordinates for α-Neu5Ac are generated using standard bond length, bond angle, and dihedral angles. AMBER9 force fields gaff is used to generate force field parameters for α-Neu5Ac.[30,31] To neutralize the negative charge carried by carboxylate group, a sodium ion is added to the system. To mimic the biological environment, TIP3P water molecules from AMBER solvent library are added into the box. The total system comprising of α-Neu5Ac and the water is simulated using NAMD over a period of 10 ns and the trajectories are collected once in every pico second resulting in 10,000 structures.[32] During the simulation, number of particles (*N*), pressure (*P*) and temperature (*T*) of the system are kept constant. Information on the conformational dynamics of α-Neu5Ac is derived by analyzing the MD trajectories using the graphical software VMD and the in-house FORTRAN programs. QM calculations on the MD derived conformational models are performed using Hartree–Fock theory with polarized split-valence 6-31G*[33] basis set in Gaussian03. This basis set is widely used for molecular structure optimization purposes.[34–41] MOLSCRIPT is used to render the conformational models.[42]

3. Results

3.1. Ring, acetamido and carboxylate group conformations of α-Neu5Ac

The conformations of α-Neu5Ac can be described by ring flexibility, acetamido group, carboxylate group, and the glycerol side chain. The analysis on the MD trajectories (10,000 structures) reveals that the ring torsional angles C-2–C-3–C-4–C-5, C-3–C-4–C-5–C-6, C-4–C-5–C-6–O-6, C-5–C-6–O-6–C-2, C-6–O-6–C-2–C-3 and O-6–C-2–C-3–C-4 favor the values around 54°, −53°, 52°, −58°, 56° and −51°, respectively, with a mean deviation of ±5° and this result confirms that the pyranose ring prefers a rigid chair conformation ($^2C_5$) in aqueous solution and is consistent with the earlier results.[26–28] The corresponding dihedral values of β-Neu5Ac as reported by Flippen are 54°, −55°, 58°, −61°, 58°, and −54°. Very similar ring conformation had been reported by the NMR experiments.[27,28] A MD simulation study by Spiwok has also confirmed that the $^2C_5$ chair conformation is the stable conformation for α-Neu5Ac in solution.[43] The acetamido group (χ_n) lies predominantly in trans conformation (≈90% population). The preferred dihedral value for carboxylate group (χ_c) is 138°.

3.2. Glycerol side chain conformation and hydrogen bonding schemes

The conformation of the glycerol side chain can be described in terms of three dihedral angles χ_1, χ_2, and χ_3 (Fig. 1). χ_1 adopts trans conformation during the entire 10 ns simulation. The conformational behavior of χ_2 reveals that it exists in two distinct conformations, g+ ($\chi_2 = 76°$) with 63% population and trans ($\chi_2 = 172°$) with 24% population. The conformational transition of χ_2 has occurred more than 20 times during the 10 ns duration. χ_3 tends to adopt trans ($\chi_3 = 173°$) and g+ ($\chi_3 = 64°$) conformations almost equally and the transition has occurred more than 10 times in the simulation. It shows that the 10 ns MD simulation is sufficient for the glycerol side chain to sweep over the entire conformational space.

During the entire simulation, the structures are stabilized by water mediated hydrogen bond between (i) the carboxylate group and the O-8/O-9 hydroxyl oxygen of glycerol side chain and (ii) the acetamido group and O-7/O-8/O-9 of glycerol side chain. While the former interactions prevail over most of the simulation time, the latter interactions are found to occur in negligibly small time. When water mediated hydrogen bonding interaction is formed between the carboxylate group and the hydroxyl oxygen of O-8, χ_2 favors trans conformation (conformer 1) and is shown in Figure 2. The average life time of this conformational state is 22.23 ± 3.2 ps and the residual time of water which mediates the hydrogen bonding is 6.0 ± 1.4 ps. When χ_2 prefers g+ and χ_3 prefers trans conformation, water mediates a hydrogen bonding interaction between carboxylate group and O-9 (conformer 2) and is shown in Figure 3. The average life time of the conformer 2 is 6.91 ± 2.3 ps and the residual time of water mediating the interaction is found to be 4.8 ± 1.2 ps. Based on the conformational propensity, life time of conformations, and residual time of water conformer 1 is the most preferred structure of α-Neu5Ac.

The minimum energy conformer of α-Neu5Ac in vacuum has been proposed by Veluraja using HSE calculations. It showed that χ_2 can exist in trans and g+ conformations with relative energy values of 0.0 and 3.4 kcal/mol, respectively, along with the global minimum conformation being the trans conformation. The same conformational preferences are reprised by the 10 ns MD simulation on α-Neu5Ac in aqueous solution with the relative energy values of 0.0 and 1.2 kcal/mol. The difference in the relative energies between HSE calculations and MD simulations can be accounted for the presence of water molecules. The role of water is crucial

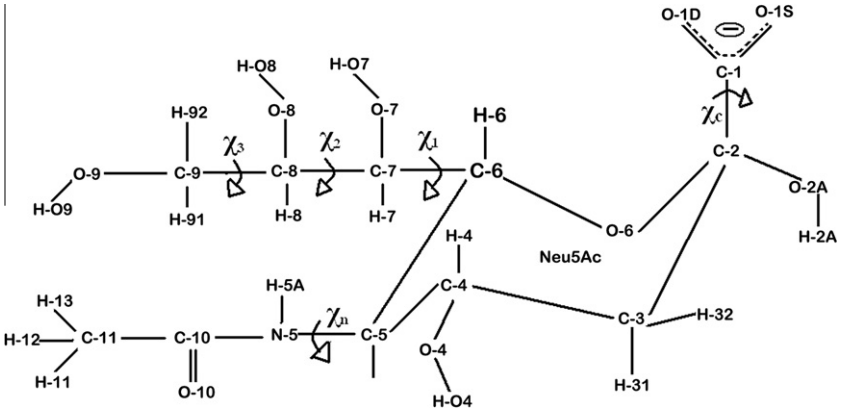

Figure 1. Schematic representation of Neu5Ac (α-anomer) [χ_n = H-5–C-5–N-5–C-10; χ_1 = C-5–C-6–C-7–C-8; χ_2 = C-6–C-7–C-8–C-9; χ_3 = C-7–C-8–C-9–O-9 and χ_c = O-1S/O-1D–C-1–C-2–O-6].