Studies on the conformations of sialyloligosaccharides and implications on the binding specificity of neuraminidases

K. VELURAJA and V. S. R. RAO

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

Abstract. Theoretical investigations, using semi-empirical potential functions have been carried out to predict the favoured conformations of the terminal dissaccharide fragments of various sialyloligosaccharides. The proposed conformational similarity for these fragments has been correlated to the binding specificity of neuraminidases. These calculations predict that bacterial neuraminidases have a binding site which can accommodate only two sugar residues and virus neuraminidases have a binding site which can accommodate more than two sugar residues.

Keywords. Conformation: N-acetylneuraminic acid; neuraminidases; sialic acid; sialyloligosaccharides.

Introduction

Sialic acid (N-acetylneuraminic acid – NeuNAc) which is an important constituent of many of the cell surface carbohydrates plays a dominant role in the biological functions of cells (Jeanloz and Codington, 1976; Schauer, 1982). It mainly occurs as the terminal sugar residue in glycoconjugates. The predominant linkages that have been observed for sialic acid (SA) in glycoconjugates are

SAα(2-3)Gal-R

SAα(2-3)GalNAc-R

SAα(2-6)Gal-R

SAα(2-6)GalNAc-R

SAα(2-8)SA-R

where R is the carbohydrate core structure. Neuraminidase enzymes of bacterial origin (*Vibrio cholerae* neuraminidase, *Clostridium perfringens* neuraminidase and *Arthrobacter ureafaciens* neuraminidase) are widely used reagents for cleaving the SA from sialyloligosaccharides. These enzymes are nonspecific for the type of sialyloligosaccharides which serve as substrates (Uchida *et al.,* 1979: Corfield *et al.,* 1981,1983), *i.e.* their activity does not depend on whether the SA (NeuNAc) is linked to the C3 of Gal or GalNAc, C6 of Gal or GalNAc or C8 of another SA (NeuNAc). On the other hand, virus neuraminidases (Newcastle disease virus, Fowl plague virus and Influenza A_2 virus neuraminidases) cleave $2 \rightarrow 3$ but not $2 \rightarrow 6$ (Schauer. 1982; Paulson *et al.,* 1982) linkages. Newcastle disease virus neuraminidase is able to cleave $2 \rightarrow 8$ linkages

---

Abbreviations used: NeuNAc, N-Acetylneuraminic acid; SA, sialic acid.

whereas Fowl plague virus neuraminidase and Influenza A_2 virus neuraminidase do not. To understand the binding specificity of neuraminidase enzymes on various substrates (sialyloligosaccharides) an attempt has been made using semi-empirical potential functions to study the favoured conformations of the terminal disaccharide fragments of the sialyloligosaccharides.

Method of calculation

The various disaccharide fragments that were studied are shown in figure 1. Steric maps were constructed for all the disaccharide fragments using the contact criteria (Ramachandran and Sasisekharan, 1968). Various conformations were generated by allowing rotations about the interunit glycosidic bond C2-O (ϕ-rotation) and O-CX(ψ-rotation) from $-180°$ to $+180°$ at intervals of $10°$. All the sugar residues were
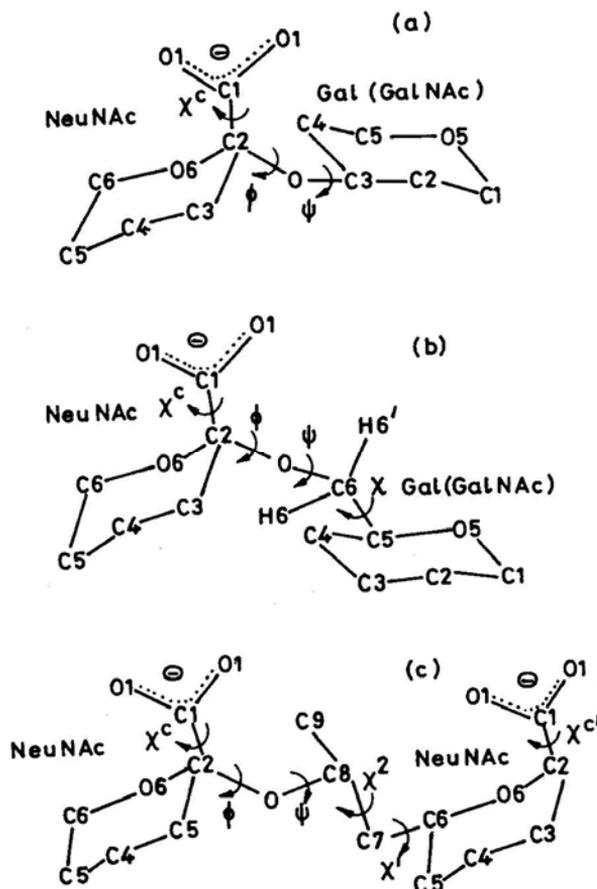

Figure 1. Numbering of atoms and possible rotational angles of the disaccharide fragment. (a)NeuNAcα(2-3)Gal   (NeuNAcα(2-3)GalNAc),   (b)NeuNAcα(2-6)Gal   (NeuNAcα(2-6)GalNAc), (c) NeuNAcα(2-8)NeuNAc. The side groups are not shown.

assumed to be in $^4C_1$ (D) chair form except NeuNAc which was assumed to be in $^2C_5$ (D) chair form. The atomic coordinates used for these residues were based on the geometry of Arnott and Scott (1972). The acetamido group of N-acetylgalactosamine was fixed using Pauling-Corey geometry (Corey and Pauling, 1953) so that C2–H2 and N–H bonds were trans. The geometry used for the NeuNAc was the same as described earlier (Veluraja and Rao, 1980,1983,1984). The side chains of NeuNAc (glycerol side chain and N-acetamido group) were fixed in their respective minimum energy conformations (Veluraja and Rao, 1980). The bond angles at the glycosidic oxygen atoms were fixed at the average value of 117·5°. The initial conformations were defined as

$$\phi = 0 \text{ when C1-C2 } cis \text{ to O-CX}$$
$$\psi = 0 \text{ when C2-O } cis \text{ to CX-HX}$$
$$\chi^c = 0 \text{ when O1-C1 } cis \text{ to C2-O6}$$
$$\chi = 0 \text{ when O-C6 } cis \text{ to C5-O5}$$
$$\chi^1 = 0 \text{ when C5-C6 } cis \text{ to C7-C8}$$
$$\chi^2 = 0 \text{ when C6-C7 } cis \text{ to C8-C9}$$

(where X represents the number of the carbon atom involved in the glycosidic linkage). In NeuNAc α(2-6)Gal (NeuNAcα(2-6) GalNAc) H6 is that hydrogen which makes an angle of H6-C6-C5-O5 = 120° when $\chi = 0°$. Clockwise rotation is taken as positive.

Steric maps for the disaccharide fragment NeuNAcα(2-8)-NeuNAc were obtained by fixing χ^1 and χ^2 in all the staggered positions. The steric map obtained when χ^1 and χ^2 were fixed in the minimum energy conformation ($\chi^1 = 150°$, $\chi^2 = 70°$) alone is shown in figure 2.
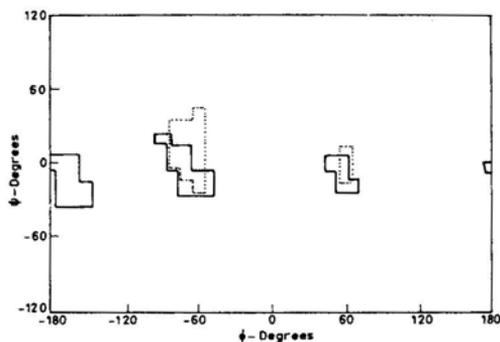

Figure 2. Steric map for the disaccharide fragment (——) NeuNAcα(2-3)Gal(. . . . . .) NeuNAcα(2-8)NeuNAc($\chi^1 = 150°$; $\chi^2 = 70°$).

The potential energy of the molecules was computed taking into account the non-bonded, electrostatic and torsional contributions. The nonbonded contribution was computed using the modified Lennard-Jones potential functions and the constants given by Momany *et al.* (1975). The σ-charge on the various atoms of the ionized form of NeuNAc (the form which is present at physiological pH) was calculated following MO-LCAO (Molecular Orbital-Linear Combination of Atomic Orbitals) method of

Del Re *et al.* (1963). π-Charges for the acetamido group and carboxylic acid group were taken from Vijayalakshrni (1972). Since the carboxylic acid group was assumed to be in the ionized state, π-charge was distributed equally on both the oxygen atoms. The total charge on each atom was obtained by summing σ-and π-charges. For the carboxylic oxygen atoms an additional charge of about −0·5 units was added as suggested by Del Re *et al.* (1963). The net charge associated with each atom was used to compute electrostatic energy.

   The disaccharide fragments can assume a large number of conformations, due to possible rotations about the interunit bonds. Hence the energy was minimized as a function of rotational angles following the minimization procedure of Fletcher and Powell (1963) and Davidon (1959).

Results and discussion

The steric maps for the disaccharides NeuNAcα(2-3)Gal, NeuNAcα(2-8)NeuNAc and NeuNAcα(2-6)Gal, (figures 2 and 3) indicate that less than 4 % of the total region is allowed in the (ϕ, ψ) plane in agreement with earlier results (Sathyanarayana and Rao, 1971). The addition of the acetamido group at the C2 position of the galactose residue does not appreciably affect the allowed region in the (ϕ, ψ) plane of the NeuNAcα(2-3)Gal and NeuNAcα(2-6)Gal. Therefore the steric maps for the disaccharide fragments NeuNAcα(2-3)GalNAcand NeuNAcα-(2-6)GalNAc are not shown.

   In the disacchardie fragments NeuNAcα(2-3)Gal and NeuNAcα(2-3)GalNAc,(ϕ, ψ) favour values around (−70°, −10°) (tables 1 and 2). When they favour values around
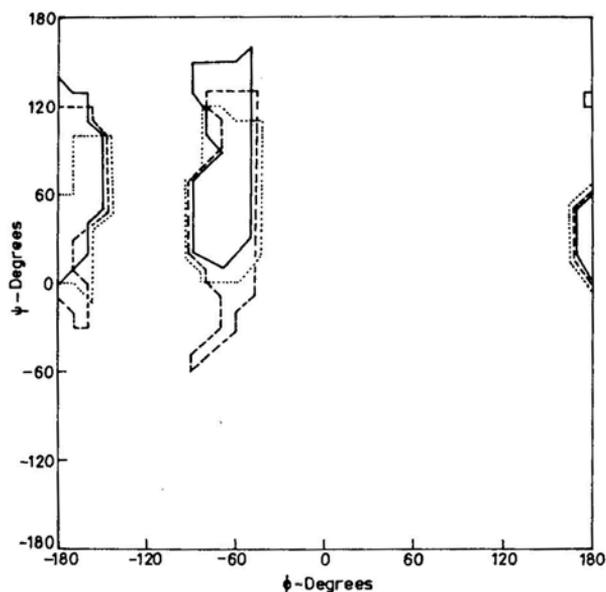

Figure 3. Steric map for the disaccharide fragment NeuNAcα(2-6)Gal. (——) (χ = 180°); (−−−)(χ= 60°);(. . . . . .)(χ=−60°).

Table 1. Minimum energy conformations of NeuNAcα(2-3)Gal.

χ^c	ϕ	ψ	Relative energy kcal mol^{-1}
−35	−67	−7	0·0
−38	−150	−20	4·0
−42	55	−8	7·9

Table 2. Minimum energy conformations of NeuNAcα(2-3)GalNAc.

χ^c	ϕ	ψ	Relative energy kcal mol^{-1}
−35	−69	−6	0·0
−41	56	−10	4·7
−38	−154	−21	6·3

(−155°, −20°) and (55°, −10°) the energy increases by more than 4 kcal mol^{-1} . This rules out the possibility of occurrence of these disaccharide fragments in these conformations. Projections of the global minimum energy conformers of NeuNAcα(2-3)Gal and NeuNAcα(2-3)GalNAc are shown in figures 4a and 5a.

Tables 3 and 4 give the probable conformers of the disaccharide fragments NeuNAcα(2-6)Gal and NeuNAcoα(2-6)GalNAc respectively. It is interesting to note

Table 3. Minimum energy conformations of NeuNAcα (2-6)Gal.q

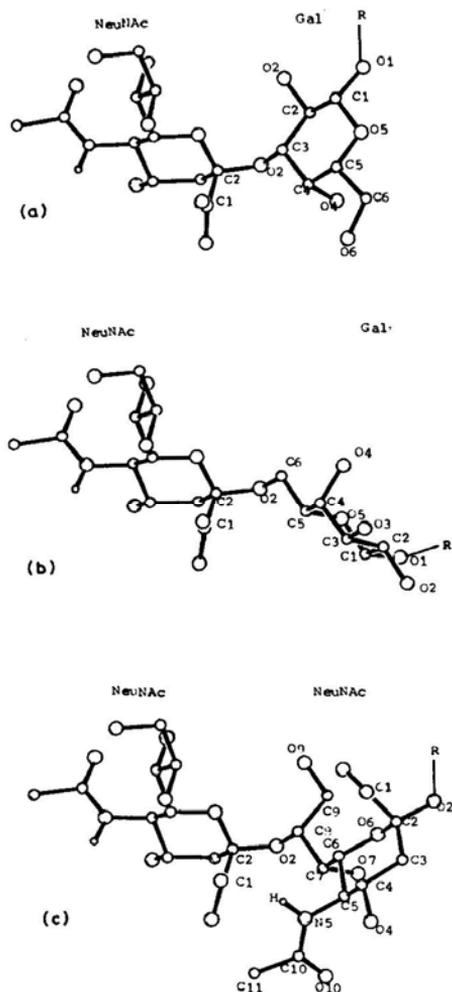| χ^c	ϕ	ψ	χ	Relative energy kcal mol^{-1}
−35	−66	−13	73	0.0
−35	−80	144	−176	0·9
−36	−82	34	65	1·0
−34	−61	80	180	1·5
−33	−68	60	63	1·5
−34	−83	52	177	1·6
−37	−147	70	178	2·5
−39	−171	17	175	2·9
−35	−61	86	−53	3·2
−38	−148	−28	83	3·3
−35	−74	130	70	3·3
−32	−141	67	69	3·4
−44	64	84	−177	4·0

Figure 4. Projection of the global minimum energy conformer of (a) NeuNacα(2-3)Gal, (b) NeuNAcα (2-6)Gal and (c) NeuNAcα(2-8)NeuNAc.

that both the disaccharide fragments favour similar conformations (conformers 1 to 4 of table 3 are equivalent to conformers 1 to 4 of table 4). The dihedral angles (ϕ, ψ, χ) which define the conformation of these disaccharide fragments favour values around $(-70°, -10°, 70°)$. When they favour values around $(-80°, 145°, -175°)$; $(-80°, 40°, 65°)$ or $(-60°, 80°, 180°)$ the conformational energy increases by about 0·9, 1·0 and 1·5 kcal mol^{-1} respectively in NeuNAcα(2-6)Gal and 1·6, 1·9 and 2·7 kcal mol^{-1} respectively in NeuNAcα(2-6)GalNAc. This indicates that the addition of the acetamido group at C2 of galactose in NeuNAcα(2-6)Gal to give NeuNAcα (2-6)GalNAc affects neither the favoured conformations nor the relative energies of these molecules significantly. Projections of the global minimum energy conformers of NeuNAcα(2-6)Gal and NeuNAcα(2-6)GalNAc are shown in figures 4b and 5b.
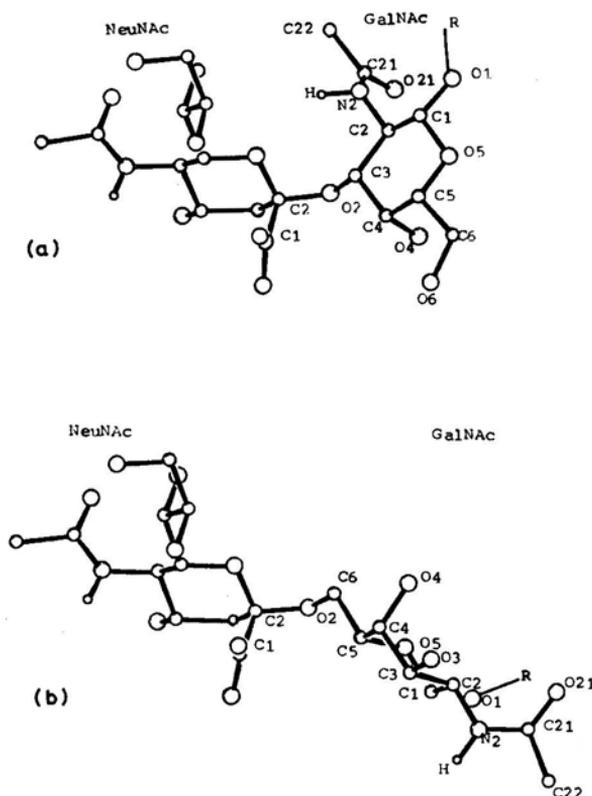
Figure 5. Projection of the global minimum energy conformer of (a) NeuNAcα(2-3)GalNAc and (b) NeuNAcα(2-6)GalNAc.

Table 4. Minimum energy conformations of NeuNAcα (2-6)GalNAc

χ^c	ϕ	ψ	χ	Relative energy kcal mol^{-1}
-35	-66	-14	73	0·0
-35	-81	145	-176	1·6
-32	-85	48	64	1·9
-36	-60	87	180	2·7
-34	-84	52	175	2·9
-38	-148	70	177	3·4
-38	-146	-29	85	3·5
-39	-170	14	172	3·7
-39	-142	67	70	4·1

In the disialic acid fragment NeuNAcα(2-8)NeuNAc the dihedral angles (ϕ, ψ, χ^2, χ^1) which define its conformation prefer values around (–75°, 10°, 70°, 150°) (table 5). When these dihedral angles assume values around (–130°, 45°, 160°, 115°) or (–165°, 0°, 80°, 170°) the conformational energy increases by about 3·4 and 4·6 kcal mol^{-1} respectively. This suggests the low probability of occurrence of the disialic acid fragment in a conformer other than the global minimum energy conformer. A projection of the low energy conformer of NeuNAcα(2-8)NeuNAc is shown in figure 4c.

Table 5. Minimum energy conformations of NeuNAcα(2-8)NeuNAc.

χ^c	ϕ	ψ	χ^2	χ^1	$\chi^{c'}$	Relative energy kcal mol^{-1}
–39	–76	8	70	148	–46	0·0
–42	–128	43	160	114	–36	3·4
–39	–165	–2	86	169	–44	4·6

A comparison of the minimum energy conformers of the disaccharide fragments NeuNAcα(2-3)Gal, NeuNAcα(2-6)Gal and NeuNAcα(2-8)NeuNAc (tables 1, 3 and 5 and figure 4) together with the geometry of the second residues show that the following set of dihedral angles

ϕ, ψ, O2-C3-C4-C5 and C3-C4-C5-C6 (approximately –70°, –10°, 180° and 180°) in NeuNAcα(2-3)Gal,

ϕ, ψ, O2-C6-C5-C4 (χ + 120°) and C6-C5-C4-C3 (approximately –70°, –10°, –170° ($\chi = 70°$) and 180°) in NeuNAcα(2-6)Gal

and

ϕ, ψ, O2-C8-C7-C6 (χ^2 +120°) and C8-C7-C6-C5(χ^1)

(approximately –70°, –10°, –170° ($\chi^2 = 70°$) and 150°) in NeuNAcα(2-8) NeuNAc favour approximately similar values. In other words, when the first residue (NuNAc) of these disaccharide fragments are superimposed then the fragment

O2-C3-C4-C5-C6 of Gal of NeuNAcα(2-3)Gal,
O2-C6-C5-C4-C3 of Gal of NeuNAcα(2-6)Gal and
O2-C8-C7-C6-C5 of second NeuNAc of NeuNAcα(2-8)NeuNAc

can take up similar orientations. It may be due to this conformational similarity, that the neuraminidase enzymes are able to cleave NeuNAc rsidue from the sialyloligosaccharide differing in the types of linkages. It is also interesting to note that the oxygen atoms O4, O5 of the galactose residue of NeuNAcα(2-3)Gal, O5, O4 of the galactose residue of NeuNAcα(2-6)Gal and O7, O6 of the second NeuNAc residue of NeuNAcα(2-8)NeuNAc also assume similar orientations. There is thus, a possibility that these atoms may be involved in hydrogen bond formation with the active site of the neuraminidase enzymes.

As mentioned earlier, in the disaccharide fragments NeuNacα(2-6)Gal and NeuNAcα(2-8)NeuNAc the C3 atom of Gal and the C5 atom of the second NeuNAc residue occupy similar positions. However, the electronegative atoms (O3 of Gal and N5 of NeuNAc) which are attached to them differ in their orientation and are fixed becuase of the ring geometry. In the disaccharide fragment NeuNAcα(2-3)Gal, the C6 atom of galactose also occupies the same position as that of the two carbon atoms mentioned above. The electronegative atom (O6) which is attached to it can occupy all the three staggered orientation because of the possible freedom of rotation around an exocyclic bond. It is, therefore, possible for this electronegative atom (O6) to occupy, in one orientation a position similar to that of O3 in NeuNAcα(2-6)Gal and in another orientation a position similar to the N5 inNeuNAcα-(2-8)NeuNAc.Thus, if the O3 of NeuNAcα(2-6)Gal is involved in an interaction (hydrogen bond formation) with a neuraminidase enzyme then the O6 of NeuNAcα(2-3)Gal can give the same type of interaction but the N5 atom of NeuNAcα(2-8)NeuNAc can not. If on the other hand the N5 atom of NeuNAcα(2-8)-NeuNAc interacts with a neuraminidase enzyme (hydrogen bond formation) then the O6 of NeuNAcα(2-3)Gal can give the same type of interaction whereas the O3 of NeuNAcα(2-6)Gal can not.

It is also interesting to note that in NeuNAcα(2-3)Gal the O2 hydroxyl of the galactose residue (fixed because of the ring geometry) and the O9 hydroxyl (three staggered orientations are possible because of the rotation around exocyclic bond) of the second NeuNAc residue in NeuNAcα(2-8)NeuNAc can also occupy similar position. No corresponding electronegative atom is present in that position in NeuNAcα(2-6)Gal. A bulky group (acetamido group) is present on the second NeuNAc residue inNeuNAcα(2-8)NeuNAc. While no bulky group is present in that position in the other two disaccharide fragments (figure 4), when the third sugar residue (R) is attached to these three disaccharide fragments there will be some differences in the overall shape of these molecules because of the differences in the orientation of the anomeric oxygens. The third sugar residue is positioned approximately in the same direction in NeuNAcα(2-3)Gal and NeuNAcα(2-8)NeuNAc and will be different in NeuNAcα(2-6)Gal(figure 4). This would mean that the orientation of the disaccharide fragment of the latter with respect to the rest of the sugar residues in the carbohydrate chain will be different from that of the former two dissacharides. These results suggest that if the neuraminidase enzymes accommodate only two sugar residues (terminal disaccharide fragments) in its binding site, because of the conformational similarity already mentioned all the three substrates should show comparable activity. On the other hand, if the binding site accommodates more than two sugar residues, then the enzymes should show comparable binding activity with NeuNAcα(2-3)Gal and NeuNAcα(2-8)NeuNAc but not with NeuNAcα(2-6)Gal because of differences in the orientation of the terminal disaccharide fragments with respect to the rest of the carbohydrate chain.

Thus neuraminidase enzymes of bacterial origin (*Clostridium perfringens, Vibrio cholerae, Arthrobacter ureafaciens*) which show comparable binding activity with all the three substrates may be able to accommodate not more than two sugar residues in their binding sites. The slight differences in their activity can be accounted for, by the difference in the orientation of some of the side group as mentioned earlier. Neuraminidase enzymes from viruses (Newcastle disease virus, Fowl plague virus,

B — 4

Influenza A$_2$ virus) which show very good activity with NeuNAcα(2-3)Gal and negligible activity with NeuNAcα(2-6)Gal containing sialyloligosaccharides, may have large binding site which accommodate more than two sugar residues. As has been mentioned earlier, if the binding site is large, these enzymes should show comparable activity withNeuNAcα(2-8)NeuNAccontaining sialyloligosaccharides also. Newcastle disease virus neuraminidase shows good activity with NeuNAcα(2-8)NeuNAc containing sialyloligosaccharides (Schauer, 1982) in agreement with theoretical predictions. Fowl plague virus neuraminidase and influenza A$_2$ virus neuraminidase show poor activity with NeuNAcα(2-8)NeuNAc containing sialyloligosaccharides. This may be due to the presence of the bulky acetamido group at C5 position of the second NeuNAc residue (figure 4) which may be causing steric problems with the combining site of these neuraminidase enzymes.

As mentioned earlier, the substitution of an acetamido group at C2 position of the galactose residue of NeuNAcα(2-3)Gal and NeuNAcα(2-6) Gal does not affect the favoured minimum energy conformations of these disaccharides (tables 1 to 4 and figures 4 and 5). These disaccharides (NeuNAcα(2-3)GalNac,NeuNAcα(2-6)GalNAc) are also cleaved by the neuraminidase enzymes indicating that the acetamido group at the C2 position of the galactose residue does not hinder the binding of the neuraminidase enzymes.

Acknowledgement

References

Arnott, S, and Scott, W. E. (1972) *J. Chem. Soc. Perkin Trans.,* **2,** 324.

Corfield, A. P., Veh, R. W., Wember, M., Michalski, J. C. and Schauer, R. (1981) *Biochem. J.,* **197,** 293.

Corfield, A. P., Higa, H., Paulson, J. C. and Schauer, R. (1983) *Biochim. Biophys. Acta,* **744,** 121.

Corey, R. B. and Pauling, L. (1953) *Proc. Roy. Soc. London* **B141,** 10.

Davidon, W. C. (1959) *AEC Research and Development Report,* ANL 5990.

Del Re, G., Pullman, B. and Yonezawa, T. (1963) *Biochim. Biophys. Acta,* **75,** 153.

Fletcher. R. and Powell, M. J. D. (1963) *Comput. J.,* **6,** 163.

Jeanloz, W. R. and Codington, J. F. (1976) in *Biological Roles of Sialic Acid,* (eds A. Rosenberg and C. L. Schengrund) p. 201.

Momany, F. A., McGuire, R. F., Burgess, A. W. and Scheraga, H. A. (1975) *J. Phys. Chem.,* **79,** 2361.

Paulson, J. C, Weinstein, J., Dorland, L., Halbeek, H. V. and Veiegenthart, J. F. G. (1982) *J. Biol. Chem.,* **257,** 12734.

Ramachandran, G. N. and Sasisekharan, V. (1968) *Adv. Prot. Chem.,* **23,** 283.

Sathyanarayana, B. K. and Rao, V. S. R. (1971) *Biopolymers,* **10,** 1605.

Schauer, R. (1982) *Adv. Carbohydr. Chem. Biochem.,* **40,** 131 (and the references cited therein).

Uchida, Y., Tsukada, Y. and Sugimori, T. (1979) *J. Biochem.,* **86,** 1573.

Veluraja, K. and Rao, V. S. R. (1980) *Biochim. Biophys. Acta,* **630,** 442.

Veluraja, K. and Rao, V. S. R. (1983) *Carbohydr. Polym.,* **3,** 175.

Veluraja, K. and Rao, V. S. R. (1984) *Carbohydr. Polym.,* **4,** 357.

Vijayalakshmi, K. S. (1972) *Theoretical studies on the conformations of aldopyranoses and their derivatives in solution,* Ph.D. Thesis, University of Madras, India.