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Theoretical Studies on the Conformation of Monosialogangliosides and Disialogangliosides

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ABSTRACT

The preferred conformation of gangliosides GM3, GM2, GM1, GD1a and GD1b have been studied by computing their potential energies. The conformation of NeuNAc in GM3 differs from that expected for the same residue in GM2 and GM1. The NeuNAc residues in GM2 and GM1 exhibit identical conformations. Theory predicts that the terminal NeuNAc of GD1a is conformationally similar to that of GM3 and that the internal one is similar in conformation to those present in GM2 and GM1 in agreement with NMR studies. The differences in chemical shifts of the C2 and C3 carbons of the internal and terminal NeuNAc of GD1a have been attributed to differences in orientation. The present studies suggest that the binding site of cholera toxin is much smaller than that of tetanus toxin. The preferred shape of these gangliosides correlate well with their biological properties.

INTRODUCTION

Gangliosides are glycosphingolipids which are characterised by the presence of sialic acid (NeuNAc) and are classified as mono, di, tri, tetra and penta-sialogangliosides according to the number of the sialic acid residues present. They occur mainly in the plasma membrane of the central nervous system. The oligosaccharide portion of the gangliosides projects outside the surface of the plasma membrane and acts as a receptor site for cholera toxin (Cuatrecasas, 1973*a*, 1973*b*; King &

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van Heyningen, 1973; Svennerholm, 1976), tetanus toxin (Holmgren *et al.*, 1980) etc. Cholera toxin binds preferentially to GM1. It also binds to other gangliosides to a lesser extent. Though tetanus toxin specifically binds with the GD1b series of gangliosides, other gangliosides also have comparable binding activity.

The cleavage of the NeuNAc residues in gangliosides seems to depend upon their locations. *Vibrio cholerae* neuraminidase and *Clostridium perfringens* neuraminidase cleave all the NeuNAc except the internal NeuNAc in gangliosides. The resistant internal NeuNAc becomes accessible for cleavage by *Clostridium perfringens* neuraminidase only in the presence of bile salt (Schauer *et al.*, 1980). In general the activity of the gangliosides seems to depend upon the number and location of the NeuNAc. Hence an attempt has been made to study the possible conformations of the oligosaccharide portions of the gangliosides GM3, GM2, GM1, GD1a and GD1b with a view to correlating shape with biological function.

METHOD OF CALCULATION

The oligosaccharide portions of the gangliosides that have been studied in the present work are shown in Figs 1 and 2. Steric maps were constructed for all the disaccharide fragments of the gangliosides using contact criteria (Ramachandran & Sasisekharan, 1968). Various conformations were generated assuming sugar residues in the chair form and allowing rotations about the interunit glycosidic bonds C1–O (ϕ-rotation) and O–CX (ψ-rotation) (for NeuNAc linked disaccharide rotation around C2–O is taken as ϕ-rotation) from $-180°$ to $+180°$ at intervals of 10°. All the sugar residues were assumed to be in $^4C_1$(D) chair form except NeuNAc which was assumed to be in $^1C_4$(D) form. The atomic coordinates of these residues were based on the standard geometry of Arnott & Scott (1972). The acetamido group was fixed using Pauling–Corey geometry (Corey & Pauling, 1953) so that C2–H2 and N–H bonds were *trans*. The geometry used for the NeuNAc was the same, as described elsewhere (Veluraja & Rao, 1980). The carboxylic acid group was fixed in the axial orientation using standard geometry (Radom, 1977). The bond angle at the glycosidic oxygen atom was fixed at the average value of 117·5°. Except for the NeuNAc linked disaccharide the initial conformation corresponding to $(\phi, \psi) = (0°, 0°)$ is the same as

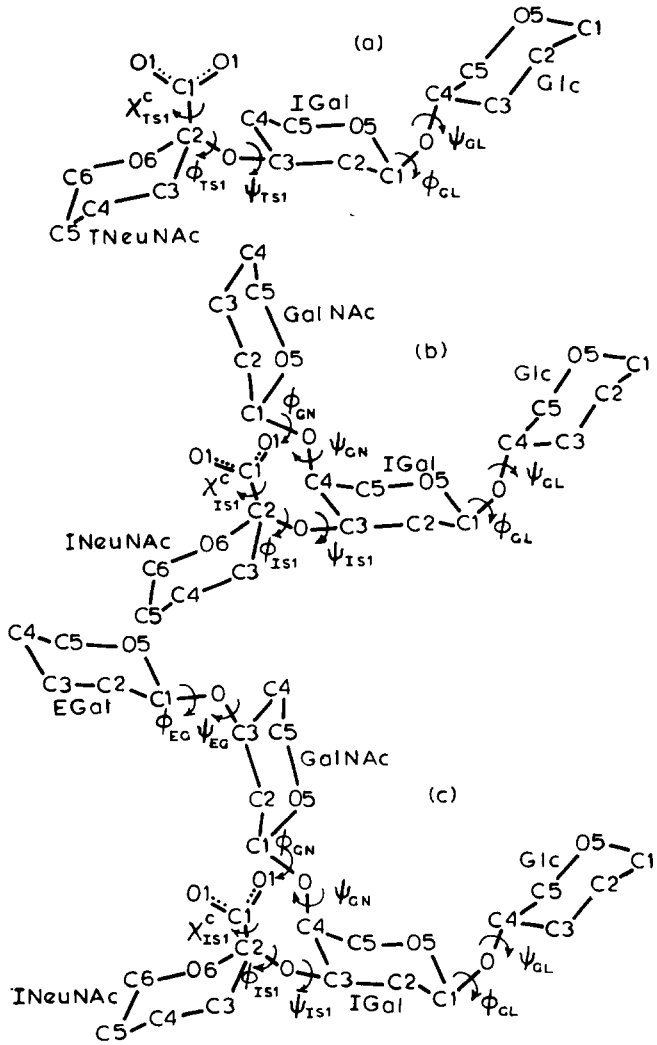

Fig. 1. Numbering of atoms and dihedral angles of the oligosaccharide portion of monosialogangliosides. (a) GM3; (b) GM2; (c) GM1. The side groups are not shown. Abbreviations: Glc, Glucose; IGal, Internal galactose; TNeuNAc, Terminal N-acetylneuraminic acid; INeuNAc, Internal N-acetylneuraminic acid; GalNAc, N-acetylgalactosamine; EGal, External galactose.

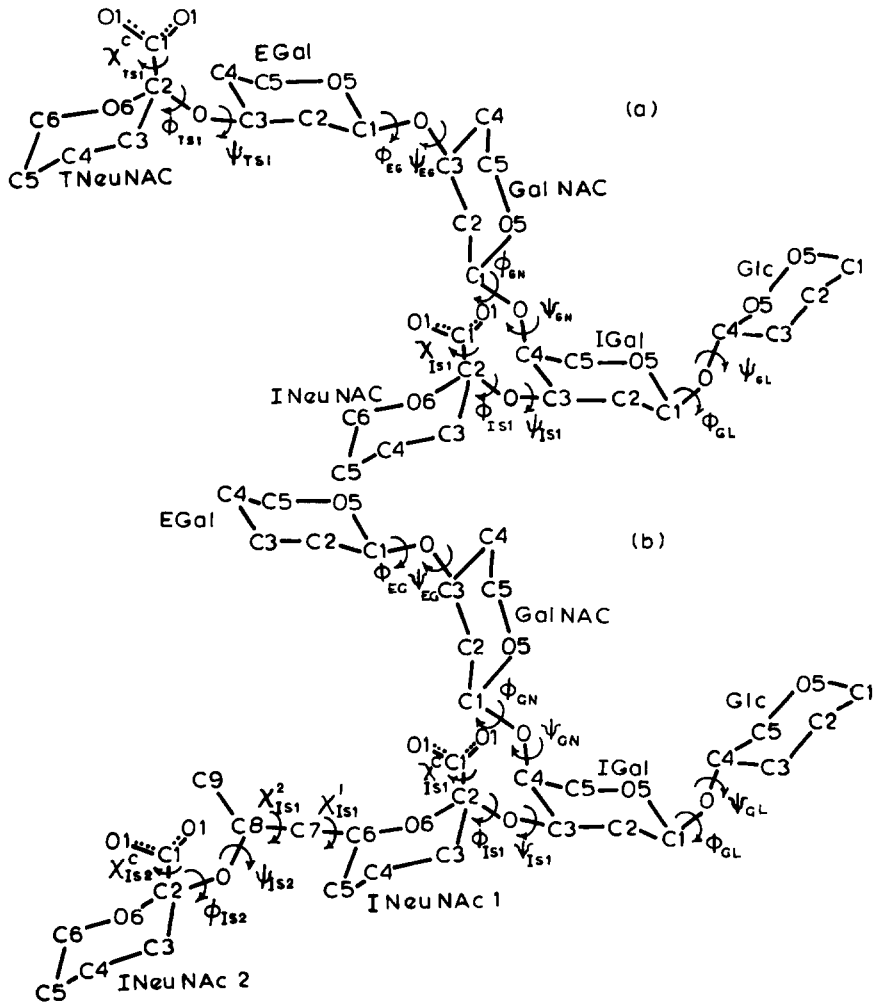

Fig. 2. Numbering of atoms and dihedral angles of the oligosaccharide portion of disialogangliosides. (a) GD1a; (b) GD1b. The side groups are not shown. Abbreviations are as in Fig. 1.

that described earlier (Yathindra & Rao, 1970). For NeuNAc-linked disaccharides (Figs 1 and 2)

$$\phi = 0 \text{ when C1–C2 } \textit{cis} \text{ to O–CX}$$

$$\psi = 0 \text{ when C2–O } \textit{cis} \text{ to CX–HX}$$

$\chi^c = 0$ when C1-O1 *cis* to C2-O6

$\chi^1 = 0$ when C5-C6 *cis* to C7-C8

$\chi^2 = 0$ when C6-C7 *cis* to C8-C9

(X – represents the number of the atoms at which the NeuNAc is linked.) Clockwise rotations were taken as positive.

Steric maps for the disaccharide fragment of NeuNAc α(2-8) NeuNAc were obtained by fixing χ^1 and χ^2 in all the staggered positions. The one corresponding to χ^1 and χ^2 in the minimum energy position ($\chi^1 = 150°$; $\chi^2 = 70°$) alone is shown in Fig. 3.

The potential energy of the molecules was computed considering nonbonded, electrostatic, and torsional contributions. The form of the

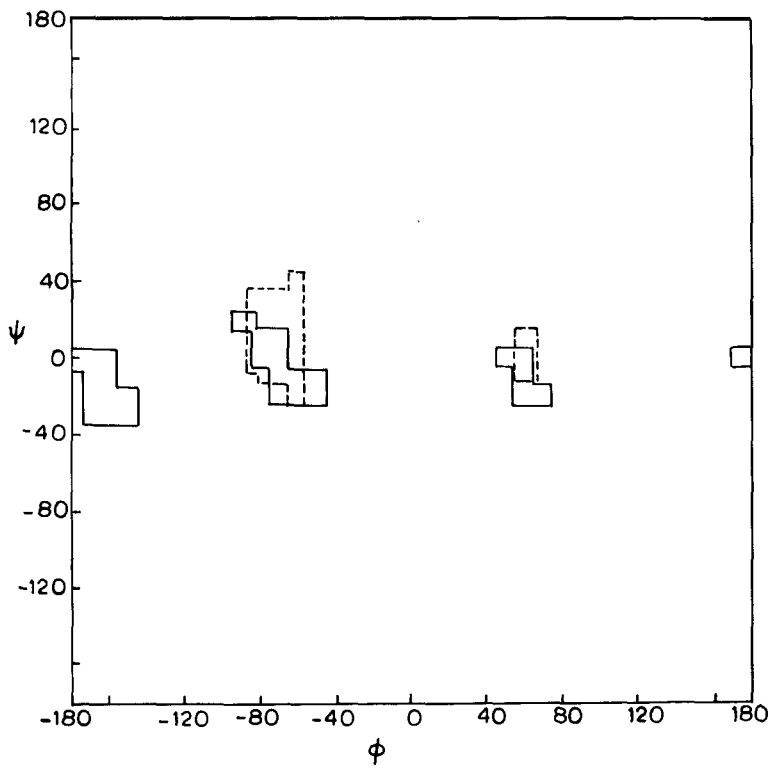

Fig. 3. Steric map for the disaccharide fragments (——) NeuNAc α(2-3) Gal; (– – –) NeuNAc α(2-8) NeuNAc.

function and constants used are those reported by Momany *et al.* (1975). The σ charges on various atoms were calculated using MO–LCAO method of Del Re (Del Re *et al.*, 1963). π-charges for acetamido groups and carboxylic acid groups were taken from Vijayalakshmi (1972). Since the carboxylic acid group of NeuNAc will be in the ionised state (normal form of occurrence in the living system) π-charges were 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 $-0{\cdot}5$ units was added as suggested by Del Re *et al.* (1963). The net charges were used for computing electrostatic energy. However these calculations do not take into account the free energy of solvation of different conformations and the entropy associated with their individual flexibility. Conformational analyses of carbohydrates have shown that in general these molecules are highly rigid. Solvent (water) accessibility studies have also indicated that it would not affect significantly the preferred conformations (Kaliannan & Rao, unpublished results). Hence the neglect of the above two factors in the present calculations may not affect the results significantly.

Gangliosides can assume a large number of conformations, due to the possible rotations about the inter-unit bonds. Hence a systematic analysis by varying the dihedral angles at discrete intervals is time consuming. To reduce computer time, energy was minimised as a function of rotational angles following the Davidon (1959) and Fletcher & Powell (1963) minimisation procedure.

RESULTS AND DISCUSSION

The steric maps of the various disaccharide fragments, shown in Figs 3 and 4 indicate that less than 4% of the total region is allowed in the ϕ–ψ plane in agreement with earlier results (Sathyanarayana & Rao, 1971). The NeuNAc α(2-3) Gal segment has three distinct allowed regions (Fig. 3) unlike the other disaccharide fragments.

The various conformations that are possible for the oligosaccharide portion of GM3 are shown in Table 1. It can be seen from Table 1 that (ϕ_{TS1}, ψ_{TS1}) favour values around ($-70°$, $-10°$). When (ϕ_{TS1}, ψ_{TS1}) assume values of about ($-155°$, $-20°$) the energy increases by about 4 kcal mol^{-1} and thus rules out the possibility of the occurrence of

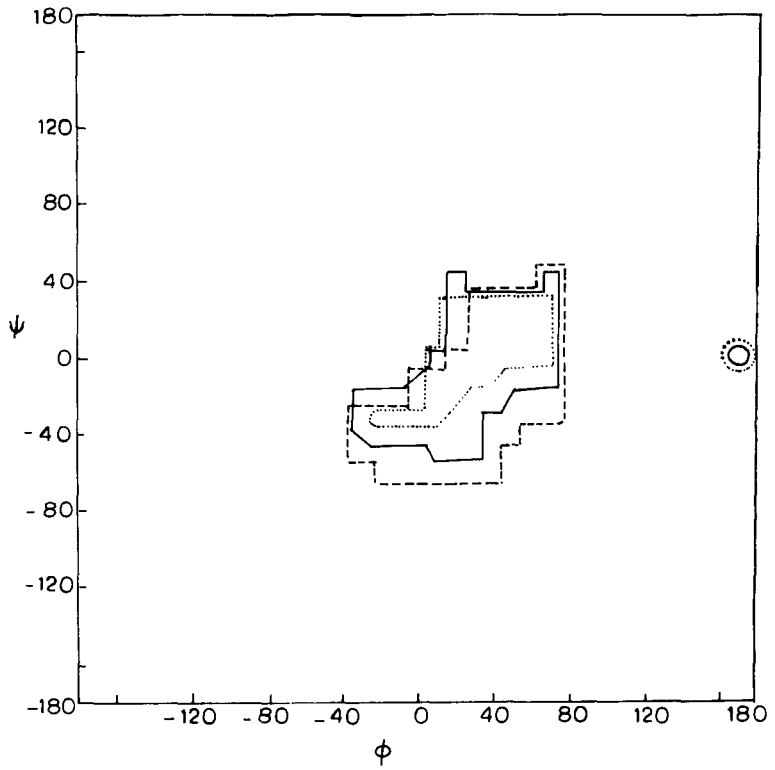

Fig. 4. Steric map for the disaccharide fragments (——) Gal β(1-4) Glc; (– – –) Gal β(1-3) GalNAc; (.....) GalNAc β(1-4) Gal.

TABLE 1
Minimum Energy Conformations of GM3

No.	ϕ_{GL}	ψ_{GL}	ϕ_{TS1}	ψ_{TS1}	χ^{c}_{TS1}	*Relative energy kcal mol*$^{-1}$
1	53	4	−67	−7	−35	0·0
2	16	−41	−68	−6	−35	0·9
3	−18	−29	−67	−6	−35	1·0
4	54	3	−155	−20	−38	4·0
5	161	8	−67	−8	−35	4·3
6	−17	−30	−155	−20	−35	4·8
7	16	−41	−155	−20	−37	4·8

NeuNAc in this orientation. In the minimum energy conformation (ϕ_{GL}, ψ_{GL}) favour values around (55°, 5°). Two more conformations around (15°, −40°) and (−20°, −30°) are also possible but the energy increases by about 1 kcal mol^{-1}. On the other hand when these angles assume values around (160°, 10°) the energy increases by about 4 kcal mol^{-1}. These results indicate that the oligosaccharide portion of GM3 is highly rigid and can assume only a very few conformations. A projection of GM3 in the minimum energy conformation is shown in Fig. 5(a).

Tables 2 and 3 indicate the probable conformers of GM2 and GM1. Generally (ϕ_{IS1}, ψ_{IS1}) prefer values around (−160°, −25°) both in GM2 and GM1. When (ϕ_{IS1}, ψ_{IS1}) assume values around (−70°, −10°) the energy increases by about 1·8 and 3·3 kcal mol^{-1} in GM2 and GM1 respectively. This indicates that the NeuNAc in GM2 and GM1 favours a similar conformation which differs from that preferred in GM3 (Fig. 5(a), (b)). It is interesting to note that (ϕ_{GN}, ψ_{GN}) in GM2 and GM1 favour values around (30°, 20°) indicating that the N-acetylgalactosamine residue favours a similar conformation in both the gangliosides.

In GM1 (ϕ_{EG}, ψ_{EG}) prefer values around (50°, 10°) (Table 3). When they assume values around (−15°, −30°), and (160°, 5°) the energy increases by about 3·1 and 5·4 kcal mol^{-1} respectively. It can also be seen from Tables 2 and 3 that the addition of galactose to the C3 position of N-acetylgalactosamine does not affect the minimum energy conformation of the rest of the fragment. The addition of N-acetylgalactosamine to the C4 of the internal galactose significantly alters the preferred conformation of the NeuNAc residue (Fig. 5).

TABLE 2
Minimum Energy Conformations of GM2

No.	ϕ_{GN}	ψ_{GN}	ϕ^{*}_{GL}	ψ^{*}_{GL}	ϕ_{IS1}	ψ_{IS1}	χ^{c}_{IS1}	*Relative energy kcal mol^{-1}*
1	29	14	60	0	−157	−25	−43	0·0
2	30	22	60	0	−68	−11	−33	1·8

* ϕ_{GL}, ψ_{GL} were fixed in the minimum energy values as shown.

TABLE 3
Minimum Energy Conformations of GM1

No.	ϕ_{EG}	ψ_{EG}	ϕ_{GN}	ψ_{GN}	ϕ^{*}_{GL}	ψ^{*}_{GL}	ϕ_{IS1}	ψ_{IS1}	χ^{c}_{IS1}	*Relative energy kcal mol*$^{-1}$
1	50	11	26	14	60	0	−158	−24	−44	0·0
2	−14	−29	27	17	60	0	−158	−23	−41	3·1
3	57	−10	27	21	60	0	−68	−9	−34	3·3
4	−20	−28	27	21	60	0	−68	−10	−33	4·5
5	162	3	27	12	60	0	−158	−24	−44	5·4

* ϕ_{GL}, ψ_{GL} were fixed in the minimum energy values as shown.

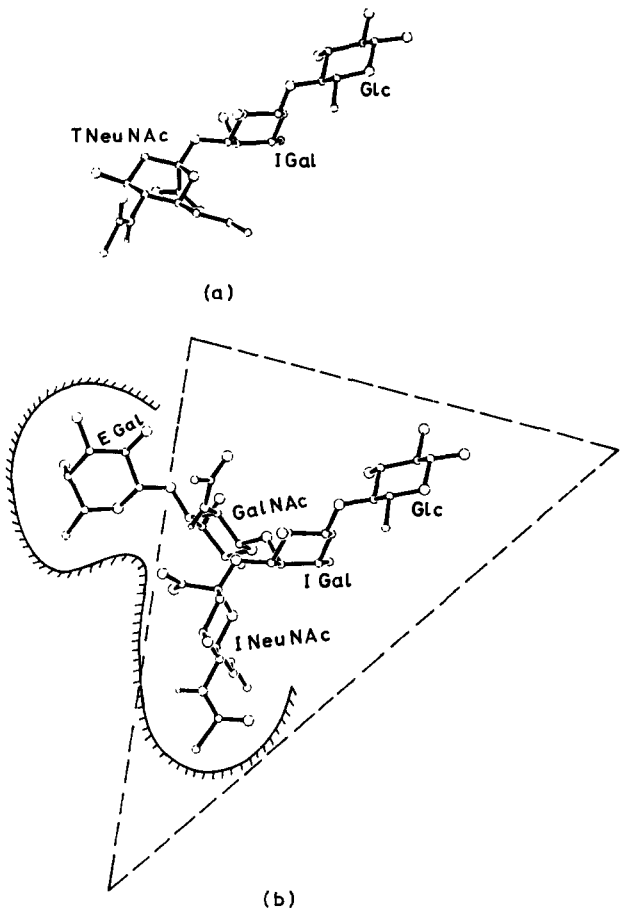

Fig. 5. Projections of the global minimum energy conformation of monosialogangliosides. (a) GM3; (b) GM1, inside the triangle GM2. The approximate binding site for cholera toxin is indicated. Hydroxyl oxygen at the glycerol side chain is not shown. Abbreviations are as in Fig. 1.

Table 4 shows that in the minimum energy conformation of GD1a the internal and terminal NeuNAc favour different conformations. The preferred values for (ϕ_{IS1}, ψ_{IS1}) of about ($-155°$, $-25°$) indicate that the conformation of the internal NeuNAc is conformationally similar to the same residue in GM2 and GM1. In the conformers whose energy is less than 4 kcal mol^{-1} (ϕ_{TS1}, ψ_{TS1}) favour values around ($-70°$, $0°$) indicating that the terminal NeuNAc is highly restricted as in GM3. The

TABLE 4
Minimum Energy Conformations of GD1a

No.	χ^c_{TS1}	ϕ_{TS1}	ψ_{TS1}	ϕ_{EG}	ψ_{EG}	ϕ_{GN}	ψ_{GN}	ϕ^*_{GL}	ψ^*_{GL}	ϕ_{IS1}	ψ_{IS1}	χ^c_{IS1}	*Relative energy kcal mol*$^{-1}$
1	−38	−74	0	32	−4	28	6	60	0	−156	−24	−42	0·0
2	−31	−71	−5	36	2	28	20	60	0	−68	−10	−33	2·4
3	−36	−75	11	−14	−27	34	13	60	0	−71	−11	−33	2·7
4	−36	−73	6	25	−35	28	21	60	0	−67	−9	−33	2·9
5	−38	−154	−21	60	−12	28	6	60	0	−156	−25	−43	4·1
6	−36	−71	1	165	0	30	0	60	0	−155	−24	−42	4·6

* ϕ_{GL}, ψ_{GL} were fixed in the minimum energy values as shown.

internal NeuNAc in GD1 can also assume a conformation similar to that of terminal NeuNAc (ϕ_{IS1}, $\psi_{IS1} = -70°, -10°$; conformer 2 of Table 4) but the energy increases by about 2·4 kcal mol^{-1}. On the other hand if both the internal and terminal NeuNAc take up a conformation around ($-155°, -20°$) (conformer 5 of Table 4) the conformational energy increases by about 4·1 kcal mol^{-1} indicating that the probability of both the terminal and internal NeuNAc occurring in the same conformation is negligibly small. In the minimum energy conformation of GD1a (ϕ_{EG}, ψ_{EG}) prefer values of about (30°, 0°) indicating that the conformation of the external galactose is affected slightly due to the addition of terminal NeuNAc. When (ϕ_{EG}, ψ_{EG}) assume values around ($-15°, -30°$) and ($25°, -35°$) the energy increases by about 2·5 kcal mol^{-1}. In this ganglioside also the favoured conformation for the N-acetylgalactosamine is the same as that in monosialogangliosides. A projection corresponding to the minimum energy conformation of GD1a is shown in Fig. 6(a).

Table 5 shows the minimum energy conformers of GD1b. It can be seen from Table 5 that the fragment constituting external galactose up to internal NeuNAc1 has conformational similarity to that of GM1. In GD1b the disialic acid fragment favours very few conformations. In the minimum energy conformations the dihedral angles ϕ_{IS2}, ψ_{IS2}, χ^2_{IS1}, χ^1_{IS1} (Fig. 2(b)) which define the disialic acid conformation prefer values around $-70°, -5°, 70°, 150°$) respectively. The next nearest conformation has about 3·2 kcal mol^{-1} higher energy. The internal NeuNAc1 always prefers a conformation similar to that of the internal NeuNAc of GM2, GM1 and GD1a (ϕ_{IS1}, $\psi_{IS1} = -160°, -20°$). When it assumes the other possible conformation (ϕ_{IS1}, $\psi_{IS1} = -70°, -10°$) the energy increases by about 5·5 kcal mol^{-1} which rules out the possibility of the occurrence of internal NeuNAc1 in this conformation. A projection of the minimum energy conformation of GD1b is shown in Fig. 6(b).

The conformational energy calculations thus suggest that there is a difference in the favoured conformations of internal and terminal NeuNAc in GD1a. The terminal NeuNAc has conformational similarity to that in GM3 whereas the internal NeuNAc is conformationally similar to that of GM2 and GM1. From similarities in the observed chemical shifts of the C2 and C3 atoms of NeuNAc in ^{13}C NMR spectra of GD1a, GM3 and GM1, Harris & Thornton (1978) suggested that the terminal and internal NeuNAc of GD1a are conformationally similar to those of

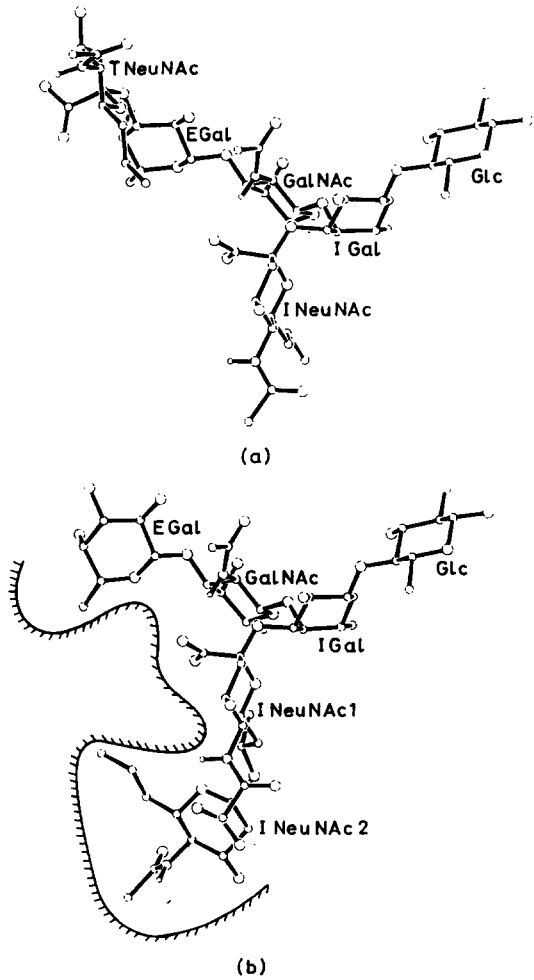

Fig. 6. Projections of the global minimum energy conformation of disialogangliosides. (a) GD1a; (b) GD1b. The approximate binding site for tetanus toxin is indicated. Hydroxyl oxygen at the glycerol side chain is not shown. Abbreviations are as in Fig. 1.

GM3 and GM1 respectively. Our theoretical results also lead to this conclusion. The observed downfield chemical shift of C2 and upfield chemical shift of C3 of internal NeuNAc relative to terminal NeuNAc has been explained by these authors by invoking the anisotropy effect of the carbonyl group of N-acetylgalactosamine. The minimum energy

TABLE 5
Minimum Energy Conformations of GD1b

No.	ϕ_{EG}	ψ_{EG}	ϕ_{GN}	ψ_{GN}	ϕ^{*}_{GL}	ψ^{*}_{GL}	ϕ_{IS2}	ψ_{IS2}	χ^{2}_{IS1}	χ^{1}_{IS1}	ϕ_{IS1}	ψ_{IS1}	χ^{c}_{IS2}	χ^{c*}_{IS1}	*Relative energy kcal mol*$^{-1}$
1	50	10	25	14	60	0	−69	−6	71	150	−157	−21	−37	−40	0·0
2	−12	−29	27	18	60	0	−69	−7	71	150	−157	−21	−38	−40	3·2
3	48	10	26	12	60	0	−127	41	161	115	−156	−26	−42	−40	3·4
4	54	7	24	15	60	0	−151	12	122	168	−155	−24	−40	−40	3·6
5	52	8	23	22	60	0	−69	−5	70	148	−71	−9	−37	−40	5·4

* ϕ_{GL}, ψ_{GL}, χ^{c}_{IS1} were fixed in the minimum energy values as shown.

conformations from the present studies differ significantly from the values proposed by Harris & Thornton (1978). The present energy calculations clearly suggest that in the minimum energy conformation, the carbonyl group of N-acetylgalactosamine is not in close proximity to the C2 and C3 atoms of the internal NeuNAc (Fig. 6(a)). Hence the observed differences in the chemical shifts of C2 and C3 may be due to differences in the preferred conformations of terminal and internal NeuNAc rather than the anisotropy effect of the carbonyl group of N-acetylgalactosamine.

CORRELATION OF CONFORMATION WITH BINDING ACTIVITY

Cholera toxin

Cholera toxin specifically binds with ganglioside GM1 through its B protomer. Other gangliosides – GM2, GM3, GD1a and GD1b – bind very weakly. Both GM2 and GM1 favour similar conformations (Fig. 5(b)). But GM1 contains an extra galactose residue attached to C3 of N-acetylgalactosamine. Hence the high activity of GM1 compared to GM2 suggest the active involvement of the external galactose of GM1 in binding. The addition of NeuNAc to the external galactose of GM1 as in GD1a, slightly affects the orientation of the external galactose but the activity decreases drastically. Such a loss in activity cannot be attributed to the slight change in conformation. It thus seems that the much reduced activity of GD1a may be due to unfavourable interactions of the terminal NeuNAc with cholera toxin. Addition of another NeuNAc at C8 of the NeuNAc of GM1 (to give GD1b) does not affect the conformation of the rest of the fragment (conformation similar to GM1) but its binding with cholera toxin is very much reduced. These results suggest that the binding site of cholera toxin is very small and can accommodate only two residues, galactose and NeuNAc as shown in Fig. 5(b). Hence removal of any one of the residues makes one of the important binding pockets unoccupied and hence leads to very weak binding. This perhaps explains the weak activity of GM2 and GM3.

Tetanus toxin

Holmgren *et al.* (1980) studied the binding activity of various gangliosides with tetanus toxin and established the order of binding activity of

GD1b > GM1 > GD1a > GM2 > GM3. Both GD1b and GM1 prefer identical conformations (Figs 5(b) and 6(b)) with the only difference, the absence of NeuNAc2 in the latter. This clearly indicates the active involvement of NeuNAc2 of GD1b in binding since GM1 has less binding activity than GD1b. Addition of terminal NeuNAc at the C3 position of the terminal galactose of GM1 (structure of GD1a) affects the preferred conformation of the external galactose residue. Comparison of Tables 3 and 4 suggests that in conformer 5 of GD1a (Table 4) the common fragment Gal–GalNac–Gal–NeuNAc has nearly the same conformation as that of GM1. But this conformer of GD1a is about 4·1 kcal mol^{-1} higher in energy than the global minimum conformation. Hence tetanus toxin has to spend part of its binding energy (about 4 kcal mol^{-1}) to push the external galactose of GD1a to a similar conformation as in GM1. This perhaps explains its low activity compared to GM1. It is interesting to note from Figs 5(b) and 6(b) and Tables 2, 3 and 5 that the common fragments in GM2, GM1 and GD1b assume identical conformations. The activity seems to increase with the increase in the size of the oligosaccharide fragment. These results suggest that tetanus toxin may have a larger binding site and can accommodate at least five to six sugar residues. The approximate binding site is indicated in Fig. 6(b).

CORRELATION OF CONFORMATION TO NEURAMINIDASE ACTIVITY

The neuraminidases of *Vibrio cholerae* and *Clostridium perfringens* cleave the NeuNAc from all the gangliosides except the 'internal NeuNAc' (Schauer *et al.*, 1980). The terminal NeuNAc in GM3 and GD1a prefers a conformation with (ϕ_{TS1}, ψ_{TS1}) close to (−70°, 0°) indicating that this may be the conformer that the enzyme recognises for cleaving the terminal NeuNAc which is linked to the third position of the galactose. The projection corresponding to the above (ϕ, ψ) value of the NeuNAc α(2–3) Gal fragment is shown in Fig. 7(a). In GD1b the disialic acid fragment (which is also cleaved by neuraminidase) prefers a conformation for (ϕ_{IS2}, ψ_{IS2}, χ^2_{IS1}, χ^1_{IS1}) close to (−70°, −5°, 70°, 150°) and the projection corresponding to this conformer of the NeuNAc α(2–8) NeuNAc fragment is shown in Fig. 7(b). Since in both the fragments the (ϕ, ψ) value is the same, this brings about the exact

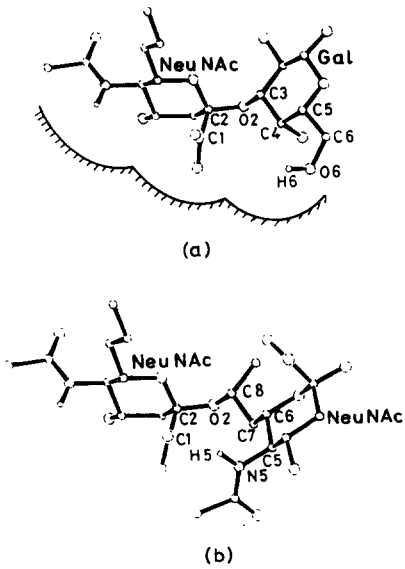

Fig. 7. Projections of the global minimum energy conformation of: (a) NeuNAc α(2-3) Gal (terminal fragment of GD1a and GM3); (b) NeuNAc α(2-8) NeuNAc (fragment from GD1b). The approximate binding site for neuraminidase action is indicated. Hydroxyl oxygen at the glycerol side chain is not shown.

coincidence of C8–C7 of NeuNAc α(2-8) NeuNAc with that of C3–C4 of NeuNAc α(2-3) Gal (Fig. 7). In NeuNAc α(2-3) Gal the two dihedral angles O2–C3$\not$C4–C5 and C3–C4–C5$\not$C6 prefer values close to 180° and are fixed because of the ring geometry of the galactose. In the NeuNAc α(2-8) NeuNAc fragment of GD1b the dihedral angles O2–C8$\not$C7–C6 and C8–C7$\not$C6–C5 prefer values about $-170°$ and 150° in the minimum energy. This leads to the approximate coincidence of C6–C5 atoms of NeuNAc α(2-8) NeuNAc with that of C5–C6 of NeuNAc α(2-3) Gal and hence leads to the approximate coincidence of N5–H5 with that of O6–H6 (Fig. 7). This conformational similarity of NeuNAc α(2-3) Gal and NeuNAc α(2-8) NeuNAc fragments may be important for the action of neuraminidase enzymes and the probable binding site is shown in Fig. 7(a). The different conformational preference of the internal NeuNAc (ϕ_{IS1}, $\psi_{IS1} = -160°$, $-20°$) in GM2, GM1, GD1a and GD1b may perhaps explain the inactivity of neuraminidase enzymes in cleaving the internal NeuNAc in gangliosides.

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