Carbohydrate Polymers **4** (1984) 357–375

Theoretical Studies on the Conformations of Higher Gangliosides

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(Received: 17 November 1983)

SUMMARY

The possible conformations of higher gangliosides (GD3, GT1a. GT1b, GQ1b) have been determined by computing their potential energy using semi-empirical potential functions. The favoured conformation of the disialic acid fragment in these gangliosides is independent of its position (internal or terminal). The favoured conformations of these gangliosides have also been correlated to their biological activity. The results suggest that tetanus toxin and sendai virus may have a large binding site which can accommodate at least four sugar residues.

INTRODUCTION

Gangliosides are mainly found on the surface of the plasma membrane nerve endings and are characterized by the presence of negatively charged sialic acid (*N*-acetylneuraminic acid, NeuNAc). The various gangliosides that have been predominantly found in the plasma membranes are GM3, GM2, GM1, GA1, GD3, GD1a, GD1b, GT1a, GT1b, GQ1b. The oligosaccharide portion of these gangliosides is located on the outer surface of the plasma membrane due to its hydrophilicity, and acts as a receptor site for cholera toxin (Cuatrecasas, 1973*a*, 1973*b*; King & van Heyningen, 1973; Svennerholm, 1976), tetanus toxin (Ledley *et al.*, 1977; Morris *et al.*, 1980; Rogers & Synder, 1981; Price *et al.*, 1977; Holmgren *et al.*, 1980*a*) and sendai virus (Holmgren *et al.*, 1980*b*; Markwell *et al.*, 1981). Holmgren *et al.* (1980*a*) studied the binding of

Carbohydrate Polymers 0144-8617/84/$03.00 – © Elsevier Applied Science Publishers Ltd, England, 1984. Printed in Great Britain

various gangliosides to tetanus toxin and established the order of binding as GT1b > GQ1b > GD1b > GT1a > GM1 > GD1a > GD3 > GA1 > GM2 > GM3. On the other hand sendai virus shows very good binding activity with GT1a and GQ1b, 1-2% activity with GD1a and GT1b and 500 times less affinity with other gangliosides (Holmgren *et al.*, 1980*b*). Recent conformational energy calculations on monosialogangliosides (GM3, GM2, GM1) and disialogangliosides (GD1a, GD1b) have suggested a small binding site for cholera toxin (Veluraja & Rao, 1983). Since higher gangliosides are better receptors for tetanus toxin and sendai virus an attempt has been made theoretically to propose favoured conformations for GD3, GT1a, GT1b and GQ1b gangliosides. The minimum energy conformations of these gangliosides have been compared with those of monosialogangliosides, disialogangliosides and asialogangliosides (Veluraja, 1983; Veluraja & Rao, 1983) and have also been correlated with their binding activity to tetanus toxin and sendai virus. Such studies also throw light on the binding site size and the residues important in binding.

METHOD OF CALCULATION

The possible rotations around the various single bonds of the oligosaccharide portion of GD3, GT1a, GT1b and GQ1b are shown in Figs 1 and 2. Rotations around the C-C bonds of the glycerol side chain of the NeuNAc are considered only if they are involved in the glycosidic linkage and otherwise fixed in the theoretically determined minimum energy conformation (Veluraja & Rao, 1980). 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) chair form. The geometries used for the various sugar residues were the same as described elsewhere (Veluraja & Rao, 1983). The initial conformation corresponding to $(\phi, \psi) = (0°, 0°)$ is the same as described earlier (Yathindra & Rao, 1970). For the sialic acid linked disaccharides (NeuNAc α(2-3)Gal and NeuNAc α(2-8)NeuNAc) the initial conformations are defined as

$\phi = 0°$ when C1-C2 is *cis* to O-C*X*
$\psi = 0°$ when C2-O is *cis* to C*X*-H*X*
$\chi^c = 0°$ when C1-O1 is *cis* to C2-O6
$\chi^1 = 0°$ when C5-C6 is *cis* to C7-C8
$\chi^2 = 0°$ when C6-C7 is *cis* to C8-C9

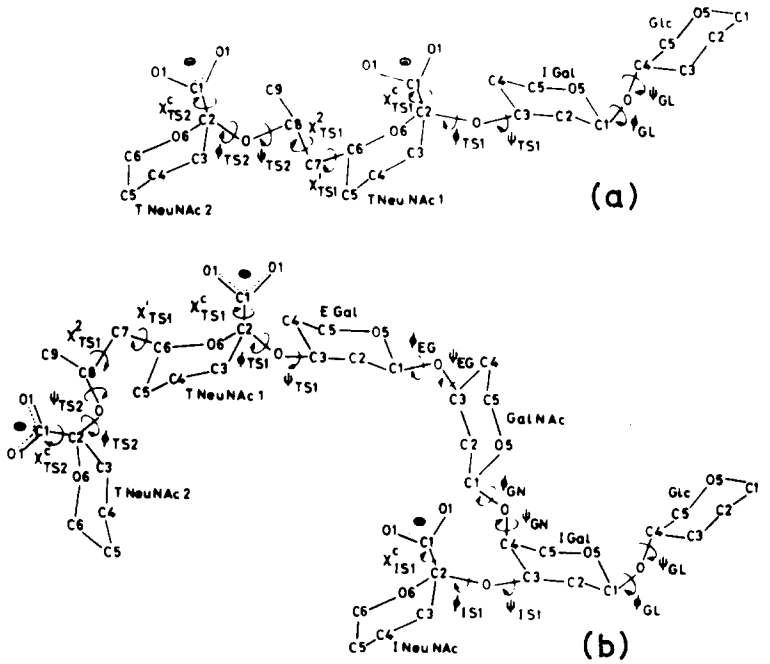

Fig. 1. Numbering of the atoms and dihedral angles of the oligosaccharide portion of gangliosides: (a) GD3 (b) GT1a. The side groups are not shown. (Glc, Glucose; IGal, internal galactose; GalNac, *N*-acetylgalactosamine; EGal, external galactose; INeuNAc, internal *N*-acetylneuraminic acid; TNeuNAc, terminal *N*-acetylneuraminic acid.)

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

The potential energy of the molecule was computed considering the non-bonded, electrostatic and torsional contributions using the functions and constants of Momany *et al.* (1975). The method used for the charge calculation is the same as described earlier (Veluraja & Rao, 1983). The net charge associated with each atom is used to compute the electrostatic energy. The 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

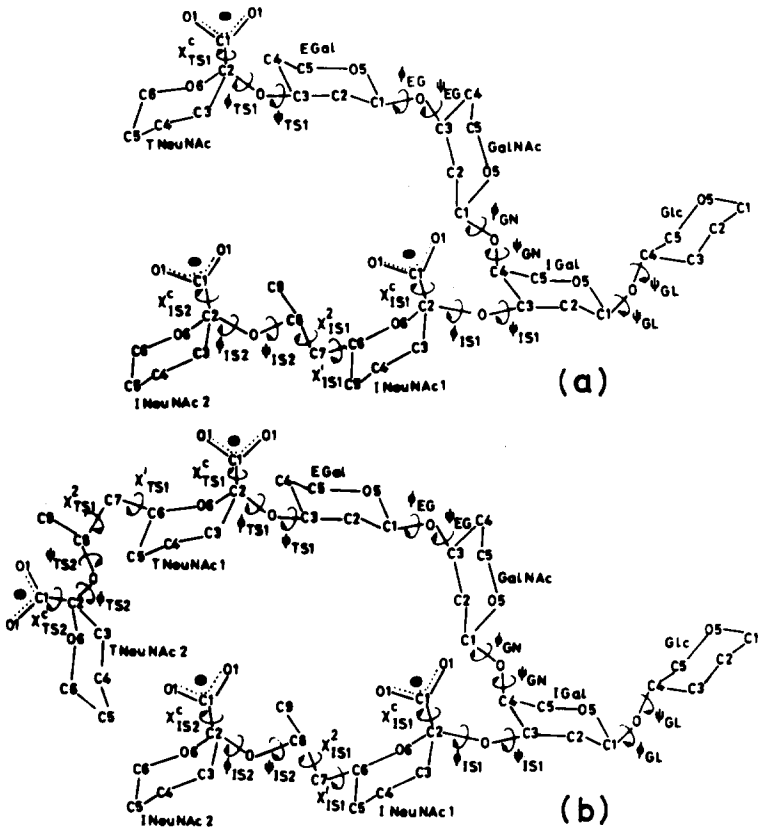

Fig. 2. Numbering of atoms and dihedral angles of the oligosaccharide portion of gangliosides: (a) GT1b (b) GQ1b. The side groups are not shown.

that it would not affect significantly the preferred conformation (Kaliannan and Rao, unpublished results). Hence the neglect of the above two factors in the present calculations may not affect the results significantly.

The oligosaccharide portion of these gangliosides can assume a large number of conformations due to freedom of rotation about the inter-unit bonds. Hence the calculation of the energy by varying the dihedral angles at discrete intervals is time-consuming. To reduce computer time, energy was minimized as a function of the rotational angles following the Fletcher & Powell (1963) and Davidon (1959) minimization procedures.

RESULTS AND DISCUSSION

The calculated low energy conformers for GD3 are shown in Table 1. The set of dihedral angles (ϕ_{TS2}, ψ_{TS2}, χ^2_{TS1}, χ^1_{TS1}), which define the conformation of the disialic acid fragment, favour values around (−70°, −5°, 70°, 150°). When they assume values around (−155°, −15°, 100°, 180°), (−120°, 40°, 95°, 180°) and (−130°, 45°, 160°, 115°) the energy increases by 3–4 kcal mol^{-1} (conformers 3, 4 and 5 of Table 1). (ϕ_{TS1}, ψ_{TS1}), which define the orientation of the disialic acid fragment with respect to the internal galactose, favour values around (−70°, −5°). When they assume the other conformation around (−160°, −20°), the energy increases by about 2·5 kcal mol^{-1}. (ϕ_{GL}, ψ_{GL}) prefer values around (50°, 10°). These results indicate that the conformation of this carbohydrate fragment is highly restricted. The projection of the minimum energy conformer of GD3 is shown in Fig. 3.

Table 2 shows the probable conformers of GT1a. The dihedral angles (ϕ_{TS2}, ψ_{TS2}, χ^2_{TS1}, χ^1_{TS1}) which define the conformation of the disialic acid fragment prefer values around (−75°, 5°, 70°, 150°). When they assume values around (−130°, 45°, 160°, 115°), (−115°, 35°, 95°, 180°) and (−155°, −10°, 105°, 175°) (conformers 7, 9 and 12) the conformational energy increases by 2·2, 2·8 and 3·7 kcal mol^{-1} respectively. The conformational angles (ϕ_{TS1}, ψ_{TS1}) which define the orientation of the disialic acid fragment with respect to the rest of the molecule favour both (−70°, −15°) and (−160°, −20°) with an energy difference of only about 0·2 kcal mol^{-1}. This suggests an equal population of these two conformers in solution. The favoured values for (ϕ_{EG}, ψ_{EG}) of around (75°, −20°) and (30°, −50°) (conformers 1–4), with equal energy, indicate the flexibility in the conformation of the external galactose residue. The *N*-acetylgalactosamine residue favours a single conformation corresponding to (ϕ_{GN}, ψ_{GN}) equal to (35°, 20°). In the minimum energy conformers, (ϕ_{IS1}, ψ_{IS1}) favour values around (−70°, −10°). When they assume values around (−155°, −25°) the energy increases by about 1·5 kcal mol^{-1}.

Because of the favoured values for (ϕ_{EG}, ψ_{EG}) around (75°, −20°) and (30°, −50°) and (ϕ_{TS1}, ψ_{TS1}) around (−70°, −15°) and (−160°, −20°) the main chain of GT1a, NeuNAc-NeuNAc-Gal-GalNAc-Glc, favours at least four different conformations (conformers 1–4 of Table 2) and the projections are shown in Figs 4 and 5. Since the energy

TABLE 1
Minimum Energy Conformations of GD3

Number	χ^{c}_{TS2}	ϕ_{TS2}	ψ_{TS2}	χ^{2}_{TS1}	χ^{1}_{TS1}	ϕ_{TS1}	ψ_{TS1}	ϕ_{GL}	ψ_{GL}	χ^{c}_{TS1}[a]	*Relative energy, kcal mol*$^{-1}$
1	−36	−71	−4	70	148	−70	−5	51	8	−40	0·0
2	−37	−70	−10	71	154	−157	−18	54	3	−40	2·5
3	−38	−154	−14	102	179	−69	−8	52	8	−40	3·0
4	−39	−118	39	95	178	−68	−9	51	10	−40	3·9
5	−42	−128	43	161	114	−69	−8	53	6	−40	4·4

(ϕ_{GL}, ψ_{GL}) can take another two sets of values (−20°, −30°) and (160°, 10°), but the energy increases by 1·2 and 4·0 kcal mol^{-1} respectively.

[a] χ^{c}_{TS1} was fixed in the minimum energy value as shown.

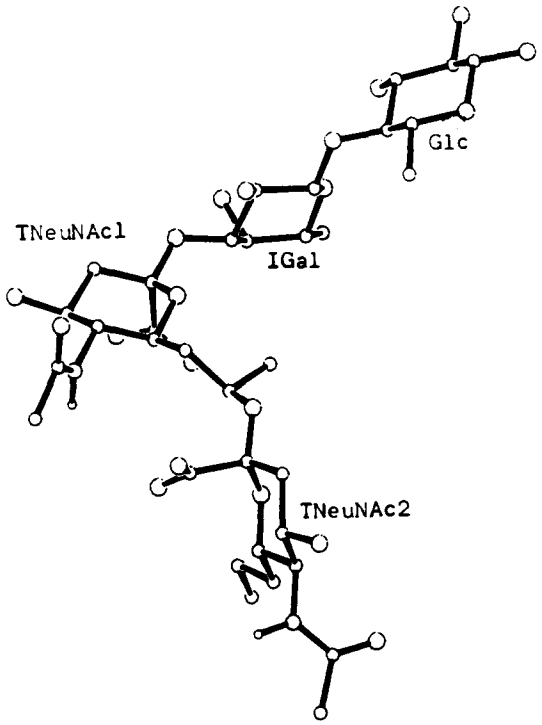

Fig. 3. Projection of the global minimum energy conformer of GD3. Hydroxyl oxygens at the glycerol side chain are not shown.

difference between these four conformers is very small (less than 0·7 kcal mol^{-1}) all can coexist in solution.

The various minimum energy conformers that are possible for the oligosaccharide portion of GT1b are shown in Table 3. In GT1b (ϕ_{TS1}, ψ_{TS1}), which define the conformation of the terminal sialic acid residue, favour values around (−70°, 0°). When they assume values around (−160°, −20°) (conformers 3 of Table 3) the conformational energy increases by about 2·8 kcal mol^{-1}. (ϕ_{EG}, ψ_{EG}) favour values around (60°, −10°) and (30°, −40°) with an energy difference of about 1·2 kcal mol^{-1} (conformers 1 and 2) indicating the flexibility in the orientation of the external galactose residue. The dihedral angles (ϕ_{IS2}, ψ_{IS2}, χ^2_{IS1}, χ^1_{IS1}) which define the conformation of the disialic acid fragment of GT1b always favour values around (−70°, −5°, 70°, 150°) (con-

TABLE 2
Minimum Energy Conformations of GT1a

Number	χ^{c}_{TS2}	ϕ_{TS2}	ψ_{TS2}	χ^{2}_{TS1}	χ^{1}_{TS1}	ϕ_{TS1}	ψ_{TS1}	ϕ_{EG}	ψ_{EG}	ϕ_{GN}	ψ_{GN}	ϕ_{GL}[a]	ψ_{GL}[a]	ϕ_{IS1}	ψ_{IS1}	χ^{c}_{IS1}	χ^{c}_{TSI}[a]	*Relative energy, kcal mol*$^{-1}$
1	−37	−74	3	70	146	−68	−14	75	−20	35	21	60	0	−67	−9	−33	−40	0·0
2	−37	−70	−10	72	154	−159	−19	61	−2	31	20	60	0	−67	−9	−32	−40	0·2
3	−38	−69	−9	72	153	−158	−20	31	−46	33	18	60	0	−68	−8	−32	−40	0·3
4	−37	−70	−4	71	149	−67	−13	36	−44	39	20	60	0	−66	−10	−33	−40	0·7
5	−39	−72	−7	72	156	−157	−21	65	−20	29	8	60	0	−155	−23	−44	−40	1·5
6	−39	−70	−8	72	154	−157	−21	32	−50	31	7	60	0	−155	−25	−44	−40	2·2
7	−42	−128	43	161	114	−67	−11	64	−17	33	21	60	0	−66	−9	−33	−40	2·2
8	−37	−70	−4	71	148	−67	−14	77	−21	34	16	60	0	−157	−22	−38	−40	2·7
9	−40	−115	35	95	−179	−59	−15	73	3	26	21	60	0	−67	−11	−31	−40	2·8
10	−42	−128	43	161	114	−65	−13	32	−47	34	20	60	0	−68	−9	−35	−40	3·1
11	−36	−80	−22	102	167	−160	−10	33	−46	37	21	60	0	−67	−9	−33	−40	3·3
12	−39	−153	−12	104	176	−66	−16	34	−51	33	20	60	0	−65	−10	−33	−40	3·7
13	−43	−128	43	159	115	−152	−24	63	−17	32	22	60	0	−67	−7	−33	−40	4·2
14	−37	−73	1	72	146	−67	−16	32	−55	33	3	60	0	−155	−25	−41	−40	4·4
15	−43	−128	43	161	114	−67	−12	69	−19	31	11	60	0	−157	−24	−43	−40	4·6
16	−43	−128	42	159	116	−153	−25	68	−17	30	10	60	0	−157	−24	−43	−40	5·0

[a] ϕ_{GL}, ψ_{GL}, χ^{c}_{TS1} fixed in the minimum energy values as shown.

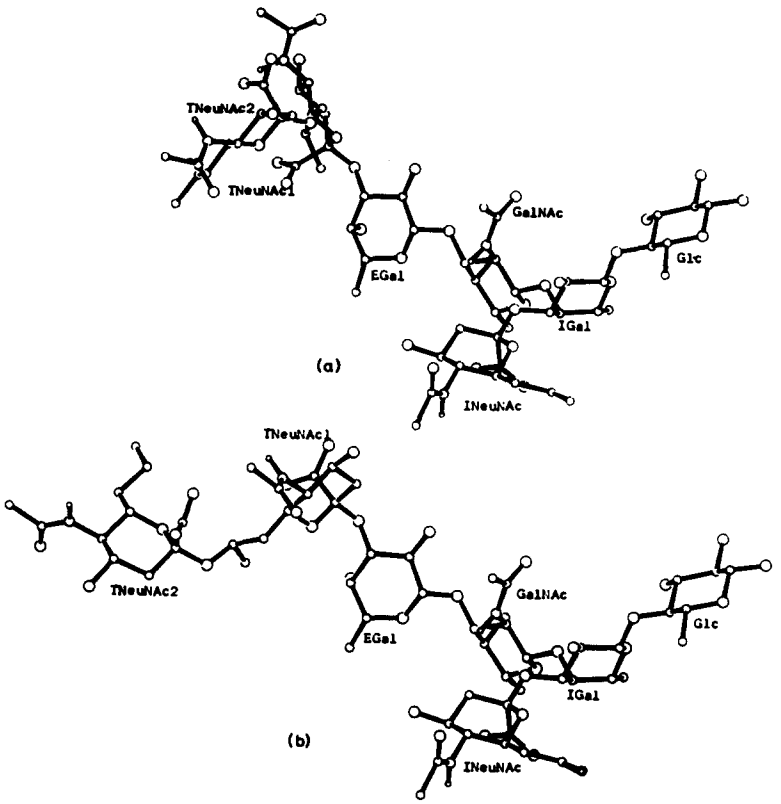

Fig. 4. Projection of (a) conformer 1 of GT1a, (b) conformer 2 of GT1a. Hydroxyl oxygens at the glycerol side chains are not shown.

formers 1–6 of Table 3). When values of around ($-130°$, $40°$, $160°$, $115°$) are favoured the energy increases by about 6·5 kcal mol^{-1} which rules out the possibility of the disialic acid fragment of GT1b occurring in this conformation. (ϕ_{IS1}, ψ_{IS1}), which define the conformation of the disialic acid fragment with respect to the internal galactose, favour values around ($-155°$, $-25°$). When they favour values around ($-70°$, $-10°$) the energy increases by about 3·7 kcal mol^{-1}. The projection of the global minimum energy conformer of GT1b is shown in Fig. 6.

A comparison of Tables 1 and 3 and an examination particularly of (ϕ_{XS2}, ψ_{XS2}, χ^2_{XS1}, χ^1_{XS1}), when X = I or T (these define the conformation of the disialic acid fragment) shows that similar values in GD3 and GT1b are favoured. This indicates that the presence of the branch at

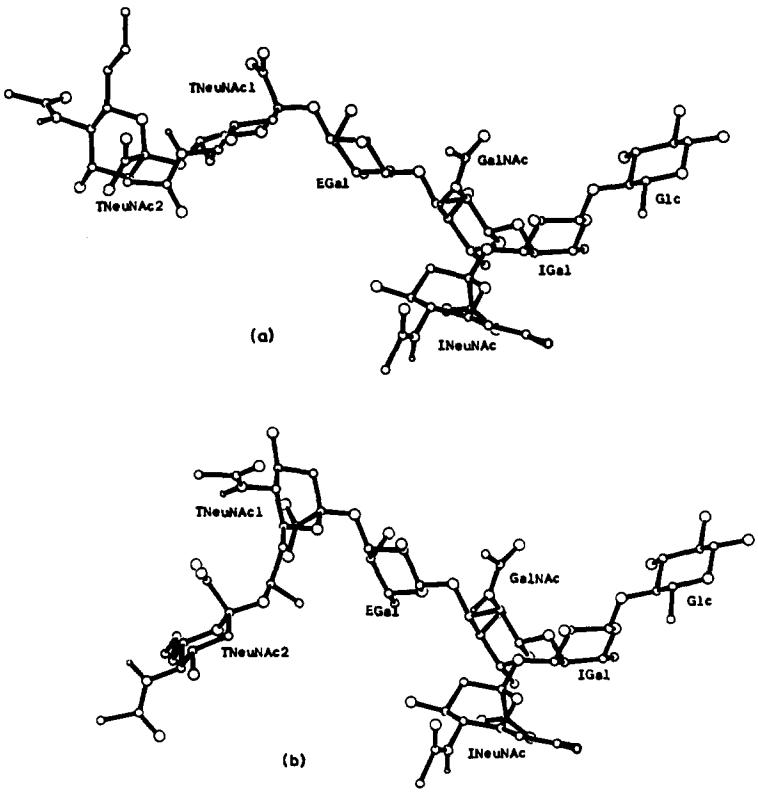

Fig. 5. Projection of (a) conformer 3 of GT1a, (b) conformer 4 of GT1a. Hydroxyl oxygens at the glycerol side chains are not shown.

the internal galactose residue in GT1b does not significantly affect the preferred conformation of the disialic acid fragment. However, the dihedral angles (ϕ_{IS1}, ψ_{IS1}), which define the orientation of the disialic acid fragment with respect to the internal galactose residue, favour different values in GT1b and GD3.

The probable conformers of the oligosaccharide portion of ganglioside GQ1b are shown in Table 4. In those conformers whose relative energy is less than 2·5 kcal mol^{-1}, (ϕ_{TS2}, ψ_{TS2}, χ^2_{TS1}, χ^1_{TS1}), which define the conformation of the terminal disialic acid fragment, favour values around ($-70°$, $-10°$, $70°$, $150°$). When they assume the other set of values around ($-130°$, $45°$, $160°$, $115°$) (conformer 11 of Table 4) the conformational energy increases by 2·7 kcal mol^{-1}. The dihedral angles

TABLE 3
Minimum Energy Conformations of GT1b

Number	χ^{c}_{TS1}	ϕ_{TS1}	ψ_{TS1}	ϕ_{EG}	ψ_{EG}	ϕ_{GN}	ψ_{GN}	ϕ_{GL}[a]	ψ_{GL}[a]	ϕ_{IS2}	ψ_{IS2}	χ^{2}_{IS1}	χ^{1}_{IS1}	ϕ_{IS1}	ψ_{IS1}	χ^{c}_{IS2}	χ^{c}_{IS1}[a]	*Relative energy, kcal mol*$^{-1}$
1	−37	−73	−2	58	−13	29	2	60	0	−69	−5	70	149	−153	−23	−34	−40	0·0
2	−36	−73	1	31	−37	27	4	60	0	−72	−5	68	148	−153	−23	−40	−40	1·2
3	−38	−155	−21	62	−11	28	6	60	0	−69	−6	70	149	−155	−23	−37	−40	2·8
4	−36	−67	−8	62	−17	29	20	60	0	−70	−4	70	148	−71	−9	−37	−40	3·7
5	−35	−66	−10	29	−47	29	20	60	0	−70	−4	70	148	−72	−7	−37	−40	4·3
6	−38	−153	−22	35	−44	26	7	60	0	−68	−6	70	148	−150	−23	−41	−40	5·6
7	−36	−71	−4	38	−36	30	1	60	0	−128	39	158	115	−153	−27	−46	−40	6·5
8	−38	−154	−20	61	−15	28	20	60	0	−70	−4	70	148	−70	−9	−37	−40	6·8

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

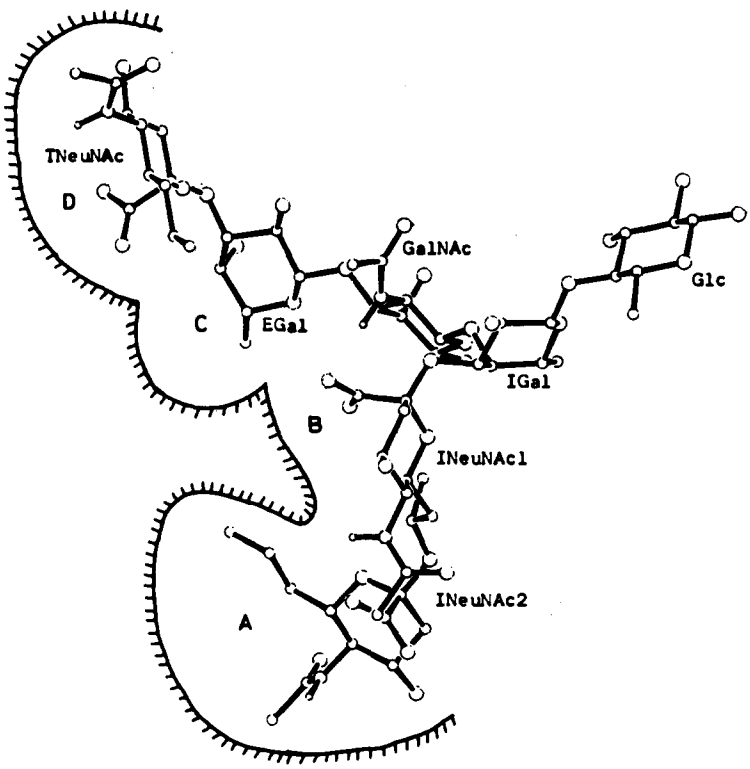

Fig. 6. Projection of the global minimum energy conformer of GT1b. The probable binding site for tetanus toxin is indicated. Hydroxyl oxygens at the glycerol side chains are not shown.

(ϕ_{TS1}, ψ_{TS1}) can assume values around (−160°, −20°) and (−70°, −10°) (conformers 1 and 3) with an energy difference of about 0·4 kcal mol^{-1} indicating that the terminal NeuNAcl can take two different orientations with respect to the external galactose residue. In the minimum energy conformers, (ϕ_{EG}, ψ_{EG}) favour values around (65°, −20°) and (35°, −50°) (conformers 1 and 2). (ϕ_{GN}, ψ_{GN}) always favour values around (35°, 20°). The dihedral angles (ϕ_{IS2}, ψ_{IS2}, χ^2_{IS1}, χ^1_{IS1}) which define the conformation of the internal disialic acid fragment favour values around (−75°, 5°, 70°, 145°) and (−155°, −15°, 100°, 180°). The energy difference of 1·1 kcal mol^{-1} between these two conformations indicates that the internal disialic acid fragment can exist in both the conformations. In the minimum energy conformers,

TABLE 4

Minimum Energy Conformations of GQ1b

Number	x^{c}_{TS2}	ϕ_{TS2}	ψ_{TS2}	x^{2}_{TS1}	x^{1}_{TS1}	ϕ_{TS1}	ψ_{TS1}	ϕ_{EG}	ψ_{EG}	ϕ_{GN}	ψ_{GN}	ϕ_{GL}[a]	ψ_{GL}[a]	ϕ_{IS2}	ψ_{IS2}	x^{2}_{IS1}	x^{1}_{IS1}	ϕ_{IS1}	ψ_{IS1}	x^{c}_{IS2}	x^{c}_{IS1}	x^{c}_{TS1}[a]	*Relative energy, kcal mol*$^{-1}$
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*
1	−39	−69	−10	71	153	−159	−20	66	−21	41	11	60	0	−75	4	71	146	−68	−8	−38	−40	−40	0·0
2	−40	−72	−7	72	153	−156	−23	33	−52	35	19	60	0	−72	−2	72	147	−70	−8	−37	−40	−40	0·0
3	−33	−81	−24	103	166	−68	−9	69	−17	53	19	60	0	−73	2	72	146	−70	−9	−37	−40	−40	0·4
4	−36	−70	−7	71	153	−160	−20	66	−19	31	17	60	0	−70	−7	73	150	−155	−20	−37	−40	−40	1·0
5	−39	−70	−10	73	155	−158	−20	64	−19	39	17	60	0	−153	−17	98	−179	−69	−9	−38	−40	−40	1·1
6	−38	−69	−5	70	147	−69	−12	59	−18	59	19	60	0	−74	3	71	145	−70	−11	−38	−40	−40	1·1
7	−38	−72	−8	72	154	−157	−22	34	−52	34	20	60	0	−153	−16	99	−179	−69	−9	−38	−40	−40	1·5
8	−38	−71	−7	72	154	−157	−24	39	−46	31	14	60	0	−68	−8	72	149	−154	−21	−36	−40	−40	1·9
9	−33	−81	−25	103	166	−162	−11	66	−20	49	17	60	0	−77	8	71	143	−69	−8	−39	−40	−40	2·1
10	−38	−75	5	72	144	−72	−10	60	−15	53	19	60	0	−154	−16	99	180	−68	−10	−39	−40	−40	2·5
11	−42	−128	43	161	113	−69	−10	68	−17	49	18	60	0	−72	0	71	146	−70	−7	−37	−40	−40	2·7
12	−34	−81	−24	104	166	−163	−11	70	−18	32	17	60	0	−70	−7	73	149	−157	−21	−37	−40	−40	3·0
13	−39	−77	18	73	134	−68	−10	58	1	56	22	60	0	−70	−9	75	152	−155	−15	−38	−40	−40	3·2
14	−35	−81	−25	104	163	−71	−10	65	−9	56	23	60	0	−69	−12	76	153	−151	−16	−39	−40	−40	3·3
15	−42	−129	43	160	115	−154	−24	66	−19	39	18	60	0	−73	0	71	146	−70	−8	−37	−40	−40	3·5
16	−32	−82	−24	104	165	−162	−13	71	−20	39	10	60	0	−148	−18	97	−179	−70	−8	−39	−40	−40	3·7
17	−41	−129	43	161	113	−69	−7	69	−21	48	19	60	0	−153	−15	100	180	−68	−9	−39	−40	−40	4·2
18	−40	−128	44	160	115	−156	−23	67	−17	30	17	60	0	−69	−7	72	150	−157	−20	−38	−40	−40	4·2
19	−38	−127	43	160	114	−69	−7	69	−15	43	17	60	0	−69	−8	73	149	−159	−19	−37	−40	−40	4·4
20	−45	−88	−19	109	166	−66	−12	36	−48	63	15	60	0	−80	13	71	143	−69	−5	−45	−40	−40	4·4

[a] ϕ_{GL}, ψ_{GL}, x^{c}_{IS1}, x^{c}_{TS1} fixed in the minimum energy values as shown.

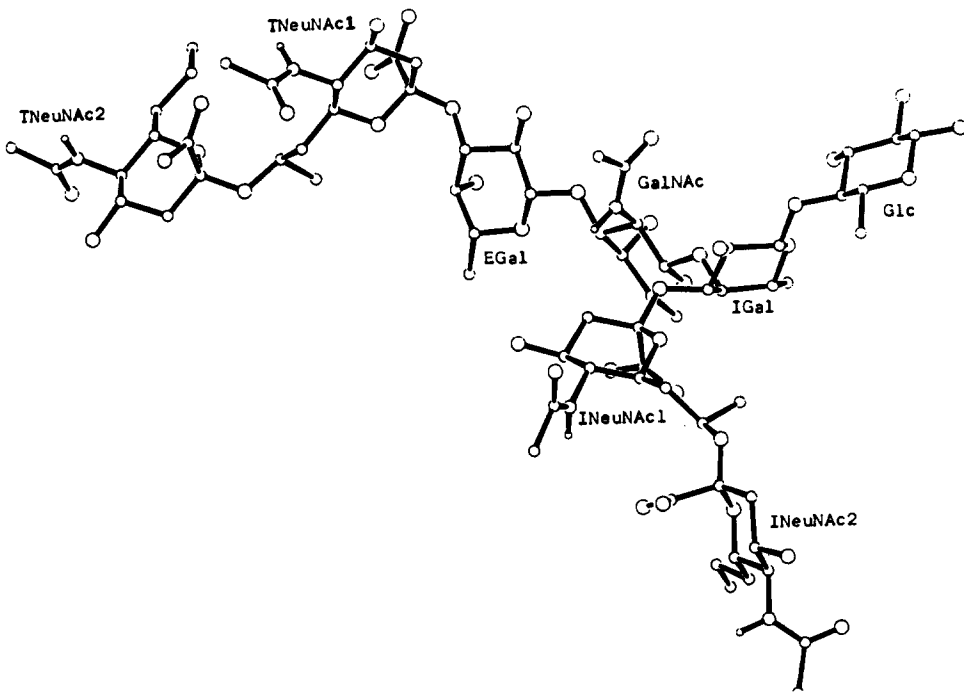

Fig. 7. Projection of conformer 1 of GQ1b. Hydroxyl oxygens at the glycerol side chains are not shown.

(ϕ_{IS1}, ψ_{IS1}) prefer values around ($-70°$, $-10°$). When they assume values around ($-155°$, $-20°$) the conformational energy increases by 1·0 kcal mol^{-1}. It is interesting to note that the terminal and internal disialic acid fragments of GQ1b favour a conformation similar to that of the same fragment in GT1a, GT1b and GD3. The projection corresponding to conformer 1 is shown in Fig. 7. In conformers 2 and 3 of GQ1b the main chain NeuNAc-NeuNAc-Gal-GalNAc-Glc resembles that of Figs 5a and 4a respectively and the internal disialic acid fragment resembles that of Fig. 7 and hence is not shown separately.

Though the only structural difference between GT1b and GQ1b lies in the presence of an additional terminal NeuNAc2 in the latter, the favoured conformations for these two gangliosides differ drastically. GQ1b can assume a conformation similar to the minimum energy conformer of GT1b by expending energy of 3·2 kcal mol^{-1} (conformer 1 of Table 3 is equal to conformer 13 of Table 4). On the other hand,

GT1b can assume conformations similar to the minimum energy conformations of GQ1b by expending 3·7–6·8 kcal mol^{-1} of energy (conformer 1 of Table 4 is equal to conformer 8 of Table 3; conformer 3 of Table 4 is equal to conformer 4 of Table 3). Similarly, the structural difference between GT1a and GQ1b lies in the presence of an additional internal NeuNAc2 in the latter. Unlike the previous case, however, GT1a and GQ1b prefer similar conformations (conformers 1, 2 and 3 of Table 4 are equal to conformers 2, 3 and 1 of Table 2 respectively).

CORRELATION OF CONFORMATION TO TETANUS TOXIN INTERACTION

Earlier conformational energy calculations on GM2, GM1, GA1 and GD1b indicated involvement of the internal disialic acid fragment and external galactose in the interaction with tetanus toxin (Veluraja, 1983; Veluraja & Rao, 1983). The present study indicates that the common carbohydrate fragments in GT1b favour conformations similar to GD1b (Veluraja & Rao, 1983), i.e. the addition of another NeuNAc residue at the external galactose of the latter to form GT1b does not alter the conformation of the original fragment. But GT1b has higher binding activity than GD1b. This indicates that the terminal NeuNAc of GT1b may also be actively involved in binding with the tetanus toxin. In all these gangliosides (GM2, GM1, GD1b, GT1b), (ϕ_{IS1}, ψ_{IS1}) define the orientation of the internal NeuNAc residue with respect to the internal galactose and favour values around ($-160°$, $-20°$) which may be important for tetanus toxin interaction. These results thus indicate that tetanus toxin may have a large binding site as shown in Fig. 6. Hence the binding site of tetanus toxin should accommodate at least internal NeuNAc2 (site A), internal NeuNAc1 (site B), external galactose (site C) and terminal NeuNAc (site D).

In the minimum energy conformers of GT1a, the external galactose and the terminal NeuNAc1 are in proper orientation for interacting at sites C and D, but the internal NeuNAc is not in a proper orientation to fit in site B. However, in conformer 8 (Table 2, Fig. 8), the internal NeuNAc is also in proper orientation to fit in site B. This suggests that the toxin has to spend about 2·7 kcal mol^{-1} to bring this residue into proper orientation to fit in site B. Moreover the sugar residue at site A

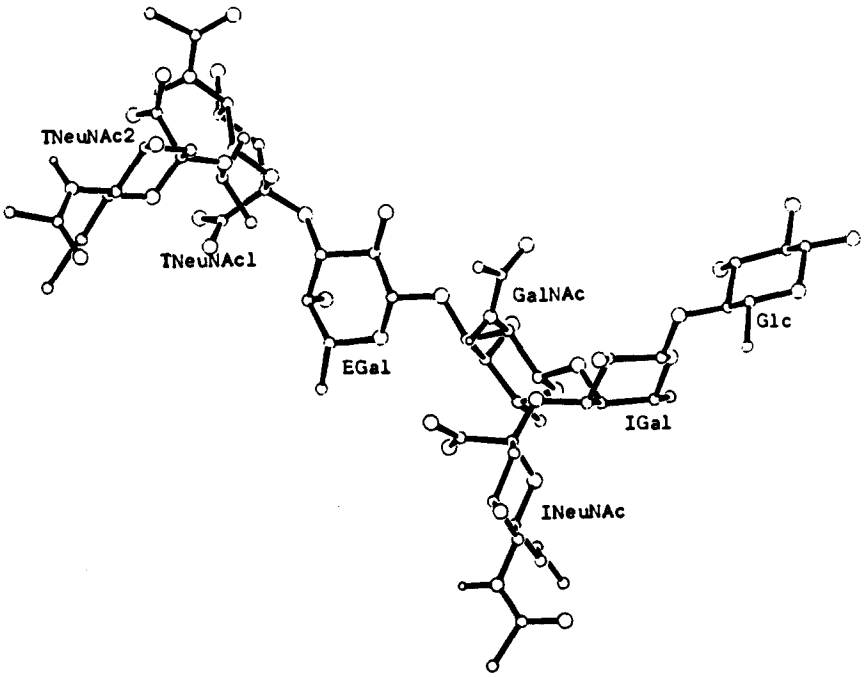

Fig. 8. Projection of conformer 8 of GT1a. Hydroxyl oxygens at the glycerol side chains are not shown.

is absent in this ganglioside. These considerations may explain the intermediate binding activity of GT1a.

In conformer 3 of GQ1b, except for the internal disialic acid fragment the rest of the molecule is in a correct orientation for interaction with tetanus toxin. In conformer 13 of GQ1b (Fig. 9) the internal disialic acid fragment is also in proper orientation to fit in sites A and B. This suggests that the toxin may initially bind with the low energy conformer by interacting with external galactose and terminal NeuNAc1 and spend part of its binding energy (3·2 kcal mol^{-1}) to reorientate the internal disialic acid fragment to fit in sites A and B. This perhaps explains the low activity of GQ1b compared to GT1b. Though the toxin has to spend about 3 kcal mol^{-1} energy to bind either to GT1a or GQ1b, in the latter, site A is also occupied by the extra internal NeuNAc2. This extra binding energy may account for the higher activity of GQ1b over GT1a. In GD3 the higher energy

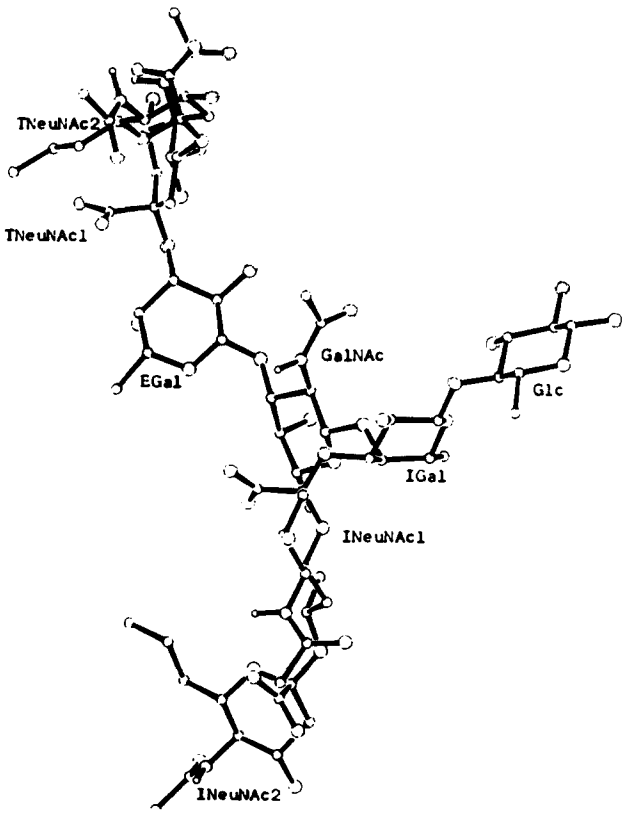

Fig. 9. Projection of the conformer 13 of GQ1b. Hydroxyl oxygens at the glycerol side chains are not shown.

conformer (conformer 2) can fit at sites A and B of tetanus toxin leaving the other two sites, C and D, vacant. This may explain its poor activity.

Sendai virus

The binding activity of sendai virus with various gangliosides has been established by Holmgren *et al.* (1980*b*). Sendai virus binds equally well with gangliosides GQ1b and GT1a. It also binds to GT1b and GD1a to some extent (1–2%). Other gangliosides have 500 times less affinity than the highly active gangliosides. These results suggest that

sendai virus binds to gangliosides which have a disialic acid fragment at the external galactose residue and disialic or monosialic acid at the internal galactose residue. A comparison of the binding specificities of sendai virus to various gangliosides shows that the removal of the disialic acid fragment from the external galactose residue affects the activity drastically. This suggests that the terminal disialic acid fragment is involved in binding. If the sendai virus binds only to the terminal disialic acid fragment then GD3 should also exhibit considerable activity, since the disialic acid fragment of GD3 favours a conformation similar to that of the one favoured in GT1a and GQ1b ((ϕ_{TS2}, ψ_{TS2}, χ^2_{TS1}, χ^1_{TS1}) favour similar values in GQ1b, GT1a and GD3). Since sendai virus binds only to a negligible extent in GD3, this indicates that the binding site might be large, accommodating more than two sugar residues.

Removal of the terminal NeuNAc2 residue from GQ1b and GT1a exposes the structures of GT1b and GD1a respectively. Such a change affects the favoured conformation of the common oligosaccharide fragment and also greatly reduces the activity of the ganglioside. A cursory look at Tables 2, 3 and 4 and the minimum energy conformation of GD1a (Veluraja & Rao, 1983) suggests that for the external galactose residue in the active ganglioside (GQ1b, GT1a), (ϕ_{EG}, ψ_{EG}) favour values close to (60°, −10°) and (30°, −50°) whereas in GD1a they favour values around (30°, 0°). In GT1b, (ϕ_{EG}, ψ_{EG}) favour values the same as those in GQ1b and GT1a. All these conformations occur in the same allowed region of the steric map (Veluraja & Rao, 1983) and hence changes from one conformation to the other conformation can take place without spending much energy. On the other hand, in GQ1b and GT1a for (ϕ_{TS1}, ψ_{TS1}), both (−70°, −10°) and (−160°, −20°) (Tables 2 and 4) are favoured equally, whereas in GT1b and GD1a the (−70°, 0°) conformation is highly favoured. Similarly, the sialic acid attached to the internal galactose residue favours a conformation in which (ϕ_{IS1}, ψ_{IS1}) are equal to (−70°, −10°) in GQ1b and GT1a and (ϕ_{IS1}, ψ_{IS1}) are equal to (−160°, −20°) in GT1b and GD1a. These two conformations occur in two different allowed regions of the steric map (Veluraja & Rao, 1983). In fact the ring of the sialic acid residue has to flip over to go from one orientation (−160°, −20°) to the other (−70°, −10°). Such a change in the orientation of the sialic acid residue may affect the probable sites of attachment of this residue with the sendai virus combining site. If (−160°, −20°) and (−70°, −10°)

for (ϕ_{TS1}, ψ_{TS1}) and (ϕ_{IS1}, ψ_{IS1}), respectively, are associated with their biological activity (binding of sendai virus) then to bring the sugar residues of GT1b and GD1a to overlap with the corresponding sugar residues in GQ1b and GT1a the virus has to spend a large amount of energy (6·8 kcal mol^{-1} in GT1b (conformer 8 of Table 3) and GD1a (Veluraja, 1983)). This and the lack of terminal NeuNAc2 may perhaps explain its reduced activity.

ACKNOWLEDGEMENT

The authors thank the Department of Science and Technology, Government of India for their financial support.

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