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CONFORMATIONAL STUDIES ON THE ABH AND LEWIS BLOOD GROUP OLIGOSACCHARIDES*

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ABSTRACT

The possible conformations for the ABH and Lewis blood group oligosaccharides have been studied by an energy-minimisation procedure using empirical potential functions. It has been found that the conformation of the core structure is not altered significantly by the addition of L*-fucose, galactose or* N*-acetyl galactosamine residues at the non-reducing end. Correlation of the preferred conformations with their known binding properties suggests that the differences between type 1 and type 2 structures become significant only when a large enough fragment of the determinant is considered. It is suggested that non-specific reagents may have small binding sites while the reagents that are specific for type 1 or type 2 structures may have larger binding sites. A two-pocket model has been proposed for antibodies and lectins which can distinguish the A1 and A2 antigens.*

1. INTRODUCTION

The chemical structures of the ABH(O) and Lewis antigens have been determined using water soluble blood group substances isolated from secretions (ovarian cyst/mucin, gastric mucin, etc.). Immunochemical investigations, most frequently by quantitative precipitation and quantitative hapten inhibition assays as well as by radio and enzyme immunoassay, have made it possible to explore the nature and size of the specific combining sites of antibodies and lectins. Such studies have indicated that the

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binding sites of antibodies and lectins as well as a large number of enzymes may be between 0·6 nm and 3·4 nm (Kabat, 1978). However, these studies have also led to the accumulation of much data which could not be rationalised.

Many of the anti-H reagents cannot distinguish between type 1 and type 2 structures (Tables 1 and 2) while the goat anti-H serum binds only to type 1 structures (Marcus & Cass, 1967) and a lectin from *Lotus tetragonolobus* binds only to type 2 structures (Miercio *et al.*, 1974). In some of the anti-Le/Le systems the type 1 and type 2 structures have comparable binding properties (Rovis *et al.*, 1973), although the Lewis determinants are generally considered to be confined to type 1 structures alone. Further the molecular basis for the existence of the two A serotypes, A1 and A2, has yet to be understood.

It is now well established that the specificity of the various blood group substances lies mainly in the terminal portion of the carbohydrate chains which do not differ from source to source (Figs 1 and 2). Thus, from a study of the possible conformations of the carbohydrate fragments, some attempts have been made to obtain information about the specificity of the binding of antibodies and lectins. This has been done using both space-filling models (Kabat, 1976) and by empirical energy calculations (Lemieux *et al.*, 1980; Thogersen *et al.*, 1982) considering only a di- or trisaccharide fragment from the terminal ends. These models could not, however, explain the observed binding properties of the various antigens, satisfactorily. This may be due to the fact that these studies did not include the complete invariant fragments and

TABLE 1
Specificity of Some Blood Group Specific Antibodies

Antigen	*Specific for type 1*	*Specific for type 2*	*Non-specific*
H-antigen	Goat anti-H[1]		Human anti-H[2] Rabbit anti-H[2]
Le^a-antigen	Goat anti-Le^a*[3] Human anti-Le^a*[3]		
Le^b-antigen	Human anti-Le^b[3]		Goat anti-Le^b[3]
B-antigen			Human anti-B[4]
A1-antigen			Human anti-A[5] Human anti-A1[5]
A2-antigen			Human anti-A[5] Human anti-A2[5]

* Also recognised type 2 structures but are fourfold less active.
[1] Marcus & Cass (1967).
[2] Lloyd & Kabat (1968).
[3] Rovis *et al.* (1973).
[4] Kabat (1970).
[5] Moreno *et al.* (1971).

TABLE 2
Specificity of Some Blood Group Specific Lectins

Antigen	*Specific for type 1*	*Specific for type 2*	*Non-specific*
H-antigen		*Lotus tetragonolobus*[1] *Ulex europeus*[2]	*Euonymus europeus*[3] Eel anti-H[2]
Le^a-antigen		*Lotus tetragonolobus*[1]	
Le^b-antigen		*Lotus tetragonolobus*[1]	
B-antigen			*Euonymus europeus*[3] *Banderaea simplicafolia**[4] Lima bean[5]
A1-antigen			*Banderaea simplicafolia**[4] *Dilochus biflorus*[5] Lima bean[5] *Helix pomatia*[5]
A2-antigen		*Lotus tetragonolobus*[1] *Ulex europeus*[2]	*Euonymus europeus*[3] Eel anti-H[2] *Dilochus biflorus*[5] Lima bean[5] *Helix pomatia*[5]

* Subunit A is inhibited by methyl-α-D-galactosamine; subunit B is inhibited by methyl-α-D-galactose.
[1] Miercio *et al.* (1974).
[2] Lloyd & Kabat (1968).
[3] Petryniak *et al.* (1977).
[4] Hayes & Goldstein (1974).
[5] Hammarstrom *et al.* (1977).

hence may not reflect all the differences of type 1 and type 2 structures. Hence, the preferred conformations of the terminal invariant carbohydrate moieties have been computed by theoretical methods to thereby postulate the probable conformations of the antigens and to correlate these with their biological properties.

2. METHOD OF CALCULATION

The various carbohydrate moieties described in the present work are shown in Figs 1 and 2.

All sugar residues were assumed to be $^4C1$ (D) form except for the L-fucose residue which was assumed to be in $^1C4$ (L) form (Stoddart, 1971). The atomic coordinates of each residue were based on the standard residue of Arnott & Scott (1972), compiled from crystal structure data. The acetamido group was fixed using Pauling-Corey geometry (Corey & Pauling, 1953) so that the C2—H2 and N—H bonds were *trans*.

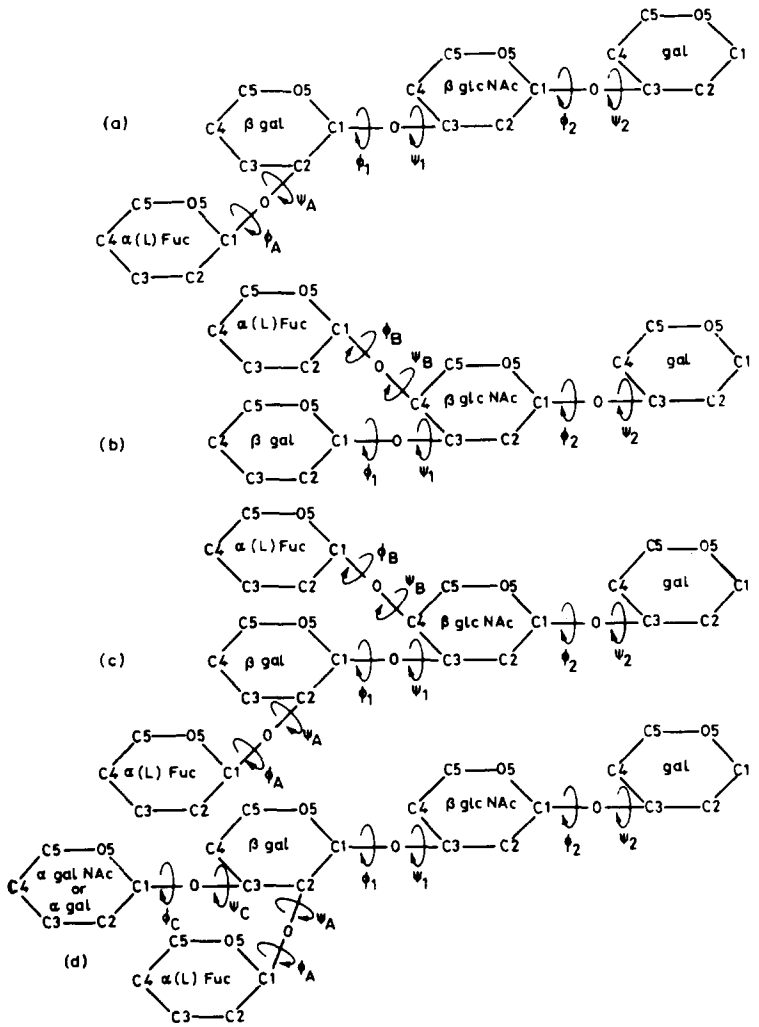

Fig. 1. Numbering of atoms and dihedral angles in the blood group oligosaccharides. Type 1 structures of (a) H, (b) Le^a, (c) Le^b, (d) A/B. For simplicity the side groups are not shown. Abbreviations: gal, D-galactose; glcNAc, N-acetyl-D-glucosamine; (L)Fuc, L-fucose; galNAc, N-acetyl-D-galactosamine.

The bond angle at the glycosidic oxygen was fixed at the average value of 117·5°. All possible conformations of the di- and oligosaccharides were generated by making rotations about the interunit glycosidic bonds C1—O (ϕ rotation) and O—CX' (ψ rotation), through $-180°$ to $+180°$ at intervals of 10°. The initial conformation corre-

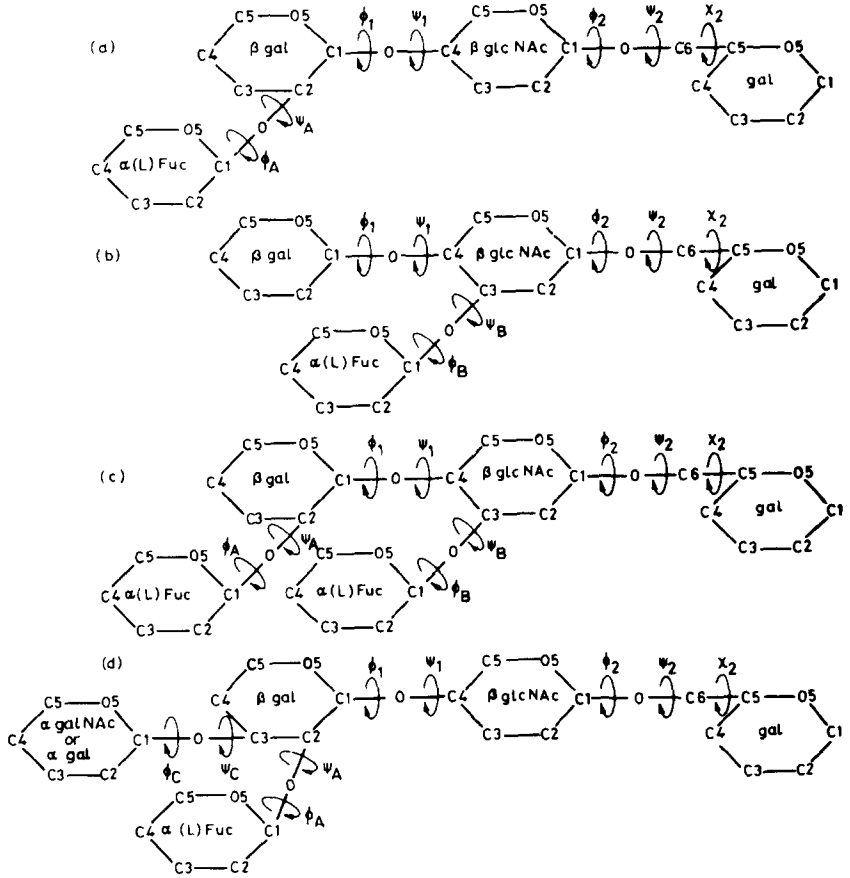

Fig. 2. Numbering of atoms and dihedral angles in the blood group oligosaccharides. Type 2 structures of (a) H, (b) Le^a, (c) Le^b, (d) A/B. For simplicity the side groups are not shown. Abbreviations as in Fig. 1.

sponding to $(\phi, \psi) = (0°, 0°)$ has been described earlier (Yathindra & Rao, 1970). A clockwise rotation was taken as positive.

Steric maps were constructed using the contact criteria (Ramachandran & Sasisekharan, 1968). The potential energy of the molecules were computed considering non-bonded, electrostatic and torsional contributions. The form of the functions and the constants used were those reported by Momany *et al.* (1975). To compute the electrostatic contribution the fractional charges on the various atoms were taken from Yathindra & Rao (1972).

The energy minimisation procedure used was that described by Fletcher & Powell (1963) and Davidson (1959). Except where branching occurs, a systematic analysis

was carried out by varying all the glycosidic torsional angles in the particular oligosaccharide simultaneously. Branches were minimised separately. Subsequently, these residues were fixed, in their minimum energy conformations, to the rest of the molecule which was then minimised.

3. RESULTS AND DISCUSSION

Figure 3 shows that in the steric maps for the type 1, galβ(1-3) glcNAc and type 2, galβ(1-4) glcNAc disaccharides, the allowed region in the (ϕ, ψ) plane is restricted to 4% or less of the total area, in general agreement with the earlier results for disaccharides. The figure also shows that the addition of an L-fucose residue to obtain H(α(1-2) linkage to gal) and Le^a (α(1-3 or 4) linkage to glcNAc) trisaccharides further restricts the allowed conformations of the type 1 and type 2 disaccharides but does not push them to totally new conformations. Thus one of the roles of the L-fucose residue seems to be to limit the flexibility of the 'core' disaccharide fragments.

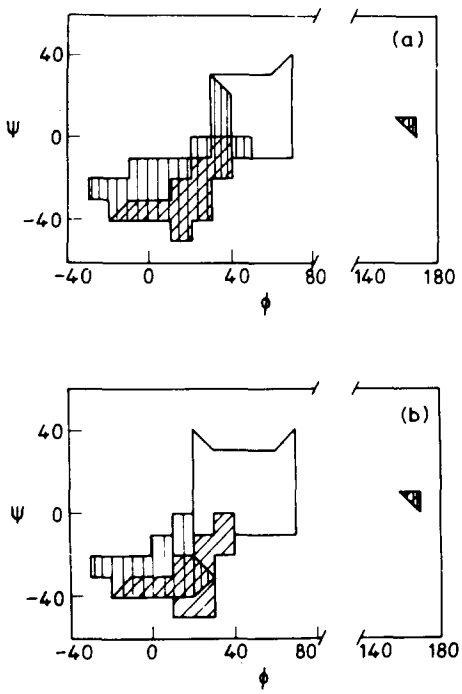

Fig. 3. Steric maps for (a) galβ (1-3) glcNAc and (b) galβ (1-4) glcNAc. Shaded area is disallowed due to the addition of an L-fuc residue. ▥, α(1-2) L-fuc to gal; ▨, α(1-4) L-fuc in (a) and α(1-3) L-fuc in (b) to glcNAc. Abbreviations as in Fig. 1.

TABLE 3
Minimised Conformations of the H Tetrasaccharides

Set number	*Angles (degrees)*							*Relative energy, kcal mol*$^{-1}$
	ϕ_A	ψ_A	ϕ_1	ψ_1	ϕ_2	ψ_2	χ_2	
(a) Type 1								
1	40	24	59	9	54	15	–	0·00
2	41	25	60	6	155	14	–	3·36
3	−24	−23	60	13	53	11	–	4·62
(b) Type 2								
1	39	24	57	−1	51	−65	−59	0·00
2	40	24	57	−1	56	−50	−174	0·57
3	40	24	57	−1	58	−48	68	1·85
4	−16	−27	50	5	52	−64	−59	1·88
5	40	24	57	−2	147	−65	−62	2·04
6	40	24	57	−1	150	−78	−174	2·17
7	−17	−27	50	4	56	−50	−174	2·45
8	40	24	57	−1	150	−75	69	3·32
9	−17	−27	50	4	58	−48	68	3·70
10	−17	−27	49	7	145	−72	−58	4·00
11	−16	−27	51	5	150	−78	−174	4·09

Table 3 shows that very few conformations are possible for the type 1 and type 2 H tetrasaccharides. It is interesting to note that the type 1 structure can favour only one conformation. The next nearest conformation has at least 3 kcal mol^{-1} higher energy and thus the possibility of this fragment occurring in this higher energy conformation is negligibly small. However, due to greater flexibility in the β(1-6) linkage defined by (ϕ_2, ψ_2, χ_2) the type 2 H tetrasaccharide is less rigid than the type 1 H tetrasaccharide. Projections of the minimum energy conformations for the H tetrasaccharides are shown in Fig. 4. Both the trisaccharide moieties, fucα(1-2) galβ(1-3) glcNAc and fucα(1-2) galβ(1-4) glcNAc, assume similar conformations suggesting that the difference in the linkage does not affect the overall shape of these fragments. The main difference between the preferred conformations in the two trisaccharide fragments lies in the orientation of the side groups on the glcNAc residue; the acetamido group and the hydroxymethyl group interchange their positions. On the other hand, if the tetrasaccharide fragment as a whole is considered there is a significant difference in the overall shape of the two fragments due to the different orientation of the gal residue at the reducing end (Fig. 4).

The conformations that fall within 5 kcal mol^{-1} of the minimum for the type 1 and type 2 Le^a structures are shown in Table 4. Projections of the minimum energy conformations are shown in Fig. 5. The conformations possible for the 'core' structures, defined by the dihedral angles (ϕ_1, ψ_1), (ϕ_2, ψ_2) and χ_2 in the type 2 structure,

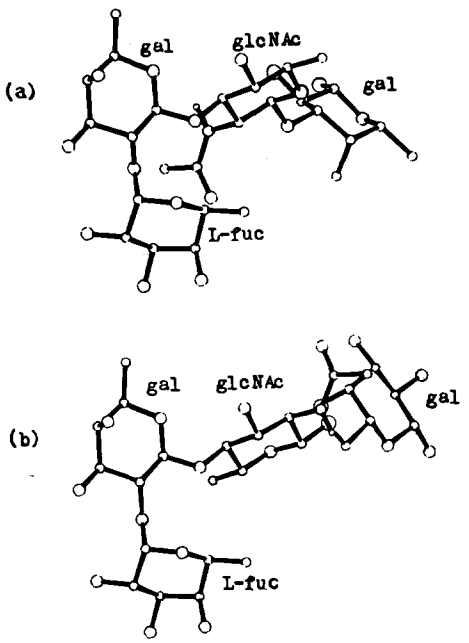

Fig. 4. Projections of the H tetrasaccharides (a) type 1 and (b) type 2 in minimum energy conformations. For simplicity hydrogen atoms are not shown. Abbreviations as in Fig. 1.

are similar in both H and Le^a substances (cf. Figs 4 and 5). Thus the addition of an L-fuc residue to gal to form the H structure or to glcNAc to form the Le^a structures does not alter the preferred backbone conformations for these molecules. This conforms with the observations made using contact criteria (Fig. 3). The differences in the linkage between the type 1 and type 2 structures in Le^a do not affect the overall shape of the terminal trisaccharide fragments but, as in the case of the H structures, these differences become significant if the tetrasaccharide fragment as a whole is considered. Even in the case of the Le^b oligosaccharides in which two L-fuc residues are attached to the 'core' structure, the preferred conformations for the backbone are similar to those which are preferred in the H and Le^a structures (Table 5). The projections of the Le^b structures in the minimum energy conformations (Fig. 5) also show that the overall shape of the terminal tetrasaccharide fragments are unaffected by the change from a $\beta(1\text{-}3)$ to a $\beta(1\text{-}4)$ link in the 'core' disaccharide. When the structure as a whole (up to the common gal residue) is considered there is a significant difference in the overall shape of the type 1 and 2 oligosaccharides.

A similar trend is also observed for the A and B pentasaccharides (Tables 6 and 7; Fig. 6). The preferred conformation of the 'core' structure is unaffected by addition of galNAc (A structure) or gal (B structure) to the H structures. Also, differences in

TABLE 4
Minimised Conformations of the Le^a Tetrasaccharides

Set number	*Angles (degrees)*							*Relative energy, kcal mol^{-1}*
	ϕ_1	ψ_1	(ϕ_B	ψ_B)	ϕ_2^*	ψ_2^*	χ_2^*	
(a) Type 1								
1	59	15	32	22	55	15	–	0·00
2	64	11	−20	−29	55	15	–	0·33
3	58	14	33	22	155	15	–	3·71
4	64	11	−21	−29	155	15	–	3·97
(b) Type 2								
1	59	11	−22	−26	51	−65	−60	0·00
2	56	12	−22	−27	56	−50	180	0·67
3	52	18	39	22	51	−65	−60	1·65
4	60	11	−23	−27	58	−48	68	1·69
5	52	19	41	21	56	−50	180	2·06
6	57	−3	−22	−26	150	−65	−60	2·13
7	51	−1	−24	−26	150	−78	180	2·27
8	54	20	40	21	58	−48	68	3·12
9	58	−2	−24	−26	150	−75	68	3·43
10	51	17	41	22	150	−65	−60	3·65
11	50	17	40	22	150	−78	180	3·74
12	51	4	41	21	150	−75	68	4·89

* The dihedral angles ϕ_2, ψ_2 and χ_2 were fixed in values shown.

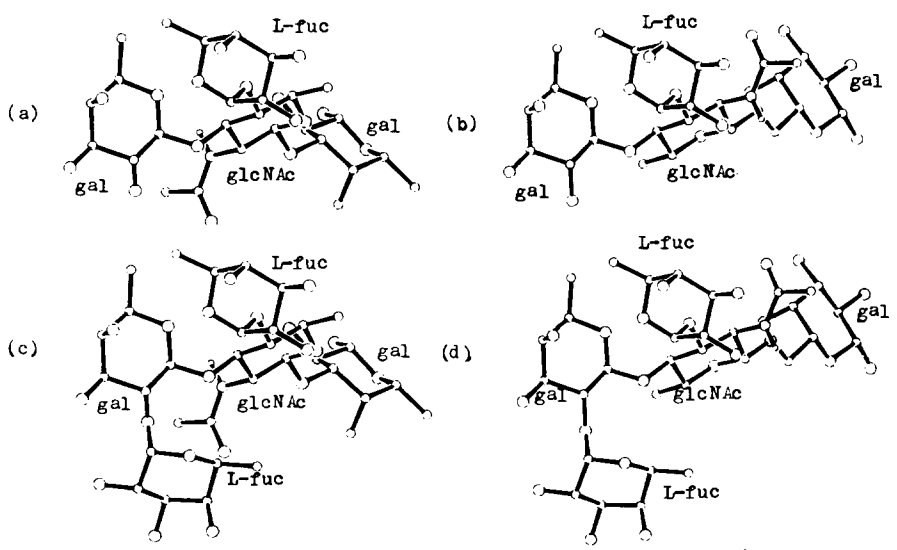

Fig. 5. Projections of the Le^a tetrasaccharides (a) type 1, (b) type 2; and the Le^b pentasaccharides (c) type 1, (d) type 2 in minimum energy of conformations. For simplicity hydrogen atoms are not shown. Abbreviations as in Fig. 1.

TABLE 5
Minimised Conformations of the Le^b Pentasaccharides

Set number	*Angles (degrees)*									*Relative energy, kcal mol^{-1}*
	ϕ_A	ψ_A	ϕ_1	ψ_1	(ϕ_B	ψ_B)	ϕ_2^*	ψ_2^*	χ_2^*	
(a) Type 1										
1	35	24	60	12	−22	−30	55	15	–	0·00
2	36	25	67	16	33	21	55	15	–	1·41
3	35	24	59	12	−22	−29	155	15	–	3·69
(b) Type 2										
1	40	23	61	11	−23	−26	51	−65	−60	0·00
2	40	23	61	11	−23	−27	56	−50	180	0·64
3	40	25	53	18	41	22	51	−65	−60	1·47
4	40	23	60	11	−23	−27	58	−48	68	1·65
5	40	25	52	19	41	21	56	−50	180	2·14
6	−17	−27	54	12	−23	−26	51	−65	−60	2·17
7	40	24	57	−2	−23	−26	150	−65	−60	2·24
8	40	25	57	−1	−24	−26	150	−78	180	2·30
9	−17	−27	56	11	−23	−26	56	−50	180	2·80
10	40	24	53	19	42	22	58	−48	68	3·05
11	40	24	57	−1	−24	−27	150	−75	68	3·43
12	−15	−28	50	19	42	22	51	−65	−60	3·47
13	40	24	51	18	41	21	150	−65	−60	3·72
14	−13	−29	54	12	−24	−26	58	−48	68	3·78
15	40	23	50	17	40	23	150	−78	180	3·82
16	−15	−29	51	18	41	22	56	−50	180	3·90
17	−17	−27	49	7	−25	−26	150	−65	−60	4·21
18	−16	−27	51	5	−23	−26	150	−78	180	4·34
19	40	24	51	4	41	21	150	−75	68	4·93

* The dihedral angles ϕ_2, ψ_2 and χ_2 were fixed in the values shown.

the overall shape of the type 1 and type 2 structures becomes important only when the residues beyond the glcNAc residue are considered.

3.1. *Glycosyl Transferase Specificity*

Most of the glycosyl transferases responsible for the sequential addition of L-fuc, galNAc or gal residues to the 'core' structures to form the H, Le^a, Le^b, A and B antigens use both type 1 and type 2 chains as acceptor molecules (Hakomori & Kobata, 1974).

The H structures are the acceptors for the α(1-3) galactosaminyl transferase and for the α(1-3) galactosyl transferase which add galNAc or gal to give the A or B structures. It should be noted (Fig. 4) that the environment around the C3 hydroxyl group of the gal to which the additional residue is to be added is similar in both type 1 and type 2 structures. Hence, the fact that experimental studies show that these enzymes

TABLE 6
Minimised Conformations of the A Pentasaccharides

Set number	*Angles (degrees)*									*Relative energy, kcal mol*$^{-1}$
	ϕ_C	ψ_C	(ϕ^*_A	ψ^*_A)	ϕ_1	ψ_1	ϕ_2	ψ_2	χ_2	
(a) Type 1										
1	−65	−57	40	25	59	9	54	15	–	0·00
2	−65	−58	40	25	60	5	155	13	–	3·43
(b) Type 2										
1	−64	−57	40	25	57	−2	51	−64	−59	0·00
2	−65	−56	40	25	57	−1	56	−51	−175	0·62
3	−65	−56	40	25	57	−1	57	−50	67	1·91
4	−66	−56	40	25	57	−2	148	−65	−61	2·14
5	−64	−57	40	25	57	−1	150	−79	−173	2·23
6	−65	−56	40	25	57	−1	149	−73	69	3·39

* The dihedral angles (ϕ_A, ψ_A) were fixed in the values shown.

TABLE 7
Minimised Conformations of the B Pentasaccharides

Set number	*Angle (degrees)*									*Relative energy, kcal mol*$^{-1}$
	ϕ_C	ψ_C	(ϕ^*_A	ψ^*_A)	ϕ_1	ψ_1	ϕ_2	ψ_2	χ_2	
(a) Type 1										
1	−65	−57	40	25	59	9	54	15	–	0·00
2	28	8	40	25	59	10	54	15	–	0·45
3	−65	−58	40	25	59	5	155	14	–	3·39
4	29	7	40	25	60	6	156	14	–	3·82
(b) Type 2										
1	−65	−56	40	25	57	−1	52	−64	−60	0·00
2	29	8	40	25	57	−1	51	−64	−61	0·43
3	−65	−57	40	25	56	−1	57	−49	−176	0·67
4	29	9	40	25	57	−2	57	−50	−176	1·19
5	−64	−56	40	25	57	−1	56	−51	68	1·90
6	−66	−56	40	25	58	−1	149	−64	−61	2·12
7	−65	−55	40	25	57	−2	149	−78	−174	2·21
8	29	7	40	25	56	−1	58	−52	67	2·37
9	28	8	40	25	57	−1	148	−63	−60	2·54
10	28	7	40	25	57	−1	150	−79	−174	2·59
11	−65	−54	40	25	57	−1	149	−72	68	3·35
12	29	10	40	25	57	−2	148	−71	69	3·86

* The dihedral angles (ϕ_A, ψ_A) were fixed in the values shown.

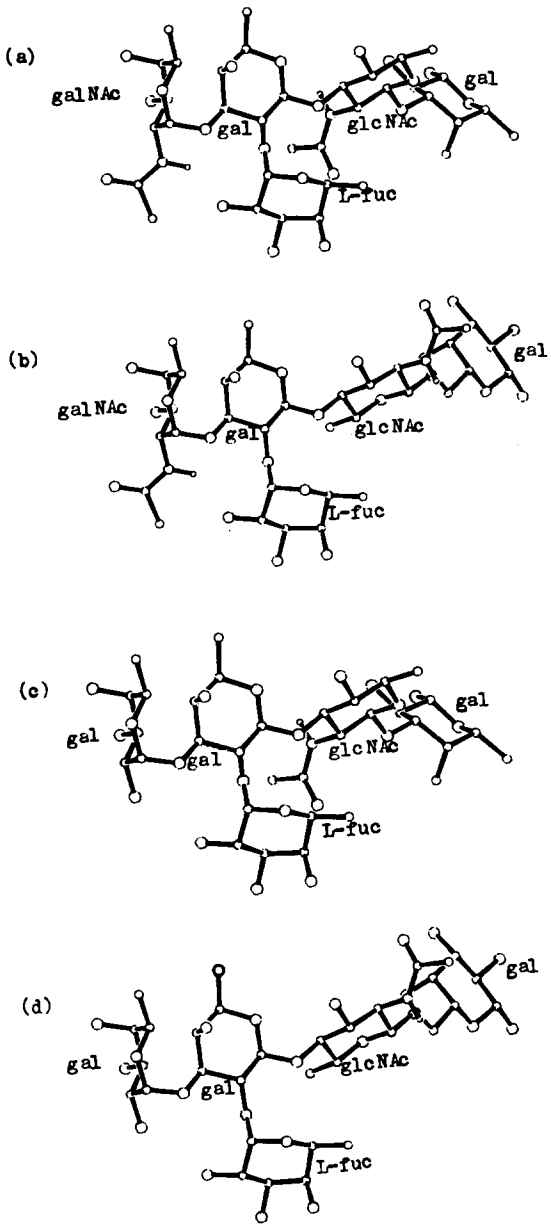

Fig. 6. Projections of the A pentasaccharides (a) type 1, (b) type 2; and the B pentasaccharides (c) type 1, (d) type 2 in minimum energy conformations. For simplicity hydrogen atoms are not shown. Abbreviations as in Fig. 1.

cannot distinguish between type 1 and type 2 structures (Hakomari & Kobata, 1974) suggests that the binding site of these two transferases is small.

The acceptors for the $\alpha(1\text{-}2)$ fucosyl transferase are the core structures gal$\beta(1\text{-}3)$ glcNAc$\beta(1\text{-}3)$ gal and gal$\beta(1\text{-}4)$ glcNAc$\beta(1\text{-}6)$ gal. This enzyme, therefore, can use both type 1 and type 2 precursors as acceptor molecules. As pointed out earlier, the overall shape of the two 'core' structures is different if the trisaccharide fragment is considered. Hence if the binding site of the $\alpha(1\text{-}2)$ fucosyl transferase is large, the same enzyme could not recognise both types of structures. However, as was previously stated, the overall shape of the type 1 and type 2 disaccharide fragments, gal$\beta(1\text{-}3)$ glcNAc and gal$\beta(1\text{-}4)$ glcNAc, is the same. There is, however, a slight difference in the environment close to the C2-hydroxyl group of the gal residue to which the L-fuc residue is to be added. In type 1 structures the acetamido group is close to the C2-hydroxyl whereas in type 2 structures this group is replaced by a hydroxymethyl group. Since these two side groups have nearly the same spatial requirements, this difference may not seriously affect the fitting of these fragments in the active site of the enzyme. Small adjustments can be made by making minor changes about the inter-unit glycosidic bonds. This suggests that if the binding site of the $\alpha(1\text{-}2)$ fucosyl transferase is small the same enzyme could add the L-fuc residue to the gal in both type 1 and type 2 structures. However, the differences in the environment (the interchange of hydroxymethyl and acetamido group) that occur on one side of the C2 hydroxyl group and which may provide a site of attachment via the hydrogen bond to the enzyme may slightly alter the efficiency of the transferase.

The core structures, gal$\beta(1\text{-}3)$ glcNAc$\beta(1\text{-}3)$ gal and gal$\beta(1\text{-}4)$ glcNAc$\beta(1\text{-}6)$ gal, are also the acceptors for the fucosyl transferases which add L-fuc to the glcNAc residue to give Le^a structures. For the type 1 structure L-fuc is attached to the C4-hydroxyl of glcNAc whereas in the type 2 structure L-fuc is attached to the C3-hydroxyl of glcNAc. It can be seen (Fig. 5) that the environment around these hydroxyl groups is quite different in the two types of structure. In type 1 structures the L-fuc is attached to the hydroxyl group next to the hydroxymethyl side group while in the type 2 structure the hydroxyl group to which L-fuc is attached is next to the acetamido side group. These changes in the acceptor molecules in the immediate neighbourhood of the site of attachment of L-fuc may affect to some extent the binding requirements of the fucosyl transferases involved. Further, unlike in the previous case, steric problems cannot easily be relieved by making rotations around the single bonds since the bonds involved (C4—C5 bond or C2—C3 bond) are part of the glcNAc ring and any rotations around these bonds to relieve bad contacts may be energetically unfavourable. Thus, even if the binding site is small, the same fucosyl transferase may not be able to convert the type 1 and 2 precursors into Le^a structures with comparable efficiency. This may explain the experimental observation that the $\alpha(1\text{-}4)$ fucosyl transferase and the $\alpha(1\text{-}3)$ fucosyl transferase are specific for type 1 and type 2 chains, respectively (Hakomari & Kobata, 1974).

3.2. *Antibody and Lectin Specificities*

Tables 1 and 2 show that in the H and Lewis systems there are many antibodies and lectins that cannot distinguish between type 1 and type 2 structures. As mentioned earlier the differences in the linkage between type 1 and type 2 structures does not significantly affect the overall shape of the terminal ends of the chains if a small fragment is considered. Thus those reagents which cannot distinguish the two types of structures may have small binding sites which can accommodate at the most the terminal residues up to the glcNAc residue.

Since differences in the overall shape of these determinants is significant only when a larger fragment is considered, it would appear that those reagents which can distinguish type 1 and type 2 structures have larger binding sites. In fact, for the lectin of *Lotus tetragonolobus* (Table 2) which is specific for type 2 structures there is some evidence that the $\beta(1\text{-}6)$ link is important for activity (Miercio *et al.*, 1974).

The lectin of *Lotus tetragonolobus* is interesting since it binds to the type 2 structures of H, Le^a and Le^b substances (Miercio *et al.*, 1974). The best inhibitor of the binding activity of this lectin is a Le^b type 2 pentasaccharide. The Le^a and H oligosaccharide fragments are somewhat less active as inhibitors compared to Le^b. It can be seen from Fig. 7, that the removal of the L-fuc residue attached to the gal residue through an $\alpha(1\text{-}2)$ link exposes the Le^a structure and removal of the L-fuc residue attached to the glcNAc residue through an $\alpha(1\text{-}3)$ link exposes the H structure. As previously mentioned, the removal of either of these L-fuc residues does not affect the preferred conformations of the remaining oligosaccharide fragment. Thus, if this lectin has a large binding site which can accommodate, at least, five residues, then removal of either of the two L-fuc residues will only reduce by one the number of residues available for binding (Fig. 7). Since in such a case, the overall shape of the remaining fragment is unaltered the sugar residues which are left can occupy their respective positions in the binding site. The lack of one L-fuc residue may slightly reduce the binding properties of these monofucosyl fragments. This may also explain the lower inhibitory properties of the H and Le^a oligosaccharide fragments compared to Le^b (Miercio *et al.*, 1974).

Although the Le^a antigen is normally considered to be of type 1 only, the goat and human anti-Le^a antibodies also recognise type 2 structures but these are fourfold less active (Rovis *et al.*, 1973). The fact that this antibody binds with both type 1 and type 2 Le^a structures suggests that it has a small binding site. However, there are some differences in the binding properties of the type 1 and type 2 structures. Although the overall shape of the trisaccharides, gal $\beta(1\text{-}3)$ glcNAc and gal $\beta(1\text{-}4)$ glcNAc,

$$\begin{array}{ll} & 4 \qquad\qquad 3 \\ \text{L-fuc } \alpha\, 1 & \qquad\qquad \text{L-fuc } \alpha\, 1 \end{array}$$

are similar, there are significant local differences in the neighbourhood of the L-fuc residue which are important for binding (Fig. 5). In the type 1 Le^a structure the neighbouring group on the glcNAc residue is a hydroxymethyl group while in the type

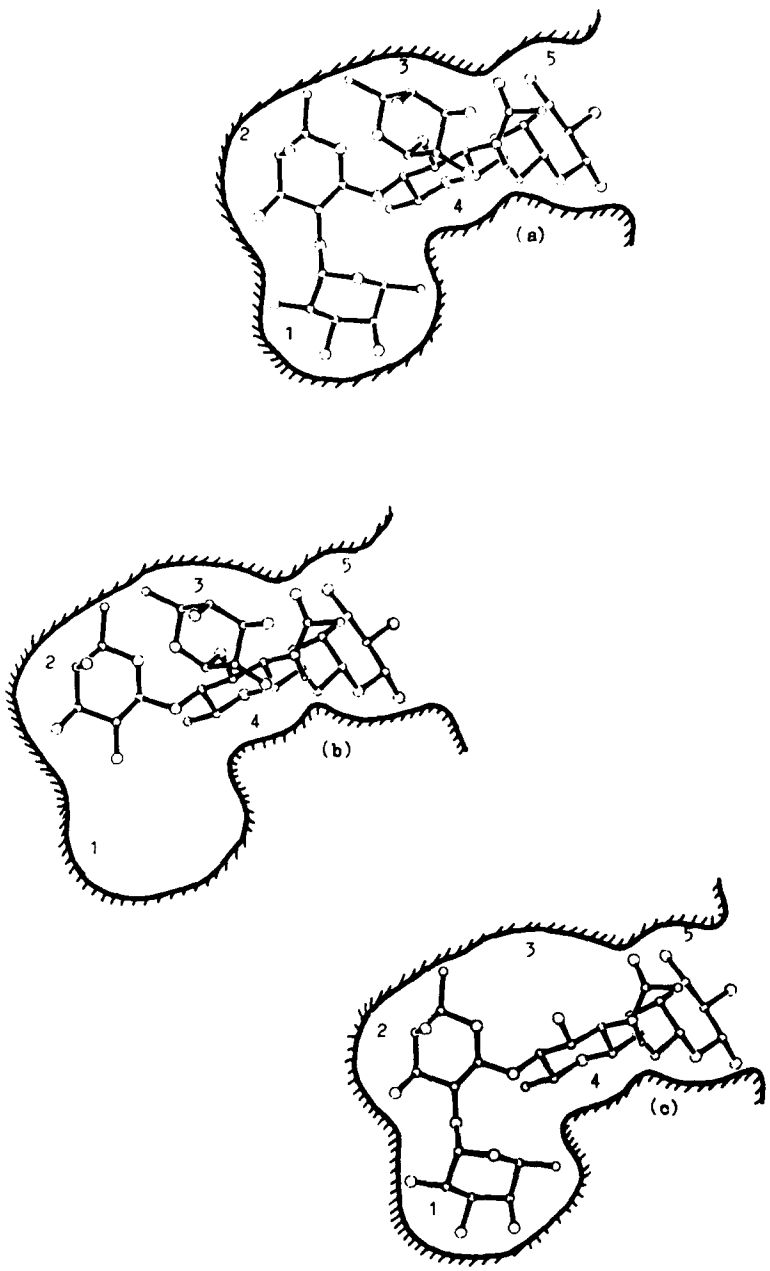

Fig. 7. Schematic representation of (a) Le^b; (b) Le^a and (c) H type 2 oligosaccharides in the lectin binding site of *Lotus tetragonolobus*.

2 Le^a structures this is replaced by the acetamido group. Though, as mentioned earlier, the spatial requirements for these two groups may be about the same, they may also provide a site of attachment with the antibody by forming hydrogen bonds. Since the strength of such a hydrogen bond depends on the direction, length and the nature of the chemical group, differences in the nature of the side group adjacent to the L-fuc residue may affect the strength of the hydrogen bond which can be formed. This may explain the observed differences in the binding properties for type 1 and type 2 Le^a structures.

3.3. *A1 and A2 Antigens*

The molecular basis for the existence of the two A serotypes, A1 and A2 is still not clear although many possibilities have been suggested. The possible variation in the number of L-fuc residues present on A1 and A2 antigens (for which there is no experimental evidence) and the possible difference between type 1 and type 2 structures have been considered as a basis for the difference between the A1 and A2 antigens. Kabat (1970) suggested that A1 had a complete A structure on both type 1 and type 2 chains, while A2 had a complete A structure on type 1 but not on type 2 chains. The type 2 chains in the case of A2 would remain as H. This seems to be the most attractive possibility at present. All the reagents which bind to the type 2 H structures also bind to A2 but not A1 antigens (Table 2) suggesting the presence of an H type 2 structure on A2 antigens.

The lectins from lima bean (Bessler & Goldstein, 1974; Hammarstrom *et al.*, 1977), *Helix pomatia* (Hammarstrom *et al.*, 1977) and *Dilochus biflorus* (Etzler & Kabat, 1970) bind to both A1 and A2 antigens (Table 2) although for A2 higher concentrations of the antigen are required for precipitation. Both the lectins from lima bean and *Helix pomatia* are inhibited by methyl-α-D-galNAc suggesting that this residue is crucial for binding. The precipitation reaction of the lectin of *Dilochus biflorus* is inhibited best by a larger oligosaccharide fragment. If Kabat's suggestion is correct and since the galNAc residue is important for binding it is expected that the A1 antigen should be precipitated at lower concentrations compared to A2 antigens since the former would contain more galNAc than the latter.

The lectin of *Banderaea simplicafolia* (Hayes & Goldstein, 1974; Murphy & Goldstein, 1977) is highly specific for A1 structures (Table 2) as is the human anti-A1 antibody (Moreno *et al.*, 1971). The existence of reagents which are A specific and which can distinguish A1 and A2 antigens suggests that these reagents are sensitive to changes at the terminal residues of both the chains. If the antibody or lectin has a large binding site which includes both the type 1 and type 2 chains the presence or absence of one residue at the end of the type 2 chain of the antigen should not make much difference to the binding properties of the antibody or lectin (cf. the lectin of *Lotus tetragonolobus*). On the other hand, if these reagents possess two small pockets each accommodating only the terminal galNAc residues, then any changes at the ends may affect their binding properties drastically (Fig. 8). Since the L-fuc residue is known to

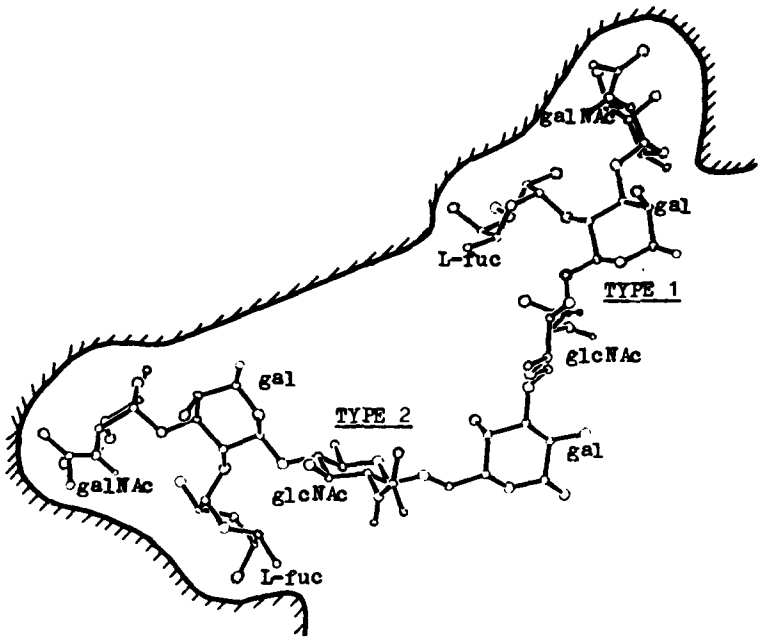

Fig. 8. Projection of the A1 antigen in minimum energy conformation. Schematic representation of the binding site of A1-specific reagents.

be important for A activity the pocket may be large enough to accommodate both the galNAc and the L-fuc residues.

The human anti-A2 antibody (Moreno *et al.*, 1971) is specific for A2 antigens. If this antibody were to bind to the single A type 1 chain then it should also bind to the A1 antigen since the A type 1 chain is present in both antigens. On the other hand, if the antibody is specific for the incomplete type 2 chain (H type 2 structure) then it would be unable to distinguish between H and A2 structures. This contrasts with the observed binding properties of this antigen (Table 1). This suggests that this anti-A2 antibody also may have two pockets in the binding site which can accommodate the galNAc residue, from the type 1 chain and the gal and/or L-fuc residue(s) from the type 2 chain and their nearby environments.

4. CONCLUSIONS

The present studies suggest the following:

1. The conformation of the core structure is not altered significantly by the addition of L-fuc, gal or galNAc residues at the non-reducing end. These residues may

restrict the possible conformations of the 'core' fragments but will not push them to totally new conformations.

2. The differences between type 1 and type 2 structures becomes significant if the binding site of the antibody, lectin or enzyme is large and the distinction between small and large binding sites can be made depending upon whether the binding site accommodates sugar residues of the determinants up to the glcNAc residue or beyond. Thus the non-specific reagents may have small binding sites and the ones that are specific for type 1 or type 2 structures may have a large binding site or be sensitive to local changes.
3. In agreement with experimental data a model with two pockets in the binding site has been proposed for the lectin from *Banderaea simplicafolia* and for the anti-A1 and anti-A2 antibodies.

REFERENCES

Arnott, S. & Scott, W. E. (1972). *J. Chem. Soc., Perkins Trans.* **2**, 324.
Bessler, W. & Goldstein, I. J. (1974). *Arch. Biochem. Biophys.* **165**, 444.
Corey, R. B. & Pauling, L. (1953). *Proc. R. Soc. (London)* **B141**, 10.
Davidson, W. C. (1959). AEC Research and Development Report, ANL-5990.
Etzler, M. E. & Kabat, E. A. (1970). *Biochem.* **8**, 869.
Fletcher, R. & Powell, M. J. D. (1963). *Comput. J.* **6**, 163.
Hakomori, S. & Kobata, A. (1974). In *The antigens*, ed. M. Sela, Vol. II, New York, Academic Press, p. 79.
Hammarstrom, S., Murphy, L. A., Goldstein, I. J. & Etzler, M. A. (1977). *Biochem.* **16**, 2750.
Hayes, L. E. & Goldstein, I. J. (1974). *J. Biol. Chem.* **249**, 1904.
Kabat, E. A. (1970). In *Blood and tissue antigens*, ed. D. Aminoff, New York, Academic Press, p. 190.
Kabat, E. A. (1976). In *Human Blood Groups, Fifth International Convocation on Immunology*, Buffalo, New York, Karger, Basel, p. 236.
Kabat, E. A. (1978). *J. Supramol. Structure* **8**, 79.
Lemieux, R. U., Bock, K., Delbaere, L. T. J., Koto, S. & Rao, V. S. (1980). *Can. J. Chem.* **58**, 631.
Lloyd, K. O. & Kabat, E. A. (1968). *Proc. Natl. Acad. Sci. USA* **61**, 1470.
Marcus, D. M. & Cass, L. E. (1967). *J. Immunol.* **99**, 987.
Miercio, E. A., Pereira, M. E. & Kabat, E. A. (1974). *Biochem.* **13**, 3184.
Momany, F. A., McGuire, R. F., Burgess, A. W. & Scheraga, H. A. (1975). *J. Phys. Chem.* **79**, 2361.
Moreno, C., Lundblad, A. & Kabat, E. A. (1971) *J. Exp. Med.* **134**, 439.
Murphy, L. A. & Goldstein, I. J. (1977). *J. Biol. Chem.* **252**, 4739.
Petryniak, J., Pereira, M. E. & Kabat, E. A. (1977). *Arch. Biochem. Biophys.* **178**, 118.
Ramachandran, G. N. & Sasisekharan, V. (1968). *Adv. Protein Chem.* **23**, 283.
Rovis, L., Kabat, E. A., Pereira, M. E. A. & Feize, T. (1973). *Biochem.* **12**, 5355.
Stoddart, J. F. (1971). In: *Stereochemistry of Carbohydrates*, New York, John Wiley & Sons, Inc., p. 50.
Thogersen, H., Lemieux, R. U., Bock, K. & Meyer, B. (1982). *Can. J. Chem.* **60**, 44.
Yathindra, N. & Rao, V. S. R. (1970). *Biopolymers* **9**, 783.
Yathindra, N. & Rao, V. S. R. (1972). *Carbohydr. Res.* **25**, 256.