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Conformational analysis of the amino termini (5 residues) of human glycophorin A_M and A_N: differentiation of the structural features of the T_N and T antigenic determinants in relation to their specificity

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ABSTRACT

The N-terminus of glycophorin A, the main transmembrane erythrocyte glycoprotein responsible for the MN blood-group specificity, has been modelled. As the minimum size of the protein recognised by the antiglycophorin A antibodies is the N-terminal glycopentapeptide, attention was focused on the T_N and T antigenic determinants of this size in order to determine wether differences in 3D structure exist and how a specific response with different antibodies is induced.

INTRODUCTION

Glycophorin A (GPA) is the major transmembrane sialoglycoprotein found in human erythrocyte membrane. The external part of the protein (70 residues) is highly glycosylated (16 oligosaccharide residues). GPA has various functions in the red cell membrane, including the display of receptor sites on the cell surface in which the carbohydrate moiety appears to be important. GPA also displays the MN (second order) blood-group determinants[1], the expression of which has been associated with the carbohydrate-containing N-terminus[2–4]. Variations in the amino acid residues at positions 1 and 5 in GPA result in glycophorins A_M (Ser/Gly) (H_2N–^{1}Ser–Ser–Thr–Thr–^{5}Gly) and A_N (Leu/Glu) (H_2N–^{1}Leu–Ser–Thr–Thr–^{5}Glu), respectively.

The T and T_N antigens are present also in GPA as a result of the removal of some of the carbohydrate residues, and these antigens have been found on the surface of human carcinoma cells[5,6]. The carbohydrate structures of the T_N and T antigenic

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determinants, namely, α-D-Gal*p*NAc→Ser (**1**) and α-D-Gal*p*NAc→Thr (**2**) are associated with the display of the T_N blood-group determinant.

Several structural studies have been performed on sialo derivatives[7–9] and these molecules have been modelled in order to assess the effect on the geometry when sialic acid is added.

β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc→Ser (**3**) and β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc→Thr (**4**) are both associated with the so-called T blood-group specificity. Such units are found at positions 2–4 of the N-terminus of glycophorin A_M and A_N. Structures **3** and **4** have been found in other glycoproteins and termed "core glycopeptides".

The results now presented are an extension of previous work on synthesis and n.m.r. studies[10,11] with the aim of gaining a better insight into the conformational properties of the N-terminus (5 residues) of glycophorins A_M and A_N, and possibly to evaluate the contribution of carbohydrate moieties to the conformation of the peptide chain.

Knowledge of the role of the sugar on the secondary structure of the peptide chain and of the differences in structure of the T and T_N antigenic determinants may provide a better understanding of their specificities.

CALCULATIONS

The glycophorin A fragments studied were Ser–Ser*–Thr*–Thr*–Gly–MM and Leu–Ser*–Thr*–Thr*–Glu–NN.

The molecular building and conformational analysis were performed by using the GENMOL program[12–17]. The results obtained were compared to other experimental data, especially those arising from the n.m.r. analysis of the conformational preference of the glycosidic bond in these compounds[11]. Molecules were built in the following sequence: (*a*) the peptide chain, (*b*) addition of α-D-Gal*p*NAc or β-D-Gal-α-D-Gal*p*NAc, (*c*) conformational analysis and optimisation of the most stable conformation.

Calculations were performed on charged molecules corresponding to their form at pH 7. The proximity of the charged extremities led to deformation of the secondary structure, which was taken into consideration whenever these structures were extrapolated to the real geometry of the N-terminal part of GPA.

RESULTS AND DISCUSSION

The atomic co-ordinates of the fragments calculated can be obtained by request from the authors.

In order to simplify the text, the GPA fragments of the MM blood group are designated M_{NG} (for non-glycosylated), M_{TN} and M_T for the principal chain carrying the

* α-D-Gal*p*NAc for the T_N antigens, β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc for the T antigens, and NeuNAc-(2→6)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc in the sialo derivatives.

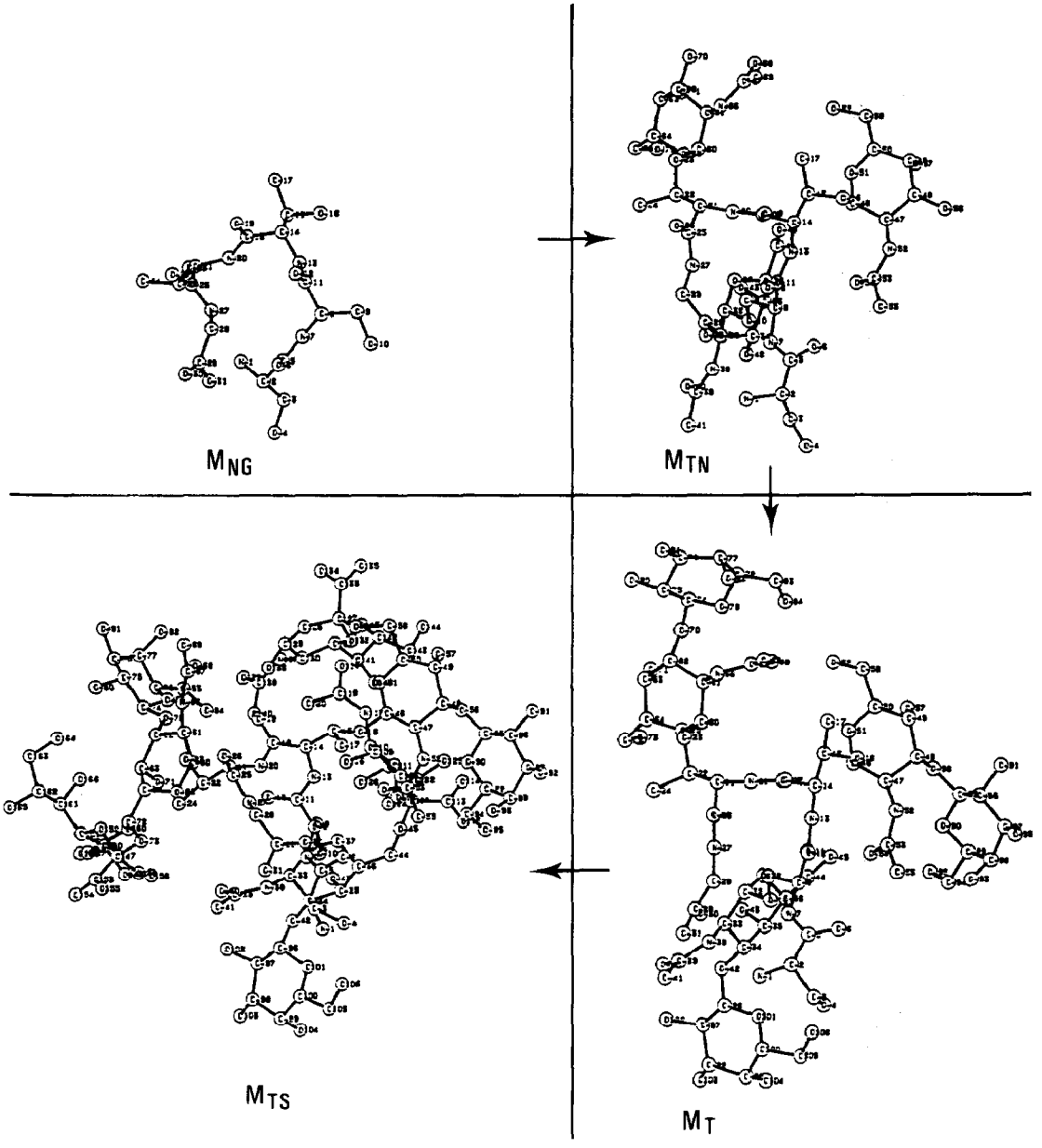


Fig. 1. Projections of M_{NG}, M_{TN}, M_T, and M_{TS} fragments derived from GPA of the MM blood group, which display the evolution of the geometry when carbohydrates are added.

TABLE I

Values (kcal.mol^{-1}) of the strain energy[a] of GPA fragments (blood group MM) (*cf.* Table VI)

	M_{NG}	M_{TN}	M_T	M_{TS}
E_{vdw}	26	72.8	119	206
E_c	1.7	19.5	78.4	220
E_H	−3.4	−18.7	−28.2	−34.7
E_{NBOND}	24.3	73.6	169.2	391.3
E_T	0.1	0.5	0.07	0.06
E_S	2.2	10.4	18.5	28.8
E_B	8.4	31.1	51.9	91.4
E_{BOND}	10.7	42	70.5	120.3
$E_{NBOND}+E_{BOND}$	35	115.6	239.7	511.6
NATOMS	31	73	106	166
$NATOMS^2/E_{TOT}$	27	45	47	54

[a] Contributions are designated as follows: E_{vdw}, van der Waals; E_c, coulombic; E_H, hydrogen bonds; E_T, torsion; E_s, stretching; E_B, bending; NATOMS, number of non-hydrogen atoms.

antigenic determinants T_N and T, respectively, and M_{TS} for the sialo derivative. For the NN blood group, the fragments are designated N_{NG}, N_{TN}, N_T, and N_{TS}, respectively.

GPA fragments of the MM blood group. — Projections in the same orientation (drawn by ORTEP[18]) of M_{NG}, M_{TN}, M_T, and M_{TS} are displayed in Fig. 1 and the corresponding strain energies are reported in Table I. The energy values are homogeneous and are proportional to the square of the number of atoms as indicated in the lower part of Table I.

Table II contains comparative values of the angles ψ (N–C–Cα–N) and φ (C–Cα–N–C) which describe the peptide secondary structure of the three molecules. The non-substituted peptide structure corresponds to a distorted α-helix. The values of ψ and φ (in the IUPAC–IUB convention[19]) are constant and close to the theoretical values[17], ψ −60° or 120° and φ −70° or −120° for an ideal α-helix. One exception is φ_5, which has a value of 149° compared to −70°. This deformation is due the proximity of the extremities. The α-helix structure is confirmed by the $C_{\alpha\text{-}1}$–$C_{\alpha\text{-}4}$ and $C_{\alpha\text{-}2}$–$C_{\alpha\text{-}5}$ distances (7.0 and 6.1 Å, respectively), which are close to the theoretical value of 5.2 Å.

In order to evaluate the deformation of the secondary structure of the peptide chain when sugars are added, the average variations of ψ and φ were computed. These values are 62°, 61°, and 92° for M_{TN}, M_T, and M_{TS}, respectively. The distances defined above between various Cα become 7.9 and 5.2 Å in M_{TN}, 7.7 and 5.5 Å in M_T, and 6.9 and 7.8 Å in M_{TS}.

The structure of the main chain is not sensitive to the addition of a second sugar molecule; the mean variation between M_{TN} and M_T is 17° for ψ and φ, whereas the deformation is larger in sialo derivatives, namely, 57° for M_T and M_{TS}.

The deformation can be observed in Fig. 2 where stereoscopic views of superimposed main chains of (M_{NG}, M_{TN}), (M_{NG}, M_T), (M_{NG}, M_{TS}), (M_{TN}, M_T), and (M_T,

TABLE II

The angles (ψ and φ) that describe the evolution of the secondary structure of the main chain when sugars are added to serine and threonine residues (*cf.* Table VII)

(*a*) Substitution of the peptide

	M_{NG}	M_{TN}	*Difference*	M_{NG}	M_T	*Difference*	M_{NG}	M_{TS}	*Difference*
ψ_1	−76	−72	4	−76	−41	35	−76	80	157
φ_2	−136	−39	97	−136	−30	106	−136	−89	47
ψ_2	−68	151	142	−68	151	141	−68	113	179
φ_3	−98	−15	83	−98	−24	74	−98	28	126
ψ_3	−47	−64	17	−47	−45	2	−47	50	97
φ_4	−63	−88	25	−63	−86	22	−63	130	67
ψ_4	−34	−52	18	−34	−71	37	−34	−56	22
φ_5	149	−100	111	149	−144	67	149	−173	38
Average			62			61			92

(*b*) Increase in the number of sugar residues[a]

	M_{TN}	M_T	*Difference*	M_T	M_{TS}	*Difference*
ψ_1	−72	−41	31	−41	80	121
φ_2	−39	−30	9	−30	−89	59
ψ_2	151	151	0	151	113	38
φ_3	−15	−24	9	−24	28	52
ψ_3	−64	−45	19	−45	50	95
φ_4	−88	−85	2	−85	−130	45
ψ_4	−52	−71	18	−71	−56	15
φ_5	−100	−144	44	−144	−173	29
Average			17			57

[a] The greatest distortions occur when sialic acid is added (*cf.* Table VII).

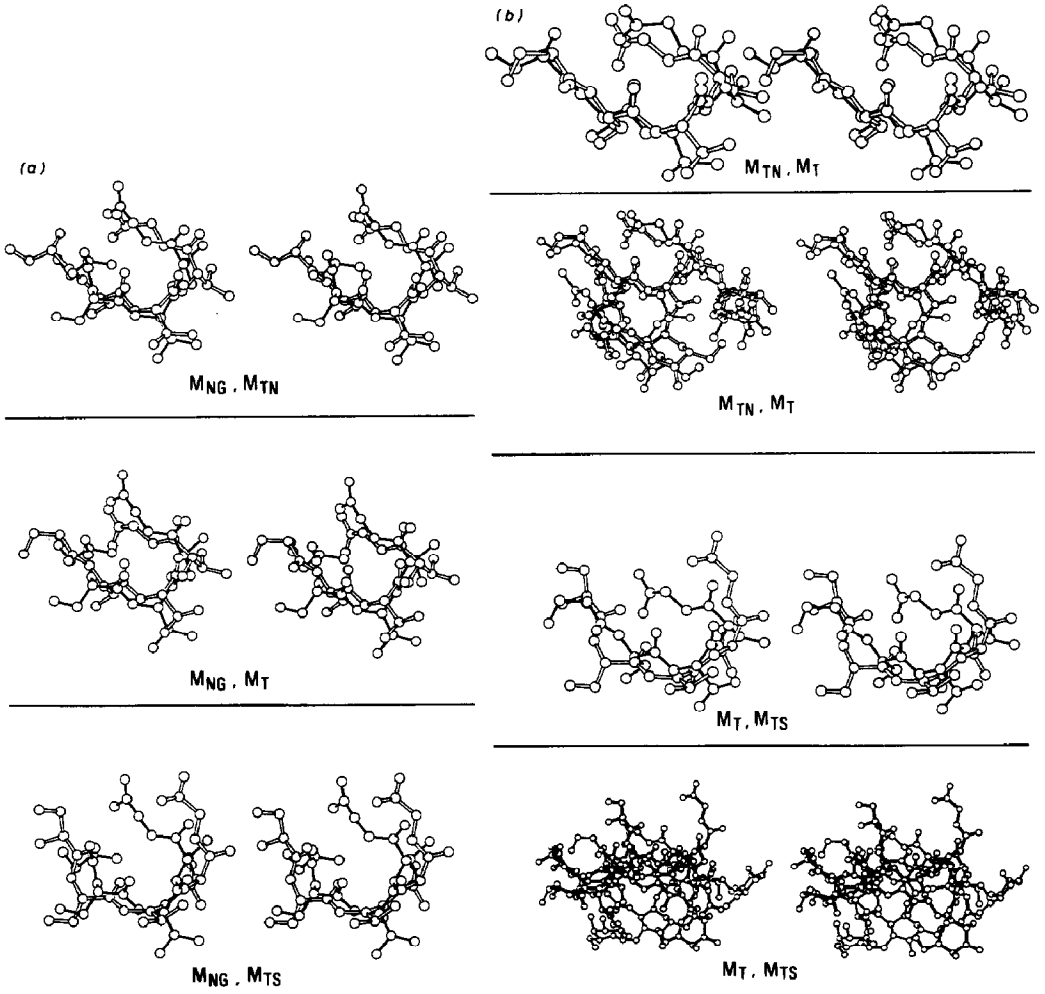


Fig. 2. Stereoscopic views of superimposed fragments belonging to GLA of the MM blood group. (*a*) The main chain of M_{NG} (filled line) superimposed on those of M_{TN}, M_T, and M_{TS} [*cf.* Table II(a)]; (*b*) the main chain and the common part of (M_{TN}, M_T), and (M_T, M_{TS}). Important deformations can be observed when sialic acid is introduced [*cf.* Table II(*b*)].

M_{TS}), are displayed, the best molecular fit being performed on the nitrogen atoms of the main chain. Views of the superimposed common part of (M_{TN}, M_T) and (M_T, M_{TS}) are given also.

The hydrogen bonds. — The hydrogen bond networks are reported in Table III. The network is strengthened when the molecular size increases as observed in Table I. Most of the hydrogen bonds are *intra-* or *inter-sugar* bonds. However, in the M_{TN} determinant, the distance between N-13 belonging to Thr^3 and the NAc atom O-54 is 3.6 Å. Since this atom is bonded to the hydroxyl group O-45 of the previous sugar (the distance O-45–O-54 is 3.1 Å), the hydrogen bond between O-54 and N-13 will be

TABLE III

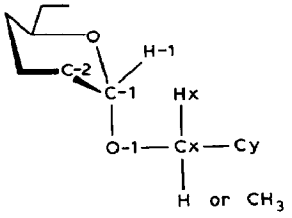
Hydrogen-bond networks (*cf.* Table VIII)

M_{NG}		M_{TN}		M_T				M_{TS}			
Atoms	D^a(Å)	*Atoms*	D(Å)	*Atoms*	D(Å)	*Atoms*	D(Å)	*Atoms*	D(Å)	*Atoms*	D(Å)
N-1 O-4	2.7	N-1 O-4	2.7	N-1 O-4	2.9	O-90 O-95	2.8	N-1 O-4	2.7	O-119 O-145	2.7
N-13 N-20	3.0	N-20 N-27	2.	N-1 N-7	3.1	O-92 O-93	2.7	N-20 N-27	3.	O-124 O-126	2.8
N-20 N-27	3.0	O-37 O-45	2.7	N-1 O-106	2.9	O-10 O-106	2.6	O-42 O-43	2.7	O-132 O-146	2.6
		O-40 O-42	2.4	N-13 N-20	3.1	O-103 O-104	2.7	O-56 O-57	2.5	O-132 O-84	2.6
		O-42 O-43	2.7	O-37 O-45	2.7	O-106 O-4	2.7	O-79 O-84	2.5	O-144 O-145	2.6
		O-51 O-59	2.7	O-51 O-59	3.			O-80 O-81	2.9	O-152 O-166	2.9
		N-52 O-56	3.0	O-56 O-57	2.7			O-81 O-82	2.7	O-164 O-80	2.7
		O-56 O-57	2.7	O-68 O-84	2.7			O-92 O-93	2.7	O-164 O-166	2.7
		O-68 O-70	2.5	O-70 O-71	2.6			O-101 O-106	2.7		
		O-70 O-71	2.7	O-71 O-73	2.9			O-103 O-104	2.7		
		O-71 O-73	2.9	O-79 O-84	2.8			O-112 O-126	2.9		
				O-81 O-82	2.7			O-115 O-95	2.7		

[a] Distance between the atoms.

strengthened when O-45 is removed. This result accords with the observation[21] of an analogous hydrogen bond in small peptides with a sugar attached to a Thr residue.

In the sialo derivative M_{TS}, hydrogen bonds are not observed between the nitrogen atoms of the main chain and the sialic acid, as suggested by others[7,8]. If distance strains are assigned in the calculation in order to obtain such hydrogen bonds, the total energy increases from 512 to 1452 kcal/mol and is not homogeneous with the other values. The value of $NATOMS^2/E_{TOT}$ becomes 19 (*cf.* 43 for the mean value).



5

The glycosidic bond. — Torsional angles around C-1–O-1 and O-1–Cx, designated χ for C-2–C-1–O-1Cx and φ for C-1–O-1–Cx-Cy (see **5**), are used to describe the conformations.

For M_{TN} and M_T, the values of χ and φ are reported in Table IV. Most of the χ values are close to the theoretical value 180°; since these bonds are soft, the values of χ

TABLE IV

The torsion parameters χ and φ around the glycosidic bond between serine or threonine and the sugar residue[a] (*cf.* Table IX)

Angle	χ_2	χ_3	χ_4	φ_2	φ_3	φ_4
M_{TN}	−168°	−168°	−166°	180°	113°	103°
M_T	−113°	−167°	−154°	175°	114°	123°
M_{TS}	−140°	−160°	−160°	160°	169°	111°

[a] The subscript numbers refer to the 2nd, 3rd, and 4th residues; χ relates to the *exo*-anomeric effect and φ to the decoupling of C-1 and CH_3-Thr.

TABLE V

Distances[a] between the hydrogen atoms of the glycosidic bond in the calculated structures of M_{TN} and M_T (*cf.* Table X)

Group	*Atoms*	*Antigens*	
		M_{TN}	M_T
Ser (2)	H-9B H-32	2.2 Å	2.2 Å
Thr (3)	H-15 H-46	2.2 Å	2.3 Å
Thr (4)	H-22 H-60	2.3 Å	2.0 Å

[a] A value of 2.1 Å was obtained by n.m.r. spectroscopy.

and φ given must be considered as averages taking into account the contribution of the *exo*-anomeric effect on the 3D structure. The values of φ for Thr-3 and Thr-4, which are close to 120°, are in good agreement with those obtained[10,11] from the chemical shifts of the resonances of C-1 and CH_3-Thr by n.m.r. spectroscopy. These values indicate the absence of warped interaction (C-1, Cγ) or (C-1, CH_3-Thr) and confirm the relative decoupling of C-1 and CH_3-Thr, in contrast to similar β compounds[11,22].

The distances between hydrogen atoms H-β(Thr) and H-1 are reported in Table V; the mean value is 2.2 Å.

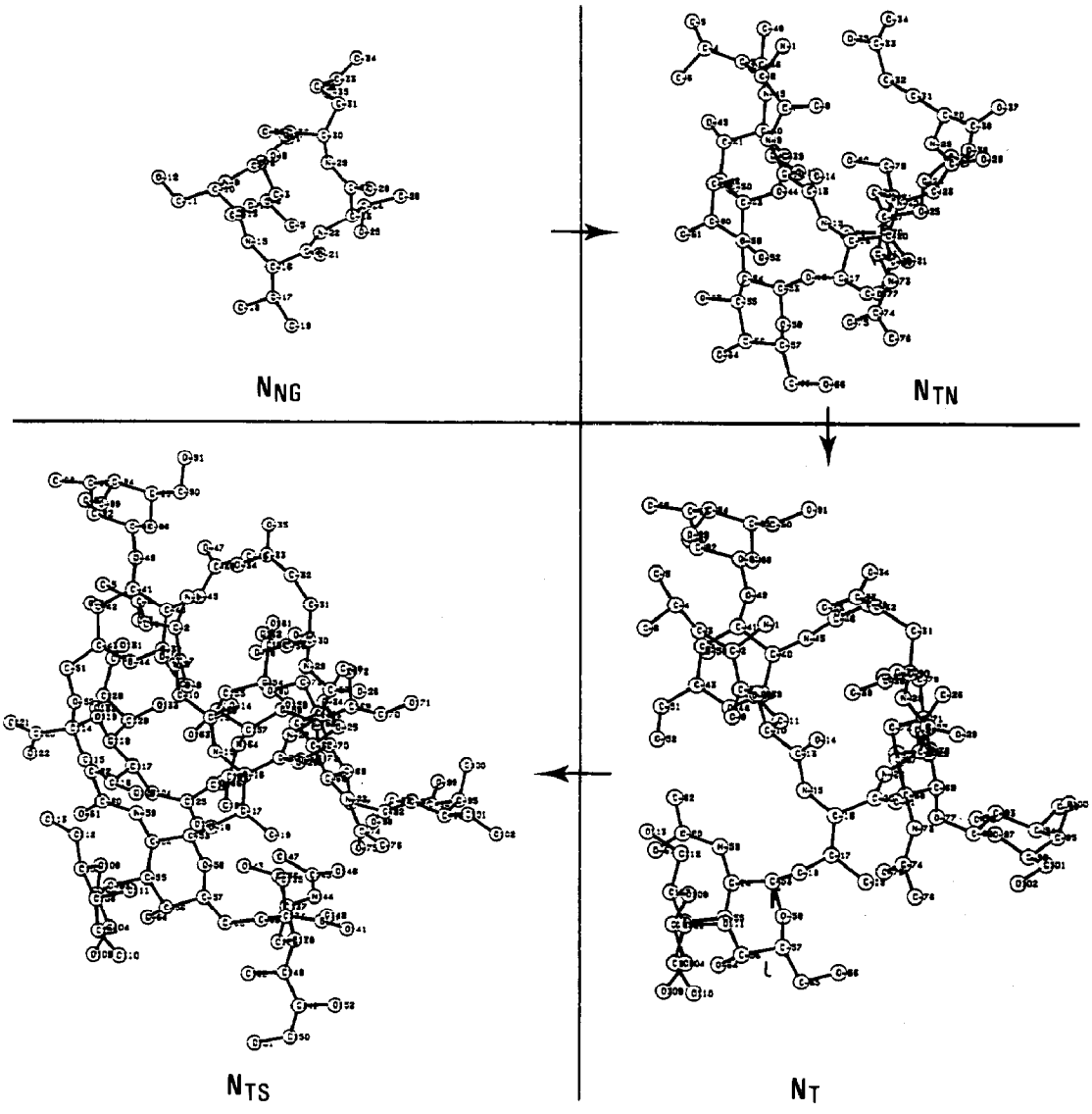


Fig. 3. Projections of N_{NG}, N_{TN}, N_T, and N_{TS} fragments of GPA of the NN blood group.

TABLE VI

Values (kcal.mol^{-1}) of the energy[a] of GLA fragments (blood group NN) (*cf.* Table I)

	N_{NG}	N_{TN}	N_T	N_{TS}
E_{vdw}	33.1	82.7	118	169
E_c	−8.2	22.8	84.7	239
E_H	−4.4	−25.4	−9.3	−16.7
E_{NBOND}	20.5	80.1	193.4	391.3
E_T	0.21	0.11	0.15	0.07
E_S	2.6	11.2	19.5	30
E_B	11.9	32	54.8	93.2
E_{BOND}	14.7	43.3	74.5	123.3
$E_{BOND}+E_{BOND}$	35.2	123.4	267.9	514.6
NATOMS	38	80	113	173
$NATOMS^2/E_{TOT}$	41	52	48	58

[a] See Table I for designation of the contributions.

The good agreement between the n.m.r. data and those reported here does not mean that the model is absolutely true, but only indicates that they are not contradictory.

GPA fragments with blood-group NN-structure. — Projections of N_{NG}, N_{TN}, N_T, and N_{TS} are displayed in Fig. 3 and the corresponding strain energies are reported in Table VI.

The values of ψ and φ that describe the peptide chain are given in Table VII. In N_{NG}, the main chain portion is a distorted α-helix (−60, 120° or −70, −120°). This deformation is probably induced by the proximity of the charged extremities. The $C_{\alpha\text{-}1}$–$C_{\alpha\text{-}4}$ and $C_{\alpha\text{-}2}$–$C_{\alpha\text{-}5}$ distances are 5.7 and 5.9 Å, respectively, assuming the α-helical structure of the peptide portion.

In contrast to the observations on the M fragments, sugars have the same effect on the secondary structure. The average variation in the torsional angles is 70° from N_{NG} to N_{TN}, 50° from N_{NG} to N_T, and 56° from N_{NG} to N_{TS}. The Cα distances become 7.5 and 6 Å in N_{TN}, 7.6 and 5.9 Å in N_T, and 7.6 and 5.8 Å in N_{TS}.

In M_{TN} and M_T, the main chain structures are similar (17° as the average difference for ψ, φ), whereas they differ considerably in N_{TN} and N_T (average difference, 71°). On the other hand, the addition of the sialic acid induces an important deformation when passing from M_T to M_{TS} (57° instead of 13° in the N derivatives). Fig. 4 shows the superimposed main chains (the best molecular fit was achieved as previously on the main-chain N atoms) in stereoscopic views of (N_{NG},N_{TN}), (N_{NG},N_T), (N_{NG},N_{TS}), (N_{TN},N_T), and (N_T,N_{TS}), and emphasises the differences described above. The common part (main chain + sugars) of (N_{TN}, N_T) and (N_T,N_{TS}) are also drawn and an important difference in geometry can be observed. For the hydrogen-bond network (Table VIII), the same conclusions as for M_{TN} and M_T can be drawn.

TABLE VII

The angles ψ and φ for the GLA fragments[a] (blood group NN) (*cf.* Table II)

(*a*) Substitution of the peptide

Angle	N_{NG}	N_{TN}	*Difference*	N_{NG}	N_T	*Difference*	N_{NG}	N_{TS}	*Difference*
ψ_1	137	160	23	137	−56	165	137	−36	173
φ_2	−7	137	144	−7	−48	41	−7	−40	33
ψ_2	−54	−177	123	−54	−175	121	−54	164	142
φ_3	−82	−79	3	−82	−61	21	−82	−39	43
ψ_3	−54	54	108	−54	−31	23	−54	−43	11
φ_4	−69	−148	79	−69	−64	5	−69	−60	9
ψ_4	−62	−55	7	−62	−61	1	−62	−54	8
φ_5	−137	−64	73	−137	−116	21	−137	−111	26
Average			70			50			56

(*b*) Increase in the number of sugar residues

Angle	N_{TN}	N_T	*Difference*	N_T	N_{TS}	*Difference*
ψ_1	160	−58	142	−58	−36	22
φ_2	137	−48	175	−48	−40	8
ψ_2	−177	−175	2	−175	164	21
φ_3	−79	−61	18	−61	−40	21
ψ_3	54	−31	85	−31	−43	12
φ_4	−148	−64	84	−64	−60	4
ψ_4	−55	−61	6	−61	−54	7
φ_5	−64	−116	52	−116	−111	5
Average			71			13

[a] The sialo (N_{TS}) and diglycosylated (N_T) structures have similar structures (*cf.* Table II).

TABLE VIII

Hydrogen-bond networks (*cf.* Table III)

N_{NG}		N_{TN}		N_T		N_{TS}			
Atoms	D^a (Å)	*Atoms*	*D* (Å)	*Atoms*	*D* (Å)	*Atoms*	*D* (Å)	*Atoms*	*D* (Å)
N-9 O-12	2.8	N-15 N-22	3.	N-1 N-9	3.1	N-1 N-9	3.	O-139 O-153	2.8
N-15 N-22	3.1	N-22 N-29	3.	N-15 N-22	2.9	N-15 N-22	3.	O-146 O-98	2.6
N-22 O-25	2.9	O-44 O-52	2.7	O-44 O-52	2.7	N-22 N-29	3.	O-151 O-153	2.8
		O-47 O-49	2.4	O-49 O-50	2.7	O-49 O-50	2.7	O-171 O-172	2.7
		O-49 O-50	2.7	O-58 O-66	2.7	O-63 O-64	2.7		
		O-58 O-66	2.7	O-63 O-64	2.7	O-77 O-78	2.7		
		O-61 O-63	2.6	O-77 O-78	2.8	O-88 O-89	2.7		
		O-63 O-64	2.7	O-78 O-80	2.9	O-99 O-100	2.7		
		N-73 O-77	3.	O-88 O-89	2.7	O-110 O-111	2.7		
		O-75 O-77	2.6	O-99 O-100	2.7	O-119 O-133	2.9		
		O-77 O-78	2.7	O-110 O-111	2.7	O-131 O-50	2.7		
		O-78 O-80	2.9			O-131 O-133	2.7		

[a] Distance between the atoms.

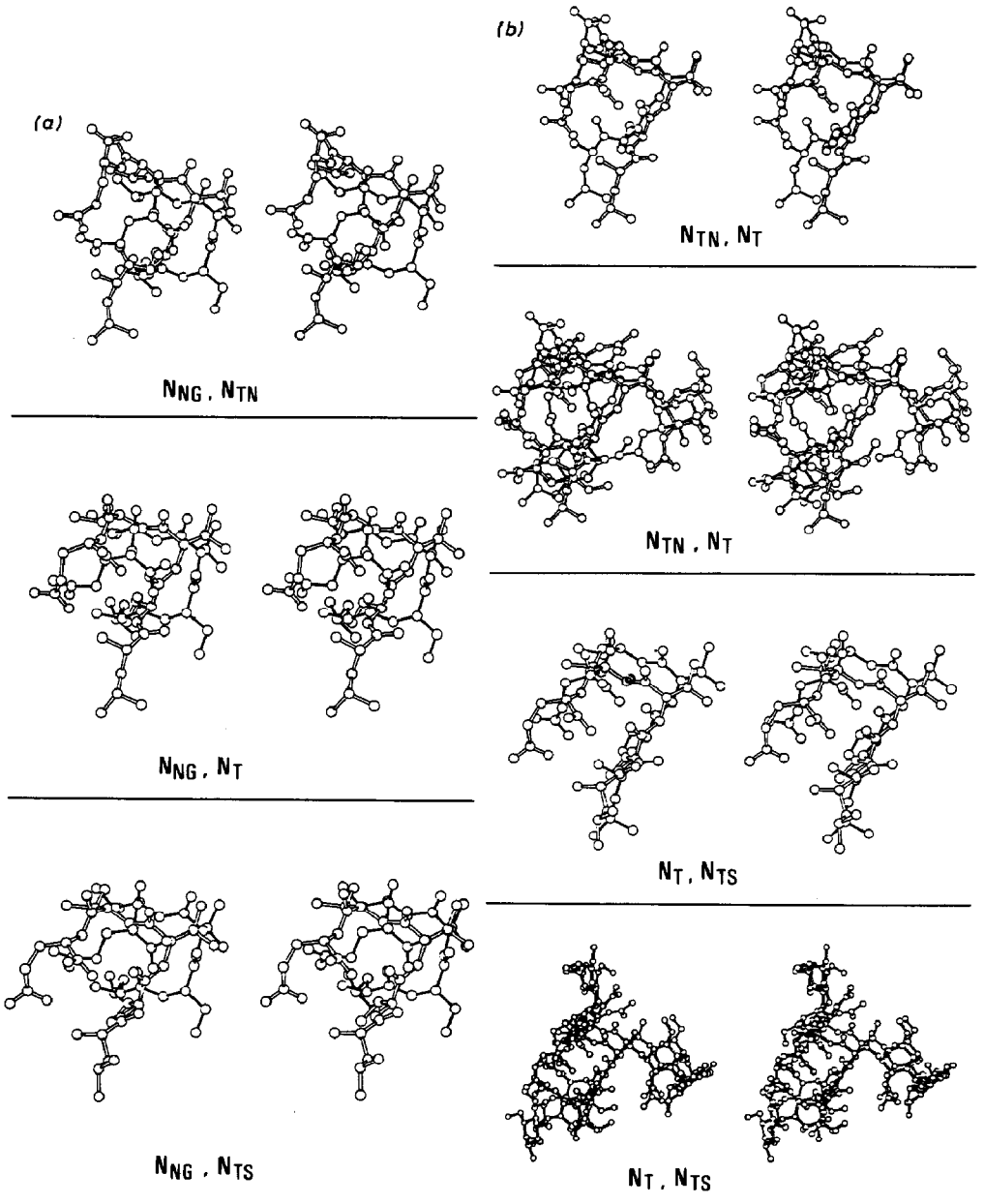


Fig. 4. Stereoscopic views of superimposed fragments belonging to GLA of the NN blood group. (*a*) The main chain of N_{NG} (filled line) superimposed on those of N_{TN}, N_T, and N_{TS}; the secondary structure is distorted [*cf.* Table VII(*a*)]; (*b*) the main chain and the common part of (N_{TN}, N_T) and (N_T, N_{TS}); the addition of sialic acids lightly perturbs the geometry of N_T.

The strain energy of N_{TS} with hydrogen bonds between the main-chain nitrogen atoms and the junction oxygen atom becomes 2910 instead of 515 kcal.mol^{-1}.

*The glycosidic bond**. — The (χ,φ) values, reported in Table IX, are similar to those observed for M_{TN} and M_T, expressing the same *exo*-anomeric effect[19] and the decoupling of C-1 and CH_3-Thr. The distances between the hydrogen atoms of the glycosidic bond in the calculated structures are shown in Table X.

Thus, the results of the modelling of the N-terminal part (5 residues) of glycophorin A_M and A_N that carry the T_N and T antigenic determinants are in good agreement with previous n.m.r. spectroscopic data, particularly at the level of the glycosidic bond. The addition of sugars considerably affects the geometry of the main chain and the distortion induced by the carbohydrates depends on the nature of the peptide.

For the MM blood-group antigens, the peptide chain maintains almost the same geometry when it is mono- or di-glycosylated (M_{TN} and M_T), whereas the addition of sialic acid considerably affects the secondary structure (M_{TS}). In contrast, for the NN blood-group antigen determinants, there are huge structural differences between mono- and di-glycosylated peptide (N_{TN} and N_T), whereas the main chain keeps the same structure when sialic acid is added to the N_T derivative to give N_{TS}.

In the sialo derivatives M_{TS} and N_{TS}, the values of the strain energies do not

TABLE IX

The torsion parameters χ, φ around the glycosidic bond between serine or threonine and the sugar residue[a] (*cf.* Table IV)

Angle	χ_2	χ_3	χ_4	φ_2	φ_3	φ_4
N_{TN}	−168°	−165°	−166°	175°	115°	104°
N_T	−113°	−157°	−166°	−176°	114°	123°
N_{TS}	−135°	−161°	−164°	−174°	120°	100°

[a] The subscript numbers refer to the 2nd, 3rd, and 4th residues; χ relates to the *exo*-anomeric effect and φ to the decoupling of C-1 and CH_3-Thr.

TABLE X

Distances[a] between hydrogen atoms of the glycosidic bond in the calculated structures of N_{TN} and N_T (*cf.* Table V)

Group	*Atoms*	*Antigens*	
		N_{TN}	N_T
Ser (2)	H-11B H-39	2.2 Å	2.3 Å
Thr (3)	H-17 H-53	2.2 Å	2.1 Å
Thr (4)	H-24 H-67	2.3 Å	2.1 Å

[a] A value of 2.2 Å was obtained by n.m.r. spectroscopy.

* Lemieux *et al.*[24] applied such an approach to the conformation of oligosaccharides relevant to the ABH and Lewis human blood-group determinants, using a very simplified interaction potential, and similar results were obtained.

parallel the formation of hydrogen bonds between the main-chain nitrogen atoms and the oxygen atom between the sugar and the sialic acid as suggested by others[7,8]. Most of the hydrogen bonds are intra- or inter-sugar bonds.

These results indicate clearly how the presence of sugar is necessary to induce the antigenic property of GPA. In a glycosylated peptide, the molecular envelope comprises only the sugars, which therefore play an essential role in the molecular recognition mechanisms, and the peptide chain acts only as a framework. Most of the peripheral atoms are oxygen atoms, which must be essential for the antigenicity.

EXPERIMENTAL

GENMOL[12] is a fast program for molecular building and performs conformational analysis simultaneously in order to give the preferred conformation. The program automatically determines the nature of each bond (σ, π, etc.) and takes into account the π systems.

Stretching and bending parameters were derived from those given[13] in MMP_2, or issued from molecular geometries coming from X-ray analysis.

The torsion, van der Waals, and hydrogen-bond parameters used were derived from ECEPP[14]. Coulombic interactions were computed in a monopole approximation with net atomic charges given by Del Ré's method[15] for the σ part with a new set of atomic parameters[16], whereas the Π charges were generated in an empirical way in order to obtain the Pariser and Parr values[17].

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