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Molecular modeling of a disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type

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The conformations of a disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type were analysed using the Tripos 5.3 force field from the Sybyl software currently used for molecular modelling. The conformation of each glycosidic linkage was calculated when included in oligosaccharide structures of up to 5 units and the influence of the glycosidic environment on the overall structure was measured. The study clearly shows that the conformation of a branched glycan cannot result from the simple addition of the different low energy conformers of each of the glycosidic linkages constituting the glycan structure. The asymmetrical conformation of the two antennae was demonstrated. The lowest energy conformations of the overall glycan structure were built and classified into 5 main models: the Y, T, bird and broken wing conformations already described and a new one called the 'back folded wing conformation'.

Keywords: molecular modeling, glycan, glycoprotein, three dimensional structure

The primary structure of *N*-glycosidically linked glycans from numerous glycoproteins has been determined [1–4], but up to now and since the pioneering work of Montreuil [1, 5–8], only few glycan conformations have been resolved. However, the knowledge of the three dimensional structure of glycan moiety is important as it may lead to a better understanding of the molecular basis of the biological role [9] of glycoproteins.

X-ray diffraction and nuclear magnetic resonance spectroscopy (NMR) are the two important tools which permit the resolution of the three dimensional structure of molecules having a stable conformation [10–12]. However, until now their use in the determination of the conformation of a glycoprotein biantennary glycan failed to give any definitive solution. In fact, X-ray diffraction of crystallized glycoproteins has allowed only the resolution of the conformation of the glycan moiety of the Fc fragment of human IgG [13] and the partial resolution of the glycan moiety of the F1 fragment of bovine prothrombin [14] and of human leukocyte elastase [15]. The available crystallographic data related to the glycan moiety of glycoproteins concern mainly di- and trisaccharides. The lack of crystallographic data can be due either to the difficulty encountered in the crystallization of glycoproteins or in the low resolution of the glycan moiety of the crystallized glycoproteins. Another possibility involves the mobility of the glycan structure which precludes any organized conformation except in some particular cases where the polysaccharide chains are immobilized due to strong interactions with polypeptide chains [13, 16].

The use of ^{1}H NMR in association with semi-empirical calculations such as HSEA [17, 18], or calculations only using empirical potential functions [19] or specialized force fields as MM2CARB [20] or PFOS [21, 22] provides some information. The conformation of short oligosaccharides or glycopeptides [23–29] has been intensively studied. From earlier studies, the three dimensional structure of glycans was considered to be the result of the combination of rigid and flexible regions [28] with flexibility mainly associated with the Man(α1-6)Man(β1-) linkage. The transitions between two conformational states were described as being under the control of some key residues such as Xyl(β1-2) or bisecting GlcNAc(β1-4) [28, 30, 31]. Taking into account the work of Homans *et al.* [32] and of Cumming and Carver [33], it appears that the conformation of oligosaccharides in solution is not unique but varies between several conformational states. Thus, the ^{1}H NMR-derived conformation represents an average con-

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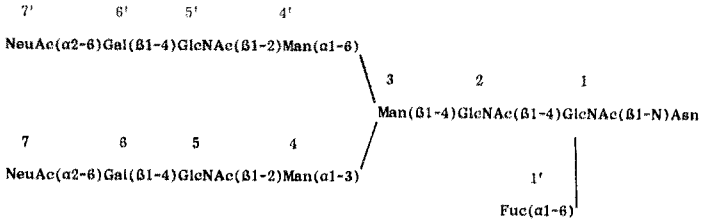

Figure 1. Structure of the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type from human lactotransferrin [36, 37].

formation which does not necessarily reflect the true picture [33].

In order to study the interactions between glycans and proteins, we have undertaken the molecular modelling of an *N*-glycosidically linked glycan using a theoretical approach. As up to now the potential function methods are of not sufficient quality [33], we have tried to investigate the performance of the empirical force field, Tripos 5.3 [34] of Sybyl software [35]. The conformational investigation was first performed on the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type (Fig. 1) found, for example, in human lactotransferrin [36, 37] and for which the ^{1}H NMR parameters of each glycosidic linkage have been described previously [24, 25, 28, 30–33, 38].

As it is not yet possible to solve a calculation on such a large structure containing 321 atoms, we first considered that the glycan moiety was composed of 12 glycosidic linkages, 8 of them being different: Fuc(α1-6)GlcNAc(β1-), GlcNAc(β1-4)GlcNAc(β1-), Man(β1-4)GlcNAc(β1-), Man (α1-3)Man(β1-), Man(α1-6)Man(β1-), GlcNAc(β1-2)Man (α1-), Gal(β1-4)GlcNAc(β1-), NeuAc(α2-6)Gal(β1-) and we have calculated their conformational parameters. Then, we have analysed the influence of the polysaccharide environment on the conformation of each of the glycosidic linkages found in trisaccharide structures and the conformation of the three following linkages Man(α1-3)Man(β1-), Man(α1-6)Man(β1-) and GlcNAc(β1-2)Man(α1-) in two pentasaccharides: Man(α1-3)[GlcNAc(β1-2)Man(α1-6)]Man(β1-4)GlcNAc(β1-) and Man(α1-6)[GlcNAc(β1-2)Man(α1-3)] Man(β1-4)GlcNAc(β1-). Finally, using the calculated data, we built and minimized one low energy conformation of the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type and we analysed the influence of the complete glycan conformation on each of the glycosidic linkage.

Methods

All calculations were carried out on an Evans and Sutherland PS 350 graphic station and on a Vax 6320 host computer. The energy calculations were performed using the molecular mechanics force field Tripos 5.3 [34] of the Sybyl molecular modelling software [35]. The three dimensional Connolly [39] representation of the surface (accessible surface of solvent) of the molecular models was obtained from Hydra [40].

Geometry of monosaccharides

The geometry and the conformation of each monosaccharide were taken from crystallographic data when available. It was assumed that *N*-acetylneuraminic acid residues were in a $^2C_5$ ring conformation and that all the other monosaccharide residues were in a $^4C_1$ ring conformation. The proton of nitrogen Nδ of the asparagine residue linked to the GlcNAc-1 residue was taken in the *trans* position of the anomeric proton of the GlcNAc-1 residue [41].

The different disaccharide and trisaccharide subunits as well as the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type were built using the following atomic labelling and torsional angles for the glycosidic linkages: $\Phi_H = \Phi(H_1\text{-}C_1\text{-}O_1\text{-}C'_x)$, $\Psi_H = \Psi(C_1\text{-}O_1\text{-}C'_x\text{-}H'_x)$ where x varies from 1 to 4 and 6, with C′ and H′ belonging to the reducing sugar. In the case of the *N*-acetylneuraminic acid residue, the definitions of the torsional angles were as follows: $\Phi_H = \Phi(C_1\text{-}C_2\text{-}O_2\text{-}C'_x)$, $\Psi_H = \Psi(C_2\text{-}O_2\text{-}C'_x\text{-}H'_x)$. The third torsional angle, Ω, was defined as: $\Omega_H = \Omega(O_1\text{-}C'_6\text{-}C'_5\text{-}H'_5)$ and then the definition was modified as: $\Psi_H = \Psi(C_1\text{-}O_1\text{-}C'_6\text{-}C'_5)$. The Φ_H, Ψ_H and Ω_H nomenclature is the most commonly adopted in the NMR studies. The sign of the torsional angle was considered as positive as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [42]. The hydroxymethyl group of each monosaccharide was assumed to be as observed in crystal structure. The value of the glycosidic valence angle was used as measured in the corresponding crystal structure when determined.

Calculations

For a given subunit, a minimization of all internal coordinates (stretchings, bendings and torsional angles) was performed. In the energy calculations, all of the above internal energy terms were added to van der Waals and Coulombic electrostatic contributions. The charge distribution used in the electrostatic term was calculated using the AM_1 quantum mechanical procedure [43].

For the relaxed structure, a conformational analysis was investigated using the Search subroutine procedure. The energy levels expressed in kcal mol^{-1} are given relative to the lowest energy found in the respective disaccharide. For larger oligosaccharides, they are given for respective conformers.

Strategy

For the eight different glycosidic linkages constituting the glycan (Fig. 1), the conformational analysis was first performed on the dissaccharide unit in a 10° by 10° grid of (Φ_H, Ψ_H) (Ω_H when existing) space. Then, the influence of

the neighbouring glycosidic linkage in both nonreducing [except for NeuAc(α2-6)Gal(β1-) and Fuc(α1-6)GlcNAc(β1-)] and reducing end on the conformation of the given linkage was analysed in the two derived trisaccharides. In such a study, the low energy conformers were calculated in a 15° by 15° grid of Φ_H and Ψ_H and Φ_H for both glycosidic linkages. The conformation of the mannotriose core was checked on the Man(α1-3)[Man(α1-6)]Man(β1-) trisaccharide; the Man (α1-3)[Man(α1-6)]Man(β1-4)GlcNAc(β1-) tetrasaccharide; the pentasaccharide I, Man(α1-3)[GlcNAc-(β1-2)Man(α1-6)]Man(β1-4)GlcNAc(β1-); and the pentasaccharide II, Man(α1-6)[GlcNAc(β1-2)Man(α1-3)]Man(β1-4) GlcNAc (β1-). The size of the tetrasacchride and pentasaccharide structures increased the time of computation considerably, so the conformations of the Man(α1-6)Man(β1-) linkage were calculated considering the Man(β1-4)GlcNAc(β1-) linkage as fixed, for the four lowest energy conformers of the GlcNAc(β1-2)Man(α1-) linkage and the three lowest energy conformers of the Man(α1-3)Man(α1-) linkage. For this analysis a 15° by 15° grid was used. In a last step, taking into account the different optimal possibilities for each linkage, a low energy glycan conformation was built and relaxed. Finally, the influence of the conformation of the glycan of low energy conformation was checked on each of the glycosidic linkage constituting the glycan structure. The values of energy levels and of torsional angles can present small errors due to the inherent limitations of any methods using empirical parameterization and due to the steps used in the grid space determinations.

Results and discussion

In order to investigate the influence of neighbouring monosaccharides on a glycosidic linkage, we calculated for the 8 different glycosidic linkages constituting the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type, the values of the torsional angles of each linkage obtained for a disaccharide, a trisaccharide and for the two pentasaccharides I and II including the mannotriose core.

GlcNAc(β1-4)GlcNAc(β1-)

The calculated values of the torsional angles of the GlcNAc(β1-4)GlcNAc(β1-) linkage were found to be restrictive to Φ_H equal to 60° and Ψ_H about −10° to −15°, in the disaccharide unit as well as in the Man(β1-4)GlcNAc(β1-4)GlcNAc(β1-) and the GlcNAc(β1-4)[Fuc (α1-6)]GlcNAc(β1-) trisaccharides. The calculated values of the torsional angles were nearly identical to the values measured for the crystalline structure of the *N,N'*-diacetylchitobiose [44] or calculated by HSEA derived-method [12, 23, 45]. In contrast, Biswas *et al.* [19] calculated two conformational states (51, 6) and (10, −40), and Imberty *et al.* [22] described variations between 25° and 145° and −50° and 210° for Φ_H and Ψ_H, respectively. It was also verified that the presence of the Asn side chain does not modify the conformation of the GlcNAc(β1-4)GlcNAc(β1-) linkage. The intramolecular H—H bond between O_5 and O'_3 probably stabilizes the conformer (60, 10–15) in the disaccharide sub-unit.

Man(β1-4)GlcNAc(β1-)

Only one family of stable conformers was detected for the 4 following analysed structures: Man(β1-4)GlcNAc(β1-) disaccharide; Man(β1-4)GlcNAc(β1-4)GlcNAc(β1-), Man (α1-3)Man(β1-4)GlcNAc(β1-) and Man(α1-6)Man(β1-4) GlcNAc(β1-) trisaccharides; and the values of dihedral angles were calculated to be within 40° to 75° and −30° to −15° for Φ_H and Ψ_H, respectively. The occurrence of an intramolecular H—H bond between O_5 and O'_3 is probably the reason why these values are not very far from the values measured by Warin *et al.* [46] (48, −0.5) for the Man(β1-4)GlcNAc(β1-) linkage found in the crystallized structure of Man(α1-3)Man(β1-4)GlcNAc(β1-) and from the values calculated by the HSEA derived-method [12, 23, 45]. Although, we did not find, like Biswas *et al.* [19] and Imberty *et al.* [22], any clearly differentiated conformational states, our data strongly suggest that some mobility can have occurred for the Man(β1-4)GlcNAc(β1-). The mobility of the Man(β1-4)GlcNAc(β1-) linkage is accentuated following the addition of the Man(α1-6)-linkage, suggesting a destabilization of the intramolecular hydrogen bond.

Fuc(α1-6)GlcNAc(β1-)

The energy calculation for the Fuc(α1-6)GlcNAc(β1-) disaccharide linkage showed the presence of four conformers: I: (60, 170, 180), $\delta E = 0$ kcal mol^{-1}; II: (60, 180, 50), $\delta E = 0.38$ kcal mol^{-1}; III: (60, 180, −60), $\delta E = 1.40$ kcal mol^{-1}, and IV (−60, 150, 170) $\delta E = 3.58$ kcal mol^{-1}. Upon addition of the Asn residue, the values of the dihedral angles of the two lowest energy conformers (I and II) remained unchanged. In contrast, the conformation of conformer III was considerably modified as the torsion angle Ψ shifted from 180° to 90° and led to conformer V (40, 90, −60). Moreover, the difference in energy level of conformer IV decreased from 3.58 to 1.32 kcal mol^{-1} and the new conformer VI (60, −60, −40) appeared showing a difference in energy level of 0.83 kcal mol^{-1}. Addition of a GlcNAc(β1-4) linkage on the GlcNAc-1 residue increased considerably the difference in energy level of conformers II–V and only conformer I remains stable in the trisaccharide unit, even in the presence of the Asn residue. The calculated values of conformer I are in good agreement with the values (60, 150, 180) measured by Brisson and Carver [24, 25] by ^{1}H NMR analysis of fucosylated oligosaccharides. The two other conformers (IV and V) possess a high energy difference. However, it is still possible that, in solution, they coexist with a minimum energy conformation. These results strongly suggest that the degree of freedom of the Fuc(α1-6)GlcNAc(β1-) glycosidic bond is restricted because

of steric hindrance interactions due to the acetamido group of GlcNAc-2.

Man(α1-3)Man(β1-)

Two low energy conformers: I (−60, −30), $\delta E = 0$ kcal mol^{-1} and II (−30, 60), $\delta E = 1.52$ kcal mol^{-1} were calculated for each studied structure: Man(α1-3)Man(β1-), Man(α1-3)Man(β1-4)GlcNAc(β1-), Man(α1-3)[Man(α1-6)]-Man(β1-), GlcNAc(β1-2)Man(α1-3)Man(β1-) and pentasaccharides I and II. The values of the dihedral angles calculated for the lowest energy conformer I (−60, −30) are in a good agreement with the corresponding values measured in the crystal structure by Warin *et al.* [46] (−57.6, −19.4) on Manα1-3Manβ1-4GlcNAcβ and by ^{1}H NMR studies: (−50, −10) [12, 23, 25, 26] on N-linked glycans or the values calculated using the force field program [45]. Conformer II, which has already been described [24], will display, when included in the disaccharide, a difference in energy level which renders its continued existence unlikely. However, its substitution by a Man(α1-6) linkage will give it a reasonable probability of existence in the mannotriose core. New conformers with positive value of Φ_H appeared consecutively to the addition of Man (β1-4)GlcNAc(β1-) linkage (conformer III (45, 30), $\delta E =$ 3.39 kcal mol^{-1}) and of Man(α1-6)Man(β1-) linkage (conformers III (40, 40), $\delta E = 0.62$ kcal mol^{-1} and IV (80, 60), $\delta E = 0.09$ kcal mol^{-1}). The addition of a GlcNAc (β1-2)Man(α1-) linkage in the GlcNAc(β1-2)Man(α1-3) Man(β1-) trisaccharide and in the pentasaccharides I and II led to an important increase in the difference in energy level of conformers II, III and IV, so that only conformer I still remains with a low energy level. Nevertheless, the existence of conformer II with a difference in energy level of about 5 kcal mol^{-1} in pentasaccharides I and II cannot be definitively excluded.

GlcNAc(β1-2)Man(α1-)

The calculation methodology we used showed directly the existence of two conformers, I (70, 55), $\delta E = 0$ kcal mol^{-1} and II (40, −55), $\delta E = 0.60$ kcal mol^{-1}, of low difference energy level in the disaccharide, while the HSEA-NMR analysis showed for the GlcNAc(β1-2)Man(α1-) linkage only one conformation (40–55, 10–30) [12, 23, 26, 45]. However, recent re-interpretation of the data by Cumming and Carver [33] using a GlcNAc(β1-2)Manα-*O*-methyl disaccharide led us to consider the NMR-derived conformation as the average between two populations: (48, 19) and (54, −3), and demonstrated that the ^{1}H NMR-derived conformation cannot only be interpreted in term of a single conformer. Moreover, the addition of either a Gal (β1-4)GlcNAc(β1-), Man(α1-3)Man(β1-) or a Man(α1-6)Man(β1-) linkage resulted in the appearance of a third conformer: (−20, −40), δE ranging from 0.09 to 0.21 kcal mol^{-1}. Conformer I was calculated with the highest probability of existence in the disaccharide structure only, while conformer II became the most favourable in all the other structures. The conformational states of the GlcNAc(β1-2)Man(α1-) linkage vary depending on the antenna on which it is located. The torsional angles are always 20°–40° less when the GlcNAc(β1-2)Man(α1-) linkage is located on the Manα1-3 antenna. Furthermore, a fourth conformer, (100, −40), $\delta E = 2.74$ kcal mol^{-1}, is predicted for the Man(α1-3)-antenna.

Man(α1-6)Man(β1-) and mannotriose core

Five conformers (I–IV and VI) were calculated in the disaccharide unit to be with negative values of around $\Phi_H = -60°$ and only one conformer (V) with a positive value of 50° (Table 1). The calculated conformations are in quite good agreement with the recent ^{1}H NMR data concerning the Man(α1-6)Manβ-*O*-methyl disaccharide [33] which allowed specification of the torsional angles to the following values: $\Phi_H = -60°$, $\Psi_H = 90°$–$200°$ and $\Omega_H = -60°$ or 180°. Similar results were obtained by Paulsen [12], Biswas *et al.* [19], Imberty *et al.* [22] and Struike-Prill and Meyer [45]. Differences were observed only concerning Ω_H, for which we calculated an additional value of about 50°. Conformer VI, calculated with 3 negative values of dihedral angles, presented the most important difference of energy level and was never observed in the other structures. The addition of Man(β1-4)GlcNAc(β1-) linkage forbids the conformer with a positive value of Φ_H and allows the existence of a new conformer, VII (−60, 90, −60).

The effect of the addition of Man(α1-3)Man(β1-) linkage (Table 2) on the Man(α1-6)Man(β1-) linkage was calculated in the Man(α1-3)[Man(α1-6)]Man(β1-4)GlcNAc(β1-) tetrasaccharide and the results are given for three conformations (−60, −40), (−40, 40) and (80, 60) of Man(α1-3)Man(β1-) linkage. For the lowest energy conformation (−60, −40) all the conformers previously described for Man(α1-6)Man(β1-) (I–V, and VII) and one additional conformer (VIII) were calculated with low difference energy. As the difference in energy level of the torsional angles of the Man(α1-3)Man(β1-) linkage increased, the number of conformational states of the Man(α1-6)Man(β1-) linkage decreased, and only three conformers (I–III) have been described for the conformation (80, 60) of the Man (α1-3)Man(β1-) linkage.

The effect of the addition of GlcNAc(β1-2) linkage on either Man(α1-3) or Man(α1-6) was studied in pentasaccharides I and II, for a constant value of Man(β1-4)GlcNAc(β1-) (60, −15). Considering, first, the optimal Man(α1-3)Man(β1-) torsional angle values (−60, −40), conformers I to III were calculated (Table 3) with different energy levels for the 4 possible conformers of the GlcNAc (β1-2)Man(α1-) linkage. In contrast, conformer VII of the Man(α1-6)Man(β1-) linkage was calculated with low difference in energy level for the only 2 conformations (60, −40) and (−20, −40) of the GlcNAc(β1-2)Man(α1-) linkage. Our results show that the distribution of the

Table 1. Calculated values of the dihedral angles Φ, Ψ and Ω and relative energy δE for the Man(α1-6)Man(β1-) linkage.

Structure	*Conformer*	Φ, Ψ, Ω (degrees)	δE (kcal mol^{-1})
Man(α1-6)Man(β1-)	I	(−60, 180, 180)	0.00
	II	(−60, 180, −50)	0.59
	III	(−70, 80, 170)	0.69
	IV	(−60, 180, 60)	1.03
	V	(50, 180, 50)	1.26
	VI	(−40, −50, −40)	1.33
Man(α1-6)Man(β1-4)-GlcNAc(β1-)	I	(−60, 180, 180)	4.08
	II	(−60, 180, −75)	0.00
	III	(−60, 90, 165)	4.62
	V	(−60, 180, 60)	4.58
	VII	(−60, 90, −60)	5.42
GlcNAc(β1-2)Man(α1-6)-Man(β1-)	I	(−60, 180, 180)	0.00
	II	(−60, 180, −60)	0.50
	IV	(−60, 180, 45)	0.86
	VII	(−60, 75, −75)	1.51

Table 2. Values of the dihedral angles Φ, Ψ and Ω and relative energy δE for the Man(α1-6)Man(β1-) linkage located in the Man(α1-3)[Man(α1-6)]Man(β1-4)GlcNAc(β1-) tetrasaccharide and calculated for the three lowest energy conformations of the Man(α1-3)Man(β1-) linkage. The torsional angles of Man(β1-4)GlcNAc(β1-) linkage were taken as (60, −15).

Conformation of Man(α1-3)Man(β1-) linkage	*Conformer*	Φ, Ψ, Ω (degrees)	δE (kcal mol^{-1})
Man(α1-3)Man(β1-) (−60, −40)	I	(−60, 180, 180)	0.33
	II	(−60, 180, −60)	0.37
	III	(−60, 90, 180)	0.85
	IV	(−60, 180, 40)	0.53
	V	(60, 180, 40)	0.32
	VII	(−60, 80, −60)	2.56
	VIII	(60, 180, −40)	1.74
Man(α1-3)Man(β1-) (−40, 40)	I	(−60, 180, 180)	0.02
	II	(−60, 180, −60)	0.04
	III	(−60, 80, 160)	1.40
	V	(60, 180, 40)	0.00
	IX	(−60, 180, 0)	1.89
Man(α1-3)Man(β1-) (80, 60)	I	(−60, 180, 180)	0.62
	II	(−60, 180, −60)	0.19
	III	(−60, 80, 160)	1.09

rotamers about the dihedral angle Ω_H depends upon the conformation of the GlcNAc(β1-2)Man(α1-), while Wooten *et al.* [38] have recently shown that the flexibility of the dihedral angle Ω_H is dependent on the oligomannosides primary sequence. The conformation (120, −40) of GlcNAc-(β1-2)Man(α1-) was only found associated with conformers I–III of Man(α1-6)Man(β1-) in pentasaccharide I, underlining the asymmetrical behaviour of the GlcNAc-5 and -5′ residues. When calculations were performed using the torsional angle values (−20, 40) for the Man (α1-3)Man(β1-) linkage, similar results were obtained for the Man(α1-6)Man(β1-) linkage with an energy shift of 4.94 kcal mol^{-1} (data not shown). Therefore, we take into consideration only the glycosidic bond (60, −40) for GlcNAc(β1-2)Man(α1-) and the conformer (−60, 180, −60) for Man(α1-6)Man(β1-).

Gal(β1-4)GlcNAc(β1-)

Only one conformer was calculated with Φ_H and Ψ_H values about 50° and −15° for Gal(β1-4)GlcNAc(β1-), Gal

Table 3. Influence of the values of the dihedral angles Φ and Ψ of the GlcNAc(β1-2)Man(α1-) linkage on the conformation of the Man(α1-6)Man(β1-) linkage included in the pentasaccharides I and II. The values of the torsional angles of Man(α1-3)Man(β1-) and Man(β1-4)GlcNAc(β1-) linkages were taken as (−60, −40) and (60, −15), respectively.

Conformation of GlcNAc(β1-2)Man(α1-)	*Conformer*	Φ, Ψ, Ω (degrees)	δE (kcal mol^{-1}) *in pentasaccharides*	
			I	*II*
GlcNAc(β1-2)Man(α1-) (60, −40)	I	(−60, 180, 180)	2.05	3.22
	II	(−60, 180, −60)	0.25	0.00
	III	(−60, 80, 160)	0.00	3.77
	VII	(−60, 80, −60)	3.91	4.93
GlcNAc(β1-2)Man(α1-) (−20, −40)	I	(−60, 180, 180)	1.70	4.70
	II	(−60, 180, −60)	2.25	0.77
	III	(−60, 80, 160)	–[a]	4.38
	VII	(−60, 100, −80)	2.87	–[a]
GlcNAc(β1-2)Man(α1-) (80, 40)	I	(−60, 180, 180)	2.65	4.42
	II	(−60, 180, −60)	2.89	1.37
	III	(−60, 80, 160)	3.34	5.09
GlcNAc(β1-2)Man(α1-) (120, −40)	I	(−60, 180, 180)	3.59	–[a]
	II	(−60, 160, −60)	2.74	–[a]
	III	(−60, 80, 160)	3.58	–[a]

[a] Difference in energy level too high.

(β1-4)GlcNAc(β1-2)Man(α1-) and NeuAc(α2-6)Gal(β1-4)-GlcNAc(β1-) structures. The values calculated for the torsional angles are in a good agreement with the values measured by Breg *et al.* [48] ($\Phi_H = 60°$, $\Psi_H = 0°$) for the oligosaccharide NeuAc(α2-6)Gal(β1-4)GlcNAc(β1-) and calculated by Paulsen [12] and Bock *et al.* [23].

NeuAc(α2-6)Gal(β1-)

Four conformers were predicted for the NeuAc(α2-6)Gal-(β1-) linkage in the disaccharide: I (−60, 180, 60), $\delta E = 0$ kcal mol^{-1}, II (−170, 180, 60), $\delta E = 0.96$ kcal mol^{-1}, III (−70, −90, 70) $\delta E = 1.09$ kcal mol^{-1}, and IV (50, 180, 60) $\delta E = 1.33$ kcal mol^{-1}. They all possessed only one value of $\Omega_H = 60°$ corresponding to the tg definition of Sundaralingam [47] formalism (O6-C6-C5-C4) used by Breg *et al.* [48]. The torsional angle Ψ_H is restricted to 2 values equal to 180° or −90°, in contrast to Φ_H which allows free rotation. Since the addition of a Gal(β1-4)GlcNAc(β1-) linkage increased considerably the difference in energy level of the 2 conformers II and IV, we did not take them into consideration. Furthermore, a new conformer, V (−60, 210, −60), $\delta E = 0.68$ kcal mol^{-1}, was generated with a gt orientation of ΩH. The values of the torsional angles we calculated for the two linkages of NeuAc(α2-6)Gal(β1-4)GlcNAc(β1-) trisaccharide structure are in a good agreement with the values (−60, 120–210, ±60) measured by ^{1}H NMR by Breg *et al.* [48] on the trisaccharide in solution. However, the gt and tg orientation of the Ω_H angle we calculated were associated with conformers I and II, respectively. In contrast, the tg and gt orientations could not be distinguished by ^{1}H NMR analysis.

Influence of the overall glycan structure on the conformation of each glycosidic linkage

We arbitrarily built a glycan conformation by adding the lowest energy conformations of each glycosidic linkage (Table 4), and we checked its influence on the conformation of each of the glycosidic linkages. The results obtained show that the low energy conformers calculated for the GlcNAc-(β1-4)GlcNAc(β1-), the Man(β1-4)GlcNAc(β1-), the Man-(α1-3)Man(β1-) and the Man(α1-6)Man(β1-) linkages were similar to the low energy conformers calculated without taking into account the influence of the overall glycan structure. In contrast, the conformations of the Fuc(α1-6)GlcNAc(β1-) linkage and of the glycosidic linkages constituting the antennae were modified. First, the stabilizing effect of the GlcNAc(β1-4) residue on the Fuc(α1-6)GlcNAc-(β1-) linkage was abolished in the biantennary *N*-acetyllactosamine structure as the difference in energy level of conformers IV and V substantially decreased. The energy level of conformer III (−30, −50) of the GlcNAc(β1-2)Man(α1-) linkage decreased and it became the most likely conformer. A new conformer ($\Phi_H = 170°$, $\Psi_H = 0°$) was predicted for the Gal(β1-4)GlcNAc(β1-) linkage located on a Man(α1-6) linked antenna. Finally, for NeuAc(α2-6)Gal(β1-), only conformers I and V were observed with low energy level. Conformer I was mainly associated with the Man(α1-3) linked antenna and conformer V with the Man(α1-6) linked antenna.

Table 4. Values of the dihedral angles Φ, Ψ and Ω and relative energy δE of the glycosidic linkages constituting the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type, calculated taking into account the influence of the overall structure of a low energy conformer.

Glycosidic linkage[a]	*Conformer*	Φ, Ψ, Ω (degrees)	δE (kcal mol^{-1})
GlcNAc(β1-4)GlcNAc(β1-) (63, −25)	I	(60, −20)	0.00
Fuc(α1-6)GlcNAc(β1-) (54, 161, 180)	I	(60, 170, 170)	0.00
	IV	(−60, 150, 170)	0.91
	V	(40, 90, −60)	1.69
Man(β1-4)GlcNAc(β1-) (63, −25)	I	(60, −15)	0.00
Man(α1-3)Man(β1-) (−61, −31)	I	(−60, −30)	0.00
	II	(−40, 30)	0.10
Man(α1-6)Man(β1-) (−64, 164, −58)	VII	(−60, 80, −60)	0.00
	II	(−60, 180, −60)	1.03
	III	(−60, 80, 180)	1.74
	I	(−60, 180, 180)	4.49
^{5} ^{4} GlcNAc(β1-2)Man(α1-) (50, −47)	III	(−30, −50)	0.00
	I	(75, 70)	0.16
	II	(45, −50)	0.67
$^{5'}$ $^{4'}$ GlcNAc(β1-2)Man(α1-) (50, −47)	III	(−30, −50)	0.00
	II	(75, 55)	1.16
	I	(60, −30)	1.93
^{6} ^{5} Gal(β1-4)GlcNAc(β1-) (45, −44)	I	(45, −30)	0.00
$^{6'}$ $^{5'}$ Gal(β1-4)GlcNAc(β1-) (45, −44)	I	(45, −30)	0.00
	II	(170, 0)	0.28
^{7} ^{6} NeuAc(α2-6)Gal(β1-) (−60, 210, −60)	V	(−60, 210, −60)	0.00
	I	(−60, 180, 60)	0.60
$^{7'}$ $^{6'}$ NeuAc(α2-6)Gal(β1-) (−60, 210, −60)	I	(−60, 180, 60)	0.00
	V	(−60, 210, −60)	0.51

[a] The data in parentheses indicate the values of dihedral angles of the glycosidic linkages of the lowest energy conformation used in the calculation.

Building and molecular modelling of the conformations of the disialylated monofucosylated biantennary glycan of the N-acetyllactosaminic type

The combination of the different conformations of each of the glycosidic linkages constituting the glycan structure will result in hundreds of conformations. However, the combination of the lowest minimum energy conformation we calculated for both pentasaccharides and Gal(β1-4)GlcNAc (β1-) and NeuAc(α2-6)Gal(β1-) linkages lead to the description of only about 40 glycan conformation families. The average conformation of each of these families was constructed and the resulting structures were minimized. The minimization always led to a decrease of the values of the dihedral angles, which was particularly important for the linkages of the Man(α1-3) linked antenna, so demonstrating the asymmetric behaviour of both antennae.

The overall shape of the glycan shows a great deal of variation, and some of the calculated conformations (Table 5), particularly ABDF for the Man(α1-6)Man(β1-) linkage and B for the GlcNAc(β1-2)Man(α1-) are in good agreement with the structures previously calculated by other theoretical and experimental methods [12, 19, 24, 25, 28, 30–33, 45]. We have selected 6 examples of conformation, as shown in Fig. 2, the torsional angles of which are given in Table 5. These examples were selected in order to show the influence on the glycan conformation of Man(α1-6)Man(β1-), Man (α1-3)Man(β1-) and GlcNAc(β1-2)Man(α1-) linkages, all the other linkages remaining constant. The variations of the GlcNAc(β1-2)Man(α1-) unit do not produce important changes in the shape of the glycan (Fig. 2b, d). On the other hand, the variations in the Man(α1-6)Man(α1-) linkage parameters induce very important conformational changes and lead to the classification in 5 main models. The 3 conformations (a), (b) and (d) (Fig. 2) were identified with

Table 5. Values of the dihedral angles Φ, Ψ and Ω of the glycosidic linkages constituting the 6 minimized conformations of the disialylated monofucosylated biantennary glycan of the *N*-acetyllactosamine type, represented in Fig. 2.

Glycosidic linkage	*Conformation*	Φ, Ψ, Ω (degrees)
GlcNAc(β1-4)GlcNAc(β1-)	(a–f)	(60, −10)
Fuc(α1-6)GlcNAc(β1-)	(a–f)	(60, 170, 170)
Man(β1-4)GlcNAc(β1-)	(a–f)	(60, −15)
Man(α1-3)Man(β1-)	(a–e)	(−60, −30)
	(f)	(−20, 40)
Man(α1-6)Man(β1-)	(a, b, d, f)	(−60, 180, −60)
	(c)	(−60, 80, −80)
	(e)	(−60, 80, 180)
^{5} ^{4} GlcNAc(β1-2)Man(α1-)	(a, c, e, f)	(20, −60)
	(b)	(60, 20)
	(d)	(−20, −40)
$^{5'}$ $^{4'}$ GlcNAc(β1-2)Man(α1-)	(a, c, e, f)	(40, −60)
	(b)	(80, 40)
	(d)	(−20, −60)
Gal(β1-4)GlcNAc(β1-)	(a–f)	(45, −30)
NeuAc(α2-6)Gal(β1-)	(a–e)	(−60, 210, −60)
	(f)	(−60, 180, 60)

the T conformation [5] and conformations (c) and (f) with the broken-wing [5, 7] and the Y [1, 8] conformations previously described. The change in the angle Ω_H of the Man(α1-6)Man(α1-) linkage from −80° to 180° is illustrated by conformations (a) and (e) (Fig. 2). The conformation (e), in which the Manα1-6 antenna is projected to the rear of the core, has not previously been described and we propose to call it the back-folded wing conformation.

Some conformations such as the T conformation (Fig. 2F) cover a large area which can indeed reach 400 Å^2 when the Man(α1-6)Man(β1-) and Man(α1-3)Man(β1-) linkages are defined as (−60, 180, −60) and (−20, 40), respectively. In contrast, the area covered by some other conformers is smaller, mainly when the Man(α1-6)Man(β1-) linkage takes the values (−60, 80, −80) and (−60, 80, 180).

The Connolly representation of the conformation reveals the hydrogen bonds. Some are common to all the structures: between O_3 of GlcNAc-1 and $O_{5'}$, of GlcNAc-2 and between O_6 of galactose and $O_{5'}$, of *N*-acetylneuraminic acid and between O of the acetamido group of GlcNAc-2 and O'_3 of Man(α1-6). Some are characteristic, as is the case of the hydrogen bond between the acetamido group of NeuAc(α2-) residue with either the GlcNAc-1 or Asn residue of conformation (e) (Fig. 2, Table 5) and of the hydrogen bond between O_3 of GlcNAc-2 and O'_5 of Man(α1-6) in structures (a) and (b). Conformation (e) has been identified as the T conformation previously described by Montreuil (3, 7).

Conclusion

The force field Tripos 5.3 [34] we used is based on molecular mechanics and is not specifically suited to the energy calculation of oligosaccharides. Nevertheless, it permits reproduction of the results obtained with HSEA derived methods. The minimization of all complete structures proceeded until the convergence criterion of the root mean square over all atoms of less than 0.1 kcal mol^{-1} Å^{-1} was obtained. We verified that the torsional barriers obtained for disaccharides Man(α1-3)Man(β1-) and Man(β1-4)GlcNAc(β1-) using Tripos 5.3 were close to the values obtained using the AM1 quantum mechanics procedure. The size (291 atoms) of the disialylated monofucosylated glycan of the *N*-acetyllactosamine type, the calculation software available and the computational possibilities presently do not permit energy calculations on the whole structure. However, the force field Tripos 5.3 [34] allowed us to study polysaccharides composed of up to 5 monosaccharides and to analyse the conformation of 1 glycosidic linkage in the environment of the overall glycan. Generally, the conformations calculated using the Tripos 5.3 [34] force field tie in well with the experimental results obtained by ^{1}H NMR and X-ray analyses. In addition, the calculations allow, in particular cases, the description of conformers which are not assessed by ^{1}H NMR analysis. This is the case for the NeuAc(α2-6)Gal(β1-) linkage, where the calculations give the precise orientation of the Ω_H torsional angle.

It is generally assumed that the flexibility of oligosaccharides in solution is mainly associated with Man(α1-6)Man(β1-) linkages [25, 26] even if some narrow torsional oscillations have already been described [29, 30] for the Man(α1-3)Man(β1-) glycosidic linkage (−20, −40). Our approach led us to define one new flexible linkage: GlcNAc(β1-2)Man(α1-) and to show, in agreement with Imberty *et al.* [22], the presence of 2 additional conformers in the Man(α1-3)Man(β1-) linkage when involved in di- and trisaccharides.

Conformation of the oligosaccharides may be under the control of some keys [28]. In the present paper, we demonstrate that the residue GlcNAc(β1-2) is a key residue as it modulates the conformation of the mannotriose core. The lower energy conformation of the Man(α1-6)Man(β1-) linkage depends upon the values of the GlcNAc(β1-2)Man(α1-) linkage. Moreover, the resulting effect of the GlcNAc(β1-2) residue is not identical for each antenna, so demonstrating the asymmetric conformation of the two antennae.

The lower energy conformers calculated for a glycosidic bond in a disaccaride unit and in a complete glycan structure are generally different. In fact, an overall glycan structure of the *N*-acetyllactosamine type can never result from summation of the lower minimum energy conformer of each of the glycosidic bonds constituting the molecule, as was assumed by Imberty *et al.* [22].

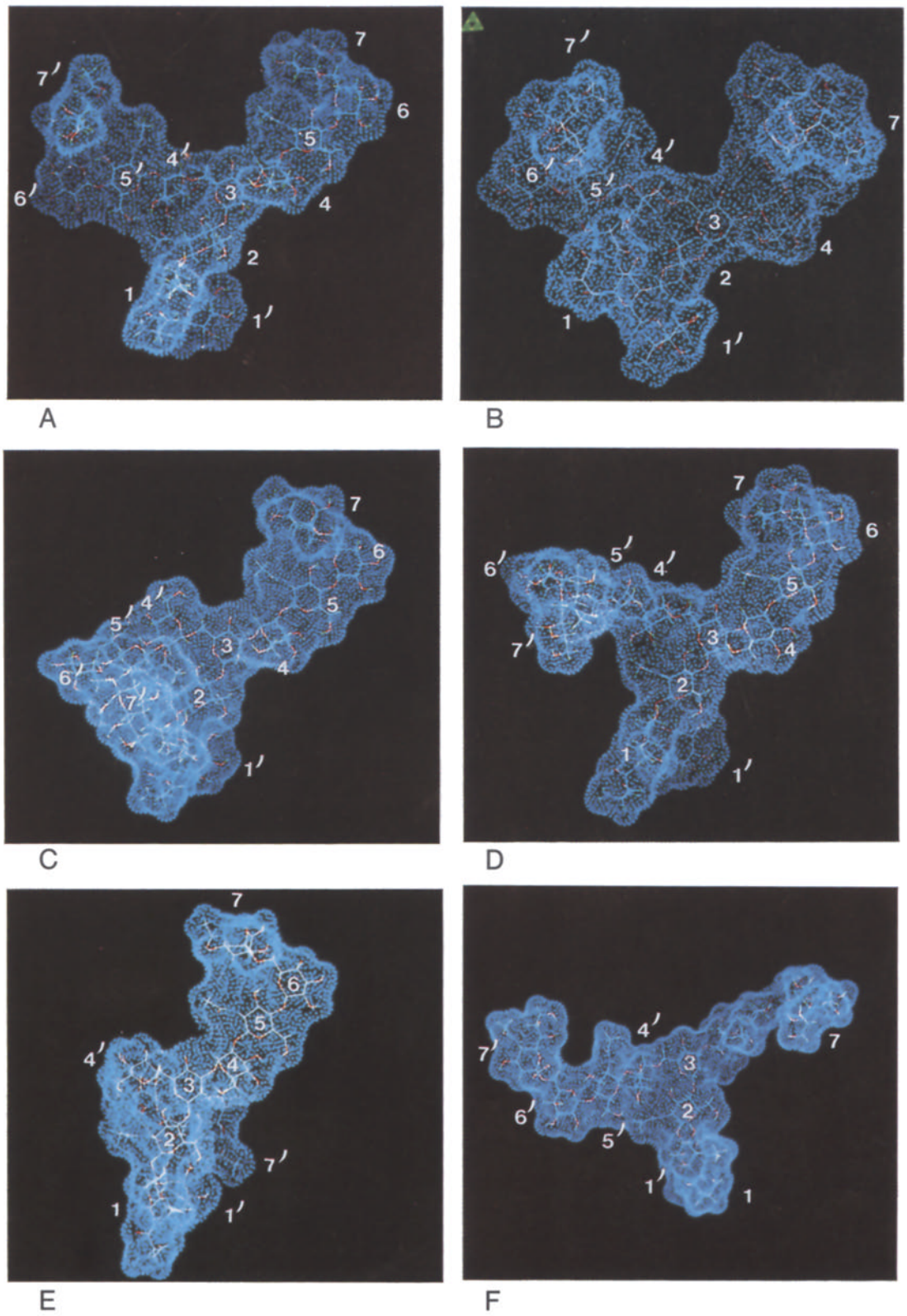

Figure 2. Connolly representation of six of the conformational states of the disialylated monofucosylated biantennary glycans of the *N*-acetyllactosamine type. The values of the dihedral angles of each glycosidic linkage are given in Table 5. Numbering refers to the glycan formula given in Fig. 1.

The energy calculations and the molecular modelling of the N-linked glycan structure we performed corroborate the pioneering observations of Montreuil [1–3, 5–7] and of Montreuil *et al.* [8], and the conformations described by Brisson *et al.* [25, 26] and by Homans *et al.* [28, 30–32], by Meyer [10] and by Paulsen [12]. Finally, the energy calculations using Tripos 5.3 force field lead to the description of new models which will be useful in the study of the interactions between glycans and proteins. This methodology at least allows testable predictions to be made which in turn will stimulate researchers in the field of physico-chemical experimentation.

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