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Articles

Conformation of the Glucotriose Unit in the Lipid-Linked Oligosaccharide Precursor for Protein Glycosylation†

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ABSTRACT: The conformation of the glucotriose unit of the protein glycosylation precursor $Glc_3Man_9GlcNAc_2$ was assessed by deuterium exchange studies on the model tetrasaccharide $\alpha Glc \rightarrow {}^2\alpha Glc \rightarrow {}^3\alpha Glc \rightarrow {}^3\alpha Man \rightarrow OCH_2CH_2CH_3$ dissolved in deuterated dimethyl sulfoxide. The hydroxyl proton on C-2 of the nonreducing end glucose and on C-4 of the glucose attached to mannose both show dramatic isotope shifts indicative of a strong hydrogen bond beween these two hydroxyl groups. Such a hydrogen bond requires a fixed conformation of the glucotriose unit that brings these hydroxyl groups within 3 Å of each other, a conformation that is supported by molecular modeling based on hard-sphere exo-anomeric (HSEA) calculations. The temperature dependence of the hydroxyl proton chemical shifts supports the postulated hydrogen bond, and the torsional angles between the three glucose units derived from the HSEA calculations are consistent with results from related studies on other saccharides. The results support a model for biochemical function in which the glucotriose unit could modulate the activity of the oligosaccharyltransferase by binding in a fixed conformation to a specific effector site in the enzyme.

The synthesis of glycoproteins that contain asparagine-linked carbohydrate is initiated in most eucaryotes by the transfer of the oligosaccharide unit from $Glc_3Man_9GlcNAc_2$-P_2-dolichol to the protein in the lumen of the endoplasmic reticulum. Immediately after transfer, the glucotriose unit and a single mannose are removed to give $Man_8GlcNAc_2$ chains that undergo further processing in the Golgi to yield more complex structures both in yeast and in higher eucaryotes [for reviews, see Kornfeld and Kornfeld (1985), Kukuruzinska et al. (1987), Kaplan et al. (1987), and Tanner and Lehle (1987)].

Several studies have dealt with the role in yeast of the glucotriose unit. This structure was found to facilitate oligosaccharide transfer to acceptor in enzymic studies with cell-free extracts (Trimble et al., 1980; Sharma et al., 1981) and yeast strains that are unable to glucosylate the precursor produce proteins that are underglycosylated (Huffaker & Robbins, 1983; Ballou et al., 1986). A yeast strain that is unable to remove the glucose units after oligosaccharide transfer to protein, however, shows no obvious growth phenotype (Tsai et al., 1984). In the kinetic studies with cell extracts, the glucotriose unit in the donor was found to increase the V_{max} for oligosaccharide transfer to endogenous acceptor but to have no effect on the K_m for the donor (Trimble et al., 1980), whereas its presence did reduce the K_m for a synthetic hexapeptide acceptor (Sharma et al., 1981). This suggests that the glucotriose unit might interact at an effector site on the oligosaccharyltransferase complex and modulate the site at which the polypeptide acceptor is bound. Such a mechanism would be consistent with the spacial relationship between the glucotriose unit and the glycosyl–pyrophosphate bond at which nucleophilic attack presumably occurs to form the *N*-glycosyl linkage.

We have previously reported the ^1H NMR spectrum for the oligosaccharide $Glc_3Man_{10}GlcNAc$, obtained from a yeast mutant defective in glucosidase I, and noted a discrepancy in the anomeric proton chemical shift for the terminal glucose unit that suggested it might be involved in an intramolecular hydrogen bond to the third glucose unit (Tsai et al., 1984). If so, we postulated that the glucotriose unit could have a fixed

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conformation that would be consistent with an effector role in regulating oligosaccharide transfer. Here we present evidence, from studies of deuterium exchange in dimethyl sulfoxide, for a strong hydrogen bond between these two glucose units in the model tetrasaccharide αGlc→$^2\alpha$Glc→$^3\alpha$Glc→$^3\alpha$Man→$OCH_2CH_2CH_3$, which result implies a defined conformation for the molecule that may be related to the biological function of the glucotriose unit. The conformation inferred from the NMR studies was supported by molecular modeling calculations.

Experimental Procedures

Materials. The oligosaccharide αGlc→$^2\alpha$Glc→$^3\alpha$Glc→$^3\alpha$Man→$OCH_2CH_2CH_3$ was prepared at RIKEN according to a published procedure (Ogawa et al., 1983). Dimethyl-d_6 sulfoxide (DMSO-d_6) (100.0 atom % D, catalog number 15,691-4) and D_2O (100.0 atom % D, catalog number 19,234-1) were from Aldrich.

For convenience in referring to specific protons in the tetrasaccharide, the hexose units are labeled A→B→C→D→OR from the nonreducing toward the reducing end. Carbon-bound hydrogens are designated H-1 to H-6 and hydroxyl hydrogens OH-2 to OH-6, so that $OH\text{-}2^A$ identifies the hydroxyl hydrogen on position 2 of the first glucose unit.

^1H NMR Spectroscopy. ^1H NMR spectra were obtained on a Bruker AM-500 spectrometer operating at 500 MHz and a Bruker AMX-600 spectrometer, both in the Department of Chemistry of this campus, with acetone as internal reference (δ = 2.217 ppm in D_2O and DMSO-d_6) (Cohen & Ballou, 1980). The temperature of the sample was controlled with a Bruker BVT-1000 variable-temperature unit that had an accuracy of ±0.5 °C. DMSO-d_6 was dried over CaH_2 (Oullette, 1965) for several days under nitrogen, filtered through a membrane filter under nitrogen, and stored in the presence of molecular sieve 3 Å under nitrogen. The addition of a few milligrams of calcium sulfate to the DMSO-d_6 solution helped to slow the rate of proton exchange and thus sharpen the signals of the hydroxyl groups. The deuterium-induced isotope shifts were measured by adding small aliquots of a standard solution of D_2O in DMSO-d_6 with a microsyringe directly to a known amount of the compound in DMSO-d_6 in the NMR tube (capped with a rubber septum) to provide a calculated ratio of OD:OH = 1.0. When the hydroxyl signals were not desired, they were suppressed by adding to the solution a small quantity of a basic ion-exchange resin (Dowex 1X2-200, deuteroxide form) that was previously stirred in D_2O and lyophilized twice. The resin promoted exchange of the acidic hydrogen atoms with the deuterium atoms of the solvent, thus suppressing the unwanted signals. The solution was kept in the NMR tube for at least 2 days with occasional stirring before the measurements were made.

The two-dimensional double-quantum filtered (DQF) COSY spectra were recorded at room temperature (22 °C) in the phase-sensitive mode according to the time proportional phase incrementation method (Marion & Wüthrich, 1983). Data matrices of 1K × 512 points were accumulated with a spectral width of 1900 Hz and relaxation delay of 1.5 s; 16 scans per FID were collected. After zero filling to 1K × 1K points, the data were multiplied by a cosine function and Fourier transformed. The three-step relayed coherence transfer spectrum was recorded in magnitude mode (Wagner, 1983; Bax & Drobny, 1985). The data matrix had 2K × 250 points with a spectral width of 1260 Hz, and 64 scans per FID were collected with a relaxation delay of 1.3 s, the first fixed delay in the pulse sequence being set at 39 ms and the other two at 29 ms. After zero filling to 2K × 512 points, the data were multiplied by a sine-bell function and Fourier transformed. The homonuclear Hartmann–Hahn (HOHAHA) spectrum (Bax & Davis, 1985) was recorded with a mixing time of 300 ms and solvent suppression using the decoupler. The low-power output of the pulse amplifier was used to generate all pulses and to provide a spin lock with an rf field strength of 1900 Hz. The data matrix had 1K × 512 points with 24 scans per FID and a relaxation delay of 1.4 s. Before Fourier transformation, the data were zero-filled to 1K × 1K and multiplied by a sine-bell window function shifted by $\pi/2$.

Spectral Assignments. Because a limited amount of sample was available and its recovery from a DMSO solution difficult, the studies in D_2O were performed first. With a DQF-COSY spectrum (Rance et al. 1983), the assignments of the H-1 and H-2 protons of the molecule were straightforward, but assignments of the remaining ring protons were complicated by overlapping resonances in the region 3.4–4.2 ppm. To overcome this problem, a three-step relayed coherence transfer COSY spectrum (Wagner, 1983) was performed. This experiment, introduced to analyze networks of coupled spins allows the observation of long-range correlations (for example, H-1 to H-3 and H-1 to H-4) in a part of the spectrum that is less crowded than the region of ring protons (Homans et al., 1984).

After the measurements in D_2O were done, the sample was recovered, exchanged three times with H_2O, and dissolved in DMSO-d_6. The 2D spectra were recorded at 22 °C because the best resolution of hydroxyl signals was observed at this temperature. A DQF-COSY spectrum allowed the measurement of the chemical shifts of the ring protons vicinal to each hydroxyl group. The hydrogen-bonding studies were then performed as discussed later. Finally, to simplify the spectrum, the solution was treated with a basic ion-exchange resin to eliminate the hydroxyl signals, after which measurement of another DQF-COSY spectrum and a HOHAHA spectrum (Bax & Davis, 1985) allowed the assignment of the ring protons. The HOHAHA spectrum, like the multiple-step relayed coherence transfer COSY spectrum, produced long-range correlations that greatly simplified the spectral assignments. In the HOHAHA spectrum, however, coherence transfer is propagated more efficiently through the entire spin system and, given a sufficiently long spin-lock period, essentially all protons in the carbohydrate ring could be correlated with the corresponding anomeric proton (Figure 1). With the ring assignments in hand, it was possible to assign the hydroxyl signals to specific positions in the rings.

Hydrogen-Bonding Studies. To detect hydrogen bonds in solution, we analyzed the shift and broadening of hydroxyl proton signals on the addition of small quantities of D_2O to a solution of carbohydrate in DMSO-d_6 (Lemieux & Bock, 1979; Bock & Lemieux, 1982). When enough D_2O is added to provide half-exchange of all hydroxyl groups, those that are hydrogen bonded will experience an isotope shift due to the deuterium atom in species HO→DO and DO→HO as compared with HO→HO, because the isotope effect is transmitted through the hydrogen bond from one deuteroxyl group to the neighboring hydroxyl group. By this technique, direct evidence for hydrogen bonds has been found in methyl α-maltoside and analogues (Alvarado, 1987), sucrose (Lemieux & Bock, 1979), and cyclohexaamylose (Christofides & Davis, 1982). Hydrogen bonding in DMSO-d_6 has also been studied by measuring the temperature dependence of hydroxyl proton chemical shifts (St. Jacques et al., 1976), the chemical shifts of exchangeable protons moving upfield owing to disruption

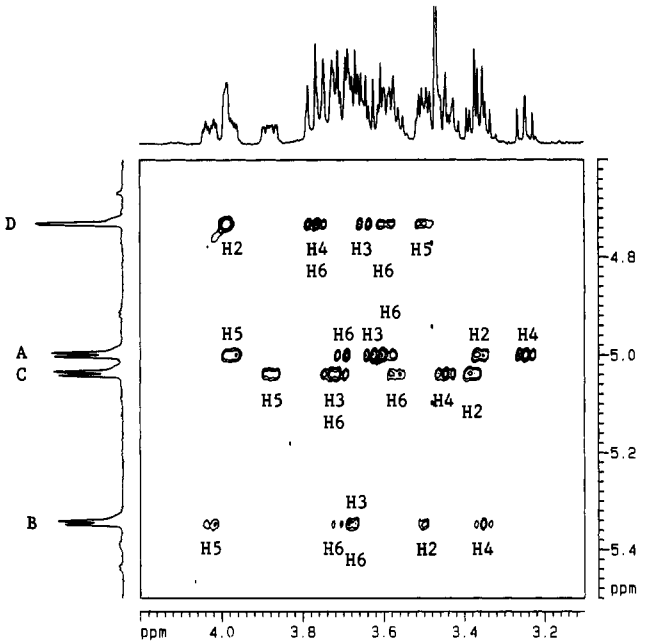

FIGURE 1: HOHAHA spectrum of the tetrasaccharide in DMSO-d_6. This figure is provided as an example of the kind of data used in making proton assignments. The dispersed two-dimensional spectrum allows correlation of all ring protons with H-1 signals of the individual hexose units. The H-1 signals were assigned previously on the basis of their coupling constants and from a comparison of the chemical shifts with reference disaccharides (Ogawa et al., 1983; Tsai et al., 1984), while the other protons were assigned herein on the bases of their chemical shifts, splitting patterns and interconnectivities of the cross-peaks. Hydroxyl proton signals were suppressed by addition of Dowex-1 (DO^-).

of hydrogen bonding with the solvent as the temperature is increased. A large dependence indicates that the proton is exposed and interacting (hydrogen bonded) with the solvent, whereas a small dependence indicates a proton shielded from the solvent or intramolecularly hydrogen bonded. On such evidence, intramolecular hydrogen bonds have been postulated in maltose, cyclohexaamylose, and amylose with OH-3 as the proton donor. The chemical shifts of the hydroxyl protons of the sample dissolved in DMSO-d_6 were measured in steps of 5 °C between 22 and 45 °C to obtain the temperature dependence.

HSEA Calculations. For an independent assessment of the conformation of the molecule and the possibility of hydrogen bond formation, an HSEA calculation was performed (Bock, 1983; Homans, 1990; Thogersen et al., 1982; Lemieux et al., 1980). The atomic coordinates of the constituent monosaccharides are from the neutron diffraction studies on methyl α-D-glucopyranoside and methyl α-D-mannopyranoside (Jeffrey et al., 1977), and the glycosidic angles were set to 117° for the calculations.

The glycosidic torsion angles ϕ, Ψ, and ω are the dihedral angles described as ϕ = H-1–C-1–O-1–C-x′, Ψ = C-1–O-1–C-x′–H-x′, and ω = O-6–C-6–C-5–H-5, where C-x′ and H-x′ are the aglyconic atoms (Lemieux & Koto, 1974). The signs of the angles are positive or negative according to the IUPAC-IUB recommendations (IUPAC-IUB, 1970, 1971). In this method, each monosaccharide ring is treated as a rigid entity and the conformational energy is calculated for different angles of ϕ and Ψ by taking into account van der Waals interactions of the substituents and the contribution of the exo-anomeric effect to the torsional movement of the glycosidic linkage. The optimum ϕ angle for α-D-glycosides, according to the exo-anomeric effect, is −60°. Therefore, the torsion angles ϕ and Ψ of the three glycosidic linkages were rotated in 5° increments between −60° and 0° to obtain the minimum values.

Table I: ^{1}H NMR Chemical Shifts[a]

proton	hexose unit A	B	C	D
		in D_2O		
H-1	5.183	5.528	5.248	4.845
H-2	3.606	3.694	3.645	4.102
H-3	3.785	3.833	3.903	3.900
H-4	3.458	3.516	3.63	3.85[b]
H-5	3.968	4.062	3.824	3.67[b]
		in DMSO-d_6		
H-1	5.000	5.345	5.040	4.735
H-2	3.360	3.500	3.380	3.985
H-3	3.625	3.675	3.73	3.650
H-4	3.248	3.355	3.450	3.77
H-5	3.975	4.030	3.880	3.50
H-6	3.59	3.66	3.570	3.60
H-6	3.705	3.73	3.72	3.77
OH-2	5.487		4.337	4.890
OH-3	4.935	4.758		
OH-4	4.950	5.023	5.422	5.086
OH-6	4.460	4.420	4.75	4.625

[a] At 22 °C referenced to internal acetone at δ 2.217. [b] Assignments uncertain owing to overlapping signals.

Results and Discussion

We have chosen to study the tetrasaccharide αGlc→^{2}αGlc→^{3}αGlc→^{3}αMan→$OCH_2CH_2CH_3$ as a model for the larger $Glc_3Man_9GlcNAc$ oligosaccharide because the smaller size allows us to assign nearly all of the protons in the molecule. That this was a valid choice is supported by the fact that the chemical shifts and coupling constants of the three glucose units in the two molecules are identical (Ogawa et al. 1983; Tsai et al., 1984).

Chemical shifts for all nonexchangeable protons of the tetrasaccharide in D_2O are listed in Table I, along with the values in DMSO-d_6 for all protons. As observed for sucrose (Lemieux & Bock, 1979), the shifts for nonexchangeable protons are similar in both solvents. Bock and Lemieux (1982) have argued that the small differences that are observed "result largely from general solvent effects" rather than gross differences in conformation. We find that, although the anomeric proton chemical shifts in D_2O are all slightly downfield from those in DSMO-d_6 (0.1–0.2 ppm), the relative shifts are similar for all protons suggesting that there is no significant effect of solvent on conformation. Certain of the hydroxyl protons, however, have anomalous chemical shifts in DMSO-d_6, notably OH-2^A (δ 5.487) and OH-4^C (δ 5.422), which are deshielded significantly relative to the other similar hydroxyl protons.

The effects of adding a limited amount of D_2O to the DMSO-d_6 solution are illustrated in Figure 2. The most dramatic changes occur with OH-2^A, which shows a pattern of two well-resolved doublets, and OH-4^C, which becomes a triplet formed by two overlapping doublets. Lemieux and Bock (1979) and Bock and Lemieux (1982) have reported isotope shifts in the signals for hydroxyl protons of sucrose in DMSO after partial exchange with deuterium, and they interpreted the effects in terms of the hydrogen-bonded formulations DMSO→HO→HO, DMSO→HO→DO, and DMSO→DO→HO. There is also evidence that the proton in the form DMSO→HO→DO undergoes a downfield shift whereas the proton in the form DMSO→DO→HO shows an upfield shift, thus providing a test for the directionality of the hydrogen bond (St. Jacques et al., 1976). Alvarado (1987) has used such isotope effects to analyze the conformation of methyl α-maltoside in DMSO and found that OH-3 and OH-3′ showed

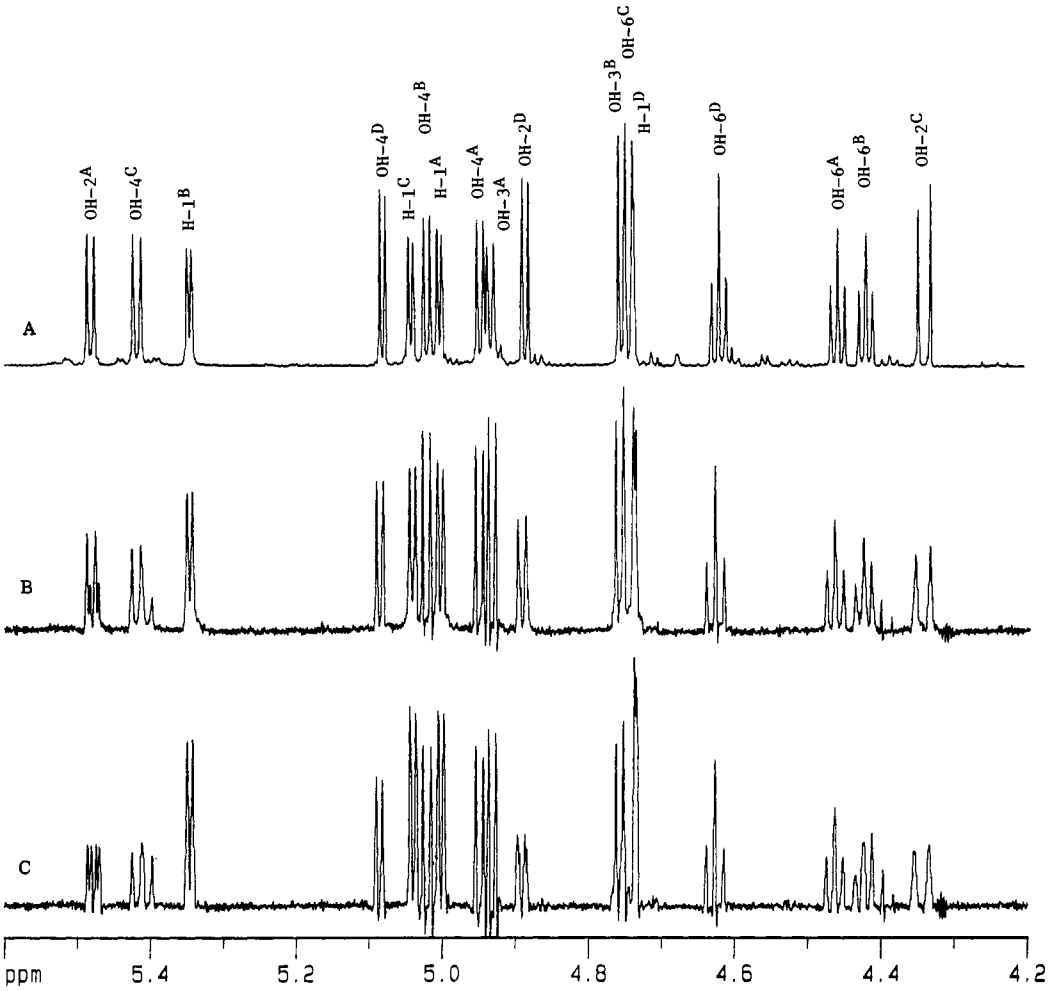

FIGURE 2: Partial ^{1}H NMR spectrum of the tetrasaccharide in DMSO-d_6. (A) The anomeric proton signals and all hydroxyl proton signals are shown. (B) After addition of D_2O to give a 1:2 ratio of DO/HO. (C) After addition of D_2O to give a 1:1 ratio of DO/HO. Major isotope shifts are seen in the signals for OH-2^A and OH-4^C, while the signals for OH-2^D, OH-6^B, and OH-2^C all show evidence of partial splitting or broadening. Spectrum A was taken at 600 MHz, while spectra B and C were at 500 MHz and were subjected to resolution enhancement, using a Gaussian function with a line broadening of 3.0 Hz and a Gaussian broadening of 0.2.

Table II: Deuterium-Induced Isotope Shifts[a]

hydroxyl group	isotope shift (ppm)[b]	hydroxyl group	isotope shift (ppm)[b]
OH-2^A	−4.6	OH-2^D	−2.3
OH-4^C	−14.3	OH-2^C	−2 (broad)

[a] In DMSO at 22 °C. [b] A negative shift is upfield.

upfield isotope shifts whereas OH-2 showed a downfield isotope shift, which results were offered as support for the hydrogen-bonded structure DMSO→HO-3→HO-2→HO-3′.

In the results we report here, the isotope shifts observed for OH-2^A and OH-4^C (Table II) are indicative of a hydrogen bond between hexoses A and C (Figure 3). Contrary to the results with methyl α-maltoside, both protons in the putative hydrogen-bonded system are shifted upfield. The strong temperature dependence of the chemical shift of OH-2^A (10.0 ppb/°C) compared to that of the other hydroxyls (4.5–7.3 ppb/°C) suggests, however, that it is the acceptor of the hydrogen bond. Some broadening of the signals for OH-2^D and OH-2^C, and perhaps OH-6^B, was also observed, but the signal for OH-4^D was unaffected, which rules out the second hydrogen bond postulated by Tsai et al. (1984).

The isotope shift on OH-2^D cannot be due to bonding to OH-2^C because these two are separated by 5.17 Å, but the large coupling constant of 9.8 Hz suggests an orientation in which OH-2^C is antiperiplanar to the C-2–H bond. If so, this

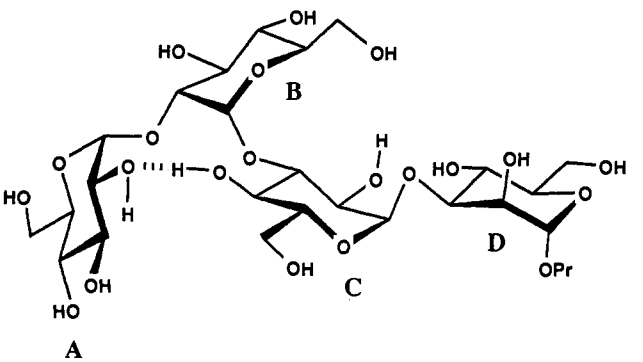

FIGURE 3: Proposed conformation of the tetrasaccharide showing the postulated hydrogen bond between OH-2^A and OH-4^C. The overall conformation approximates that required by the HSEA calculations, and the direction of the hydrogen bond is supported by the temperature dependence of the chemical shift of OH-2^A. Pr is $CH_2CH_2CH_3$.

hydroxyl group is in a position suitable for hydrogen bonding to O-1^C. Moreover, the upfield chemical shift (4.337 ppm) suggests that OH-2^C is not strongly bonded to solvent (Casu et al., 1966) and the low temperature dependence of its chemical shift (1.37 ppb/°C) suggests that it may be the donor in an intramolecular hydrogen bond. Similar features were found for methyl 4-*O*-(α-D-glucopyranosyl)-α-D-xylopyranoside and were offered in support of a hydrogen-bonded system of the type HOD←OH-2←O-1 (Alvarado, 1987). A hydrogen

Table III: Calculated Minimum-Energy Torsion Angles[a]

segment	structure	bond angle ϕ	bond angle φ
A→B	αGlc(1→2)αGlc	−45°	−30°
B→C	αGlc(1→3)αGlc	−40°	−20°
C→D	αGlc(1→3)αMan	−50°	−10°

[a] From molecular modeling (HSEA) calculations. The conformation resulting from these bond angles gives the following interatomic distances: $O\text{-}2^A$–$O\text{-}4^C$ = 3.0 Å, $O\text{-}6^B$–$O\text{-}2^C$ = 3.4 Å, $O\text{-}2^C$–$O\text{-}4^D$ = 4.1 Å, and $O\text{-}2^C$–$O\text{-}2^D$ = 5.2 Å.

bond to $OH\text{-}6^B$, however, cannot be ruled out. Finally, there is no obvious explanation for the apparent isotope shift on $OH\text{-}2^D$, since it appears to be well isolated from any other hydrogen-bonding group in the molecule.

Molecular modeling (HSEA) calculations suggest angles of ϕ and Ψ for the three interglycosidic bonds as shown in Table III. Assuming normal $^4C_1$ chair conformations and these angles, the calculated distance between $O\text{-}2^A$ and $O\text{-}4^C$ is 3.0 Å, a value that is consistent with the postulated hydrogen bond. The calculated distance between $O\text{-}6^B$ and $O\text{-}2^C$ is 3.4 Å, which is somewhat large for a hydrogen bond, but slight rotation of the C-5–C-6 bond could bring $O\text{-}6^B$ closer to $O\text{-}2^C$ to allow interaction.

It is notable that the calculated torsion angles between hexoses C and D are identical with those for the two sugars in methyl 4-*O*-(α-D-glucopyranosyl)-α-D-xylopyranoside (Alvarado et al., 1984; Alvarado, 1987). For many disaccharides, a steric interaction between H-5 of the nonreducing residue and some bulky group in the reducing residue is the limiting factor in achieving a conformation that is favorable in terms of the exo-anomeric effect. For methyl α-maltoside, the interatomic distance between H-5 and C-6′ is 2.8 Å, whereas the absence of the hydroxymethyl group in methyl 4-O-(α-D-glucopyranosyl)-α-D-xylopyranoside reduces the steric interaction and allows the substituent at C-5′ (H-5′ equatorial) to get closer to H-5 (2.3 Å) so that the preferred conformation occurs at $\phi = -50°$ and $\Psi = -10°$ (Alvarado, 1987). In the tetrasaccharide, the equatorial hydrogen at $C\text{-}2^D$, rather than an OH as in glucose, reduces the steric interaction with the proton at $C\text{-}5^C$ with the result that $\phi = -50°$ and $\Psi = -10°$, and a distance $H\text{-}5^C$ to $H\text{-}2^D$ = 2.3 Å is observed in the preferred conformation.

In the case of segments A–B and B–C of the tetrasaccharide, the controlling steric hindrance is given by $O\text{-}3^B$ and $O\text{-}2^C$, respectively. This is seen in the deshielding of $H\text{-}5^A$ (3.968 ppm) and $H\text{-}5^B$ (4.062 ppm) as compared with the chemical shift of H-5 of methyl α-glucopyranoside (3.64 ppm). The calculated interatomic distances $H\text{-}5^A$–$O\text{-}3^B$ = 2.6 Å and $H\text{-}5^B$–$O\text{-}2^C$ = 2.5 Å are similar to the sum of the van der Waals radii of hydrogen and oxygen (2.45 Å), in agreement with the postulated solution conformation of the tetrasaccharide.

The NMR data we have obtained in DMSO provide strong support for a hydrogen bond between glucoses A and C in the tetrasaccharide, and the HSEA calculations suggest a preferred conformation for the molecule that is consistent with this result. Moreover, the similarity between the spectra in water and DMSO suggests that the conformations are not dramatically different in the two solvents. We conclude, therefore, that the tetrasaccharide and the corresponding fragment in the oligosaccharide $Glc_3Man_9GlcNAc_2$ have a fixed conformation in water in which the three glucose units form a somewhat flattened trimeric unit that could serve for specific recognition by a part of an oligosaccharyltransferase complex such as that postulated by Geetha-Babib et al. (1988). Inspection of a space-filling model of the tetrasaccharide in the proposed conformation reveals a slight difference in hydrophobicity between the two faces of the glucotriose unit, which offers the possibility that one of the faces could interact preferentially with a hydrophobic cleft in the enzyme and modulate its activity. This is a testable model and one that may lead to new insights into the factors that regulate protein glycosylation.

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Conformational Transitions of Gramicidin A in Phospholipid Model Membranes. A High-Performance Liquid Chromatography Assessment†

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ABSTRACT: We have investigated the conformation of gramicidin A reconstituted in different phospholipid environments, small unilamellar vesicles, extensive bilayers, and micelles, by exploiting a recently proposed experimental approach based on high-performance liquid chromatography [Bañó et al. (1988) *J. Chromatogr. 458*, 105; Bañó et al. (1989) *FEBS Lett. 250*, 67]. The method allows the separation of conformational species of the peptide, namely, antiparallel double-stranded (APDS) dimers and $\beta^{6.3}$-helical monomers, and quantitation of their proportions in the lipid environment. Various experimental parameters (e.g., nature of organic solvent, time of incubation in organic solvent, lipid-to-peptide mole ratio, time of sonication, and temperature) commonly involved in sample preparation protocols have been analyzed independently. The results show how the peptide conformation in model membranes is exquisitely dictated by the particular nature of the reconstitution protocol. In addition, we have elucidated the nature of the slow conformational transition of gramicidin toward the channel configuration that takes place upon incubation of the model membranes. This transition has been characterized as a temperature-dependent conversion from APDS dimeric to $\beta^{6.3}$-helical monomeric forms. Analysis of kinetic data permits an accurate calculation of the rate constant for this process at different temperatures in phospholipid vesicles and micelles. Finally, an explanation is proposed for the laboratory-to-laboratory variation in the observed spectral patterns of inserted gramicidin. Evidence is presented that a given circular dichroism spectrum can be attributed mainly to the contributions of two well-defined conformational species, APDS dimers and $\beta^{6.3}$-helical monomers, coexisting in the lipid environment in a proportion which is in turn determined by the specific protocol followed for sample preparation.

The conformation of the transmembrane channel forming hydrophobic pentadecapeptide gramicidin A, in organic solvent and especially in phospholipid model membranes, has been the subject of extensive study in a number of laboratories for the last 2 decades. As a result, a variety of different models have been suggested for its structure, essentially inferred from spectroscopic techniques such as circular dichroism (CD),[1] nuclear magnetic resonance (NMR), and infrared spectroscopy (IR) [for reviews, see Urry (1985), Wallace (1986, 1990), and Cornell (1987)], diffraction studies (Wallace & Ravikumar, 1988; Langs, 1988, 1989), and theoretical considerations (Venkatachalam & Urry, 1983; Pullman & Etchebest, 1987). Among the most relevant, parallel (PDS) and antiparallel double-stranded (APDS) helical dimers and head-to-head (HH), head-to-tail (HT), and tail-to-tail (TT) β-helical dimers have been proposed. It has been thoroughly demonstrated and seems at present well established that the HH dimer (N-terminus-to-N-terminus dimer) is actually the predominant configuration of the active transmembrane cation channel (Urry et al., 1983a; Andersen, 1984; Wallace, 1986). PDS, HT, and TT dimers appear to be much less stable than APDS and HH dimers on the basis of their diminished ability for hydrogen bonding (Sung & Jordan, 1989), and, moreover, available evidence has not supported so far their presence in reconstituted model membranes. As concerning APDS dimers, however, recent experimental work by Durkin et al. (1987) and theoretical molecular mechanics studies (Sung & Jordan, 1988, 1989) have led to a reconsideration of the possible significance of this configuration by suggesting that, if formed in the membrane, it might also behave as an ionic channel.

CD spectroscopy (and to a lesser extent NMR) has demonstrated in the past to be a very sensitive technique to show that gramicidin can adopt different conformations in different lipid environments (Urry et al., 1979; Masotti et al., 1980; Wallace et al., 1981) and that the spectra can also vary considerably depending on the conditions used for reconstitution (Urry et al., 1982, 1983a,b). In this context, samples that were prepared by cosolubilization of gramicidin and phospholipid exhibited different CD patterns depending on the peptide:lipid

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[1] Abbreviations: APDS, antiparallel double stranded; CD, circular dichroism; DMSO, dimethyl sulfoxide; HH, head-to-head; HPLC, high-performance liquid chromatography; IR, infrared; LPC, egg yolk lysophosphatidylcholine; MLV, multilamellar vesicle(s); NMR, nuclear magnetic resonance; PC, egg yolk phosphatidylcholine; SUV, small unilamellar vesicle(s); TFE, trifluoroethanol; THF, tetrahydrofuran.

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