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GLYCAN CONFORMATIONAL CHANGE IN MESOMORPHIC STRUCTURES OF TERNARY SYSTEMS: LIPOSACCHARIDE/PHOSPHOLIPID/WATER

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An amphipatic liposaccharide, β16, has been synthesized by condensation of the glycoamino acid β of ovomucoid with the palmitic acid to serve as a model on which the properties of the saccharide chains can be studied. This paper reports the ternary system β16/dipalmitoylphosphatidylcholine (DPPC)/water. Using X-ray diffraction and freeze-fracture electron microscopy, it was shown that the ternary system exhibits mesomorphic structures in the temperature range over which the aliphatic chains of the DPPC are in a liquid-like conformation. A phase diagram of the system was drawn at 75°C in terms of the water concentration and of the β16 content. As the molar fraction in β16 increases from about 0.08 to 1, the ternary system displays successively two lamellar structures analogous to that exhibited by the system DPPC/H_2O, then a hexagonal structure similar to that exhibited by the system β16/H_2O. The two types of lamellar structure were shown to differ by the T or Y conformation adopted by their saccharide chains.

Introduction

Among membranous proteins, glycoproteins are of particular interest. Through their carbohydrate moieties, they are receptors for many biological effectors such as viruses, proteins, enzymes, specific antibodies and hormones and they play an important part in intercellular adhesion and recognition [1]. But membranous glycoproteins are very complicated molecules and are difficult to isolate in quantities allowing detailed physicochemical study, so it is interesting to use models on which the properties of their saccharide chains can be studied. For that purpose two types of amphipatic block copolymer were synthesized and studied [2,3]. In these copolymers the hydrophilic block is the glycoamino acid 0β from hen egg-white ovomucoid (its primary structure is given in Fig. 1) and the hydrophobic block is a peptide or a lipid block.

The glycoamino acid 0β consists of a rigid core formed by the sequence GlcNAc-Man-$(GlcNAc)_2$-Asn and two antennae branched at the mannose of the core and formed by the sequence:

$$\begin{array}{ll} \text{Man-GlcNAc-} & \text{Man-GlcNAc} \\ | & | \\ \text{GlcNAc} & (\text{GlcNAc-Gal})_{0\text{ or }1} \end{array}$$

Molecular models show that the antennae can turn around the Man-Man$\alpha_{1,3}$ and Man-Man$\alpha_{1,6}$ linkages and take up different orientations in relation to the rigid core, leading to different conformations of the saccharide block.

As a matter of fact, two types of conformation of the saccharidic 0β chains were found in block

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine.

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copolymers 0βEb with a hydrophobic polypeptide block of poly(γ-benzyl-L-glutamate) (Eb). Copolymers 0βEb exhibit in the dry state and in concentrated dimethylsulfoxide (DMSO) solution, mesomorphic lamellar structures in which the polypeptide chains adopt an α helical conformation and the saccharide 0β block takes up a T- or a Y-shaped conformation, depending on the molecular weight of the polypeptide block [2].

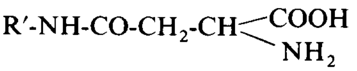
Liposaccharides $0\beta C_n$, or in a simplified expression, 0β*n*, in which the hydrophobic moiety is formed by the paraffin chain of a fatty acid containing *n* carbon atoms (*n* varying from 8 to 24), have been shown to exhibit a cylindrical hexagonal structure in the dry state and in concentrated water solutions [3]. The absence of lamellar structure for liposaccharides is probably the consequence of an unfavourable volume ratio between the hydrophilic and hydrophobic moieties of the molecule. But, by adding phospholipids to liposaccharides, one can modify at will the hydrophilic-hydrophobic balance of the system and restore the possibility of existence of lamellar structures and perhaps of conformational changes for the saccharide chains. Furthermore, this method provides model membranes that will allow the study of the effect of the conformation of the saccharide chains of glycoproteins on their specificity of interaction with biological effectors.

For all the preceding reasons we have undertaken the structural study of liposaccharide/phospholipid/water systems. This paper reports on the ternary system β16/DPPC/water. This system exhibits mesophases in the temperature range over which the aliphatic chains of the DPPC are in a liquid-like conformation [5]. Mesomorphic structures were studied using X-ray diffraction and freeze-fracture electron microscopy. We describe the phase diagram of the system at 75°C, the structure of the mesophases and the organization of the components in these structures.

Materials and Methods

Materials

Ovomucoid was extracted from hen egg white [6] and used as saccharide source. After purification [7] ovomucoid was submitted to pronase hydrolysis [8]. The resulting glycoamino acids were purified and fractionated by column chromatography [8]. Two glycoamino acids were obtained: the α fraction (containing *N*-acetylneuraminic acid) and the β fraction (an asialoglycoamino acid) [9]. The β fraction has a molecular weight of 3134 and contains 16 saccharide residues (one galactose, five mannose, 10 *N*-acetylglucosamine) [4]. It is terminated by an asparagine residue linked by its side-chain to the reducing group of an adjacent *N*-acetylglucosamine residue (Fig. 1). Amphipatic liposaccharides β*n* were obtained by the formation of an amide linkage between the α amino group of the glycoamino acid



$$R'\text{-NH-CO-CH}_2\text{-CH}\begin{matrix}\text{COOH}\\ \text{NH}_2\end{matrix}$$

and the carboxyl group of the fatty acid R-COOH [3]. As the liposaccharide used for the present study was prepared with palmitic acid, it will be called β16. DPPC was purchased from Fluka.

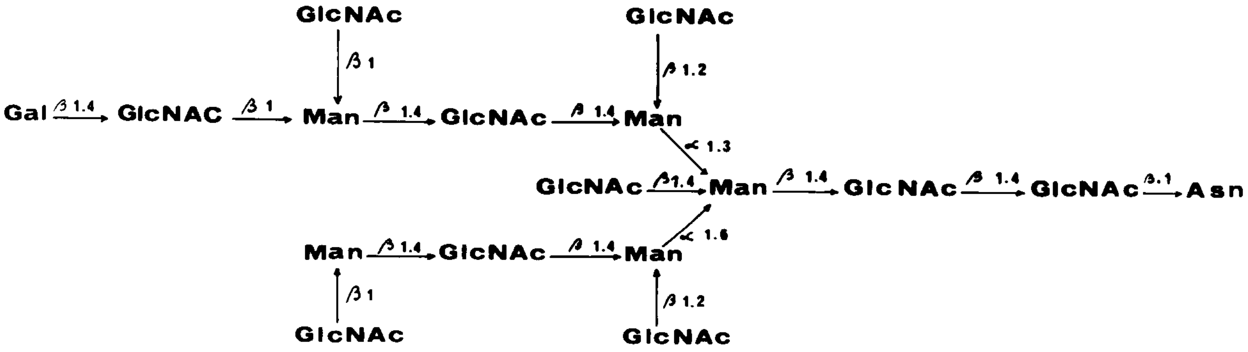


Fig. 1. Primary structure of the glycoamino acid extracted from hen egg white ovomucoid [4].

Silica-gel thin-layer chromatography showed a single spot.

Mesomorphic gels

Mesomorphic gels of systems DPPC/H_2O and β16/H_2O were prepared by swelling a known amount of the material in a known amount of water. Good homogeneity was obtained by heating. After studying the systems at 75°C, the gels were evaporated to dryness. Possible degradation of the dried material was checked by silica-gel chromatography for the DPPC and by high-pressure liquid chromatography (HPLC) for the liposaccharide β16. The results were negative.

For the preparation of the mesomorphic gels of the system β16/DPPC/H_2O, appropriate quantities of DPPC and β16 were first dissolved in an excess of solvent $CH_3OH/CHCl_3/H_2O$ (3:2:1, v/v). The solvent was carefully removed under vacuum. Then an excess of double-distilled water was added to the dry material and, after homogeneity had been realized by heating, the desired concentration was obtained by slow evaporation of water. Concentrations were checked by evaporating to dryness in vacuo at 100°C after X-ray experiments.

Freeze-fracture electron microscopy

Samples were placed in the recess of a 3 mm gold disc and equilibrated for several minutes at the desired temperature in an atmosphere saturated in water. Then they were plunged into partially solidified freon 22 and transferred to liquid nitrogen. Quenched samples were fractured at -120°C in a Balzers BAF 301 unit under a vacuum of about $2 \cdot 10^{-6}$ Torr. The fracture surfaces were replicated with platinum (Pt) and carbon (C). Pt-C replicas were cleaned with $CHCl_3$, $CHCl_3$/MeOH/H_2O (3:2:1, v/v) or EtOH/H_2O (5:100, v/v) according to the content of the gels in β16. Replicas were collected on 300-mesh copper grids and examined in a Siemens Elmiscop 102 electron microscope.

X-ray diffraction

X-ray diffraction studies were carried out under vacuum in a Guinier type camera. Strictly monochromatic X-ray (Cu, Kα_1) is isolated by a bent quartz monochromator and focussed as a sharp line. The camera is equipped for recording the diffraction patterns from samples held at various temperatures with an accuracy of ±1°C.

Structural determination

X-ray diffraction patterns provided by the ternary system β16/DPPC/H_2O in domains in which mesophases exist are characterized by the presence in the wide-angle region of a diffuse band at 4.5 $Å^{-1}$ typical of the disordered liquid-like conformation (type α) of the paraffin chains, and in the low-angle region by a set of sharp lines, the ratio of the Bragg spacings of which varies with the type of structure.

Lamellar structure. The low-angle X-ray patterns are characterized by the presence of a set of sharp lines with Bragg spacings in the ratio 1, 2, 3, 4 characteristic of a layered structure. The lamellar structure consists of plane, parallel, equidistant sheets of thickness d. Each sheet results from the superposition of two layers: one, of thickness d_B, is formed by the paraffin chains; the other, of thickness d_A, contains the β saccharide chains, the AsnCO groups of the liposaccharide β16, the polar head groups of the DPPC and the water.

The total thickness, $d = d_A + d_B$, of a sheet is given directly by the Bragg spacings of X-ray patterns.

The thicknesses d_A and d_B defined above and the average area, S_P, available to each paraffin chain at the interface of the layers can be calculated using equations based on simple geometrical considerations:

$$d_B = d_{(P_1+P_2)} = d\left[\Phi_{P_1} + \Phi_{P_2}\right] \tag{1}$$

where Φ_{P_1} represents the volume ratio of each paraffin chain of the DPPC in the solution and Φ_{P_2} represents the volume ratio of the paraffin chain of the liposaccharide β16 in the solution

$$\Phi_{P_1} = C_{DPPC} \cdot X_{P_1} \cdot V_P \cdot K^{-1} \tag{2}$$

$$\Phi_{P_2} = C_{\beta 16} \cdot X_{P_2} \cdot V_P \cdot K^{-1} \tag{3}$$

where

$$K = C_{\text{DPPC}}\left(X_{\text{P}_1} \cdot V_{\text{P}} + X_{\text{pol}} \cdot V_{\text{pol}}\right) + C_{\beta 16}\left(X_{\text{P}_2} \cdot V_{\text{P}} + X_{\text{AsnCO}} \cdot V_{\text{AsnCO}} + X_{\beta} \cdot V_{\beta}\right) + C_{\text{H}_2\text{O}} \quad (4)$$

and where: C_{DPPC} is the mass ratio of DPPC in the solution; $C_{\beta 16}$ is the mass ratio of $\beta 16$ in the solution; $C_{\text{H}_2\text{O}}$ is the mass ratio of H_2O in the solution; X_{P_1} is the mass ratio of the hydrophobic paraffin chains in DPPC; X_{P_2} is the mass ratio of the hydrophobic paraffin chain in $\beta 16$; V_{P} is the specific volume of the paraffinic chains in the α conformation. V_{P} is estimated by adding the volumes occupied by the CH_2 and CH_3 groups in liquid paraffins and corrected for temperature using the volume thermal expansion coefficient $(\partial v/\partial t)(1/v) = 7.48 \cdot 10^{-4}\ (^\circ\text{C})^{-1}$ [10]. The result is $V_{\text{P}}\ (75^\circ\text{C}) = 1.28\ \text{cm}^3 \cdot \text{g}^{-1}$; X_{pol} is the mass ratio of the polar head group of DPPC; V_{pol} is the specific volume of the polar head group of DPPC. V_{pol} is taken from Ref. 11 and equals $0.664\ \text{cm}^3 \cdot \text{g}^{-1}$; X_{AsnCO} is the mass ratio of the AsnCO group in $\beta 16$; V_{AsnCO} is the specific volume of the AsnCO group in $\beta 16$: V_{AsnCO} is evaluated by adding the molar volumes of the Asn and CO groups. The Asn molar volume is calculated from its density measured by pycnometry, the CO molar volume is obtained from the density of ketones with hydrocarbon chains of different lengths. $V_{\text{AsnCO}} = 0.57\ \text{cm}^3 \cdot \text{g}^{-1}$; X_{β} is the mass ratio of the saccharide block in $\beta 16$; V_{β} is the specific volume of the saccharide block in $\beta 16$. V_{β} is taken from Ref. 8 and equals $0.62\ \text{cm}^3 \cdot \text{g}^{-1}$.

The specific surface S_{P} of each paraffin chain given by the equation

$$S_{\text{P}} = \frac{2M_{\text{P}} \cdot V_{\text{P}}}{N\, d_{(\text{P}_1+\text{P}_2)}} \quad (5)$$

where M_{P} is the molecular weight of the paraffin chains = 211; and N is Avogadro's number.

Hexagonal structure. The low-angle X-ray diffraction patterns are characterized by the presence of a set of sharp lines with Bragg spacings in the ratio $1, \sqrt{3}, \sqrt{4}$, which is typical of a two-dimensional hexagonal array. Such a structure consists of long, parallel cylinders arranged in a two-dimensional hexagonal array. The cylinders are filled with the hydrophobic fatty acid chains. The polar head group of the DPPC, the saccharide chains and the AsnCO group of the liposaccharide $\beta 16$ and the water occupy the space between the cylinders (Fig. 2).

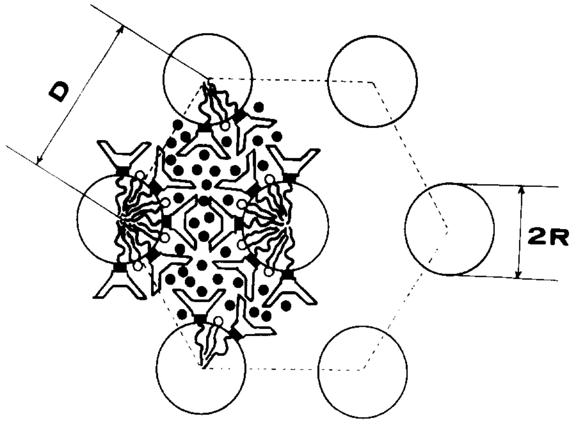


Fig. 2. Schematic representation of the hexagonal structure displayed by the system $\beta 16/\text{DPPC}/H_2O$ at 75°C. D is the spacing between two neighbouring cylinders; $2R$ is the diameter of the cylinders containing the aliphatic chains of the components (〰). The space between the cylinders contains the AsnCO groups of the $\beta 16$ (■), the saccharide chains of $\beta 16$ (Y), the polar head-groups of the DPPC (○) and the water (●).

The distance, D, between the axis of two neighbouring cylinders is calculated from the Bragg spacings on the X-ray patterns.

The diameter, $2R$, of each cylinder and the average area, S_{P}, available to each paraffin chain on the lateral surface of the cylinders are given by the following equation:

$$R^2 = \frac{D^2\sqrt{3}}{2\pi} \cdot | V_{\text{P}}\left[C_{\text{DPPC}} \cdot X_{\text{P}_1} + C_{\beta 16} \cdot X_{\text{P}_2}\right] \cdot K^{-1} \quad (6)$$

$$S_{\text{P}} = \frac{2M_{\text{P}}V_{\text{P}}}{R \cdot N} \quad (7)$$

Results and Discussion

X-ray diffraction patterns of the ternary system $\beta 16/\text{DPPC}/H_2O$ were recorded at 75°C and for

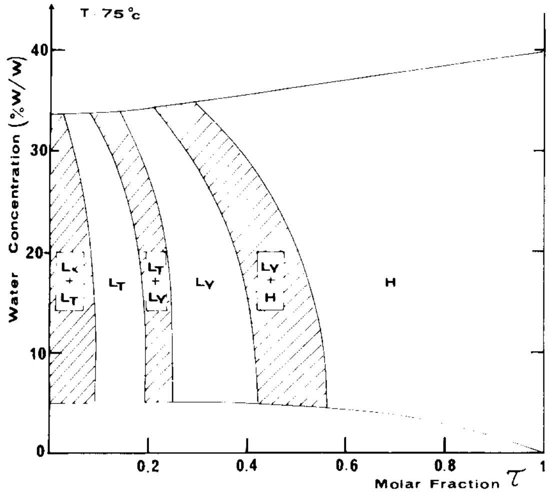


Fig. 3. Phase diagram of the ternary system $\beta 16/DPPC/H_2O$ at 75°C in terms of the water concentration and of the molar fraction τ in $\beta 16$.

various contents in each component. This study has allowed the phase diagram of the system to be drawn in terms of the water concentration and of the molar fraction, τ, in $\beta 16$ (Fig. 3). The molar fraction, τ, is defined by the ratio:

$$\tau = \frac{n_{\beta 16}}{n_{\beta 16} + n_{\mathrm{DPPC}}} \tag{8}$$

where $n_{\beta 16}$ is mol $\beta 16$ and n_{DPPC} is mol DPPC in the solution.

For $\tau = 0$, the system $DPPC/H_2O$ was shown to exhibit a lamellar structure, Lα, with aliphatic chains in a liquid-like conformation for water concentrations ranging from about 5 to 34% (unpublished results).

For $\tau = 1$, the system $\beta 16/H_2O$ was verified to exhibit a hexagonal structure for water concentrations ranging from 0 to about 40%. So in Fig. 3, lower and upper lines are drawn that represent the limits of the water concentration range studied.

The phase diagram of the ternary system $\beta 16/DPPC/H_2O$ exhibits three domains in which a pure mesomorphic structure is present. For values of τ ranging from 1 to about 0.5, a hexagonal structure, H, is displayed. As τ decreases from about 0.38 to 0.24 and then from 0.18 to 0.08, two pure lamellar structures are successively displayed. Such structures are denoted LY and LT, respectively.

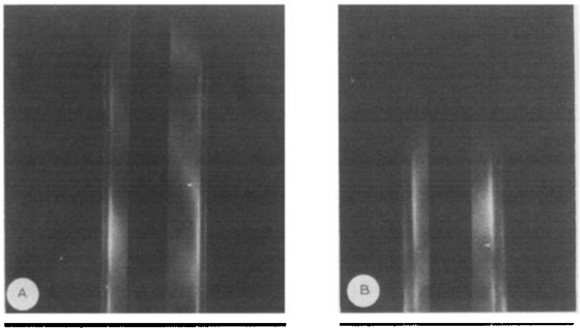


Fig. 4. Low-angle X-ray diffraction patterns of the system $\beta 16/DPPC/H_2O$. A, for values of τ under about 0.08; B, for values of τ ranging from 0.18 to 0.24.

For values of τ under about 0.08, each low-angle X-ray diffraction pattern exhibits two sets of lines with Bragg spacings typical of layered structures (Fig. 4A). Bragg spacings of one set of lines take the same values as the Bragg spacings found for the Lα structure. Bragg spacings of the second set of lines are the same as those obtained for the LT structure. Therefore, the LT lamellar structure is different from the Lα lamellar structure.

For values of τ between about 0.18 and 0.24, each negative displays again two sets of lines with Bragg spacings typical of layered structures (Fig. 4B). The Bragg spacings of one set of lines correspond to the Bragg spacings of the LT lamellar structure. The Bragg spacings of the second set of lines are the same as those obtained for the LY lamellar structure. Therefore the LT and LY are two different lamellar structures.

(A) Hexagonal structure

Freeze-fracture micrographs of the hexagonal structure (Fig. 5) are characterized by the presence of regular striations which correspond to the fracture jumps from one hydrophobic cylinder to another. The ripple repeat distance is in good agreement with the X-ray diffraction data.

The variation in the structural parameters was studied in terms of both the liposaccharide molar ratio, τ, and the water content of the system. Fig. 6 illustrates the variation with τ of the structural parameters for $C_{H_2O} = 35\%$.

As τ increases from about 0.30 to 1:

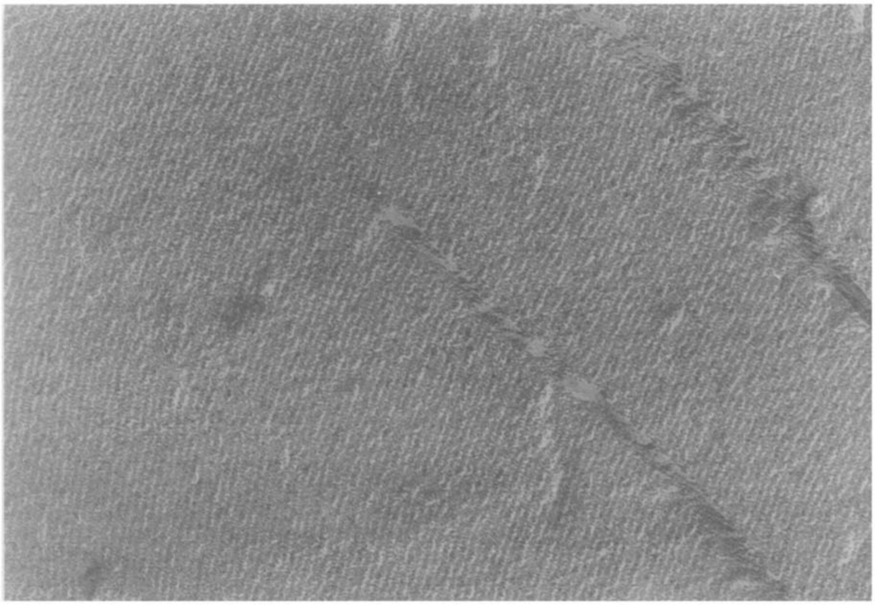


Fig. 5. Freeze-fracture electron micrograph of the hexagonal structure of the system β16/DPPC/H_2O; $\tau = 0.80$, $C_{H_2O} = 20\%$, magnification, ×137000.

the spacing, D, between the axes of two neighbouring cylinders decreases from 98 to 85 Å;
the diameter, $2R$, of the cylinders containing the aliphatic chains of both the phospholipid and the liposaccharide decreases from 48 to 23 Å;
the average area, S, available to each paraffin chain on the lateral surface of the cylinders increases from 37 to 80 Å^2;
the volume of the hydrophobic part of the liposac-

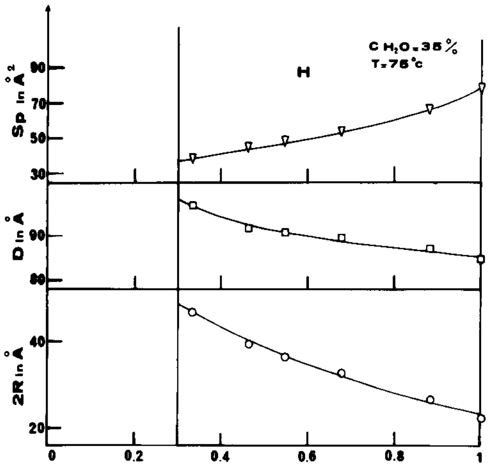


Fig. 6. Variation with τ of the geometrical parameters of the hexagonal structure of the system β16/DPPC/H_2O for $C_{H_2O} = 35\%$. □, D; ○, $2R$; ▽, S_P.

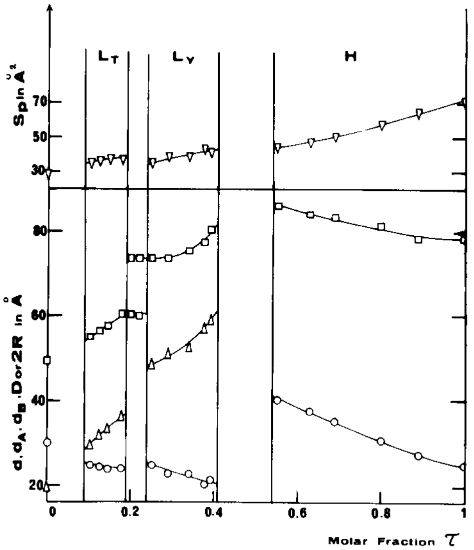


Fig. 7. Variation with τ of the geometrical parameters of the mesomorphic structures displayed by the system β16/DPPC/H_2O for $C_{H_2O} = 15\%$. Lamellar structures: □, d; △, d_A; ○, d_B; ▽, S_P. Hexagonal structure: □, D; ○, $2R$; ▽, S_P.

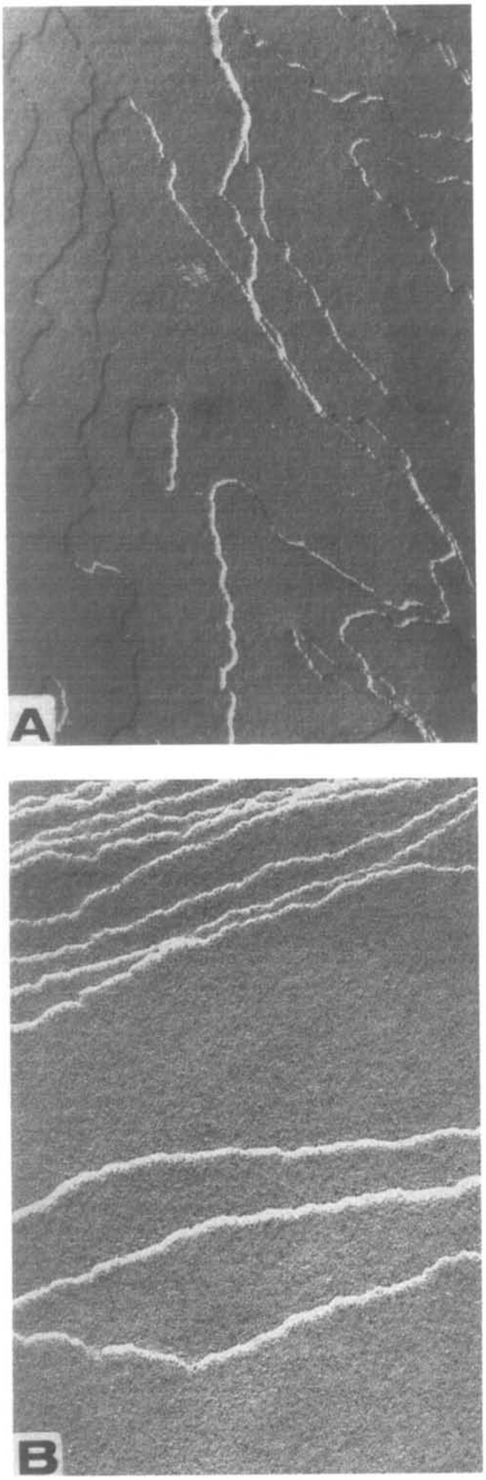


Fig. 8. Freeze-fracture electron micrographs of the lamellar structures of the system β16/DPPC/H_2O. A, lamellar structure LT, $\tau = 0.12$; $C_{H_2O} = 20\%$; magnification, ×114000. B, lamellar structure LY, $\tau = 0.28$; $C_{H_2O} = 22\%$; magnification, ×137000.

charide is higher than that of the polar head group of the DPPC, so an increase in τ involves an increase of the specific surface, S_P, of the paraffin chains. The volume of each hydrophobic paraffin chain remains constant so that the diameter of the cylinders decreases to compensate the increase in S_P.

In Fig. 7 is shown the variation of the structural parameters with τ for $C_{H_2O} = 15\%$.

As can be seen from comparison of Figs. 6 and 7, the increase in the water concentration from 15 to 35% involves an increase of about 10 Å in the spacing, D, between the axes of two neighbouring cylinders. But the effect of the water concentration on the diameter of the cylinders and on the specific surface S_P is very small.

(B) Lamellar structures

On the freeze-fracture images obtained for lamellar structures (Fig. 8) are seen stacked smooth areas like those revealed by the freeze-cleavage of lipid bilayers [12,13].

The variation in the geometrical parameters of the lamellar structures was studied as a function of the molar fraction, τ, of the liposaccharide and of the water concentration. The influence of τ is similar for all the water concentrations studied. It is illustrated in Fig. 7 for a water concentration of 15%.

The variation in all the structural parameters presents a discontinuity at a τ value of about 0.22. This discontinuity confirms the existence of two lamellar structures.

Within the two domains separated by the discontinuity, as τ increases:
the intersheet spacing, d, increases;
the thickness, d_B, of the hydrophobic layer formed by the aliphatic chains of both the phospholipid and the liposaccharide decreases slightly;
the thickness, d_A, of the hydrophilic layer containing the polar groups of the phospholipid, the saccharide chains and the AsnCO groups of the liposaccharide and the water increases;
the average area, S_P, available to each paraffin chain at the interface of the layers slightly increases.

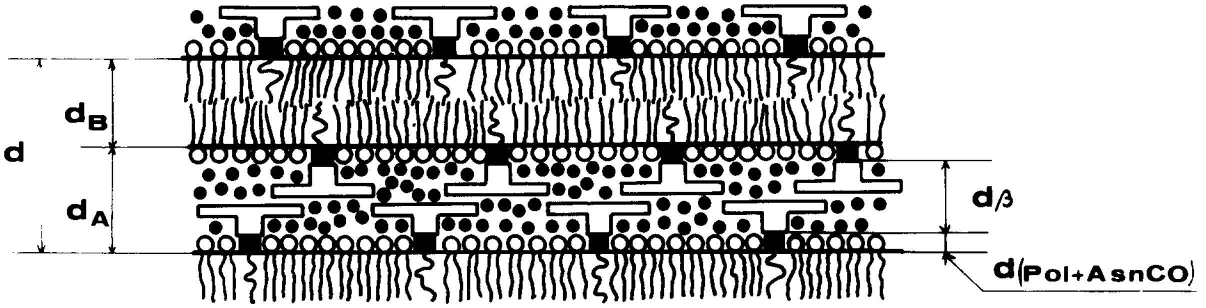


Fig. 9. Schematic representation of the LT lamellar structure. d_B = thickness of the hydrophobic layer containing the aliphatic chains of the components (〰). d_A = thickness of the hydrated layer. d_β = thickness of the hydrated lamella containing the saccharide chains of the β16 (⊤) and the water (●). $d_{(\mathrm{pol+AsnCO})} = d_A - d_\beta$ = thickness of the hydrated lamellae containing the polar head groups of the DPPC (○), the AsnCO groups of the β16 (■) and the water (●).

Values of the specific area, S_P, give some idea of the area, S_{pol}, available to each polar head group of the DPPC at the interface of the hydrophobic and the hydrophilic layers. For the values of S_{pol} to remain within the limits determined by S_P, it is necessary to suppose that the hydrated layer results from the superposition of three lamellae: two lamellae containing the polar head groups of the DPPC and the AsnCO groups of the liposaccharide are separated by a third lamella formed by the β saccharide chains (Figs. 9, 10).

The discontinuity observed at a τ value of about 0.22 corresponds to a sudden and large increase in the intersheet spacing, d, and in the thickness, d_A, of the hydrophilic layer, suggesting that the two types of lamellar structure differ by the arrangement of the components in the hydrated lamellae.

The surface, S_β, available for each saccharide chain at the interface of the hydrated lamellae was calculated from the values of S_P and τ using the equation:

$$S_\beta = S_P\left[1 + 2\frac{1-\tau}{\tau}\right] \tag{9}$$

The specific areas S_{pol} and S_{AsnCO} were calculated using the following equations:

$$S_{\mathrm{pol}} = \frac{2M_{\mathrm{pol}} \cdot V_{\mathrm{pol}}}{N \cdot d_{(\mathrm{pol+AsnCO})}} \tag{10}$$

and

$$S_{\mathrm{AsnCO}} = \frac{2M_{\mathrm{AsnCO}} \cdot V_{\mathrm{AsnCO}}}{N \cdot d_{(\mathrm{pol+AsnCO})}} \tag{11}$$

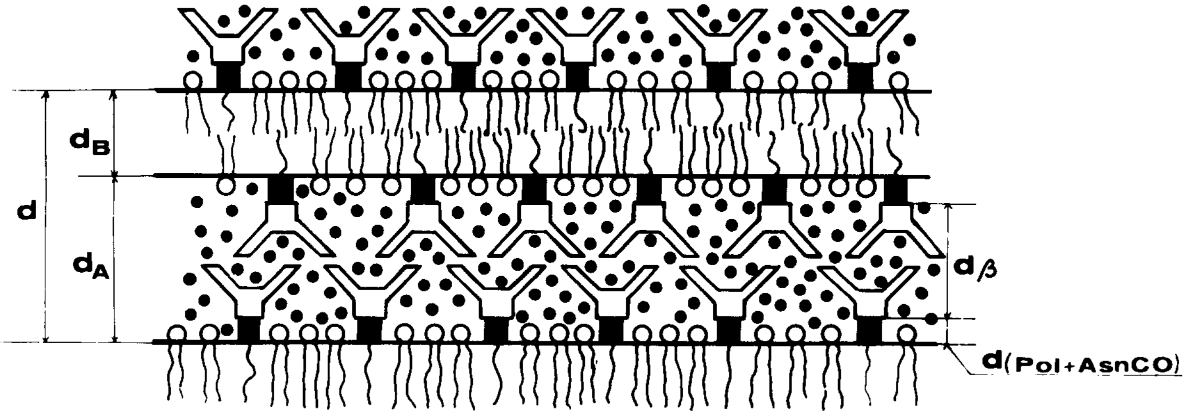


Fig. 10. Schematic representation of the LY lamellar structure. For symbols, see Fig. 2.

where $M_{pol} = 311.7$: molecular mass of a polar head of DPPC; $M_{AsnCO} = 158$: molecular mass of a AsnCO group of the liposaccharide; $d_{(pol+AsnCO)}$ is the thickness of the lamella containing the polar head groups of the DPPC and the AsnCO groups of the liposaccharide.

The thickness, $d_{(pol+AsnCO)}$, of the lamella containing the polar head groups of the DPPC and the AsnCO groups of the liposaccharide, and the thickness, d_β, of the lamella containing the β saccharide chains can be calculated using the following equations:

$$d_{(pol+AsnCO)} = d\Big[C_{DPPC} \cdot X_{pol} \cdot V_{pol} + C_{\beta 16} \cdot X_{AsnCO} \cdot V_{AsnCO} + C_{(H_2O \cdot AsnCO)} + C_{(H_2O \cdot pol)}\Big] \cdot K^{-1} \tag{12}$$

$$d_\beta = d\Big[C_{\beta 16} \cdot X_\beta \cdot V_\beta + C_{(H_2O \cdot \beta)}\Big] \cdot K^{-1} \tag{13}$$

where:

$$C_{(H_2O \cdot pol)} = C_{H_2O} \cdot \frac{f_{pol} \cdot n_{pol}}{n_{H_2O}} \tag{14}$$

$$C_{(H_2O \cdot AsnCO)} = C_{H_2O} \cdot \frac{f_{AsnCO} \cdot n_{AsnCO}}{n_{H_2O}} \tag{15}$$

$$C_{(H_2O \cdot \beta)} = C_{H_2O} \cdot \frac{f_\beta \cdot n_\beta}{n_{H_2O}} \tag{16}$$

and f_{pol}, f_{AsnCO} and f_β are the numbers of water molecules respectively associated with a polar head of the DPPC, an AsnCO group of the liposaccharide and a β saccharide chain; n_{pol}, n_{AsnCO} and n_β are the numbers of polar heads, of AsnCO groups and of β chains in the solution; n_{H_2O} is the total number of water molecules in the solution.

As it is impossible to measure the degree of hydration of each component, the calculations were made for various values of f_{pol}, f_{AsnCO} and f_β. The results were compared with the values of d_{pol} and d_β obtained when studying the binary systems DPPC/H_2O and 0βEb/DMSO, respectively. The values of f_{pol} retained are 5 for $C_{H_2O} = 15\%$ and 10 for $C_{H_2O} = 30\%$. The values of f_{AsnCO} retained are 4 for $C_{H_2O} = 15\%$ and 8 for $C_{H_2O} = 30\%$. They were assumed to increase linearly with the water concentration in such a way that for $C_{H_2O} = 15\%$, the smallest values of $d_{(pol+AsnCO)}$ and of d_β obtained are compatible with the smallest value of d_{pol} found for the DPPC without water and with the smallest thickness of the β saccharide chain lamella found for the copolymers 0βEb in the dry state.

The variation in the geometrical parameters of the soluble lamellae versus the molar fraction, τ, of the liposaccharide in the ternary system is shown in Fig. 11 for a water concentration of 15%. As τ increases from about 0.09 to 0.19:

the thickness $d_{(pol+AsnCO)}$ remains constant;

the thickness, d_β, of the saccharide chain lamella increases;

the specific surface, S_β, decreases.

The lamellar structure, LT, corresponding to τ values between 0.09 and 0.19 presents analogies with the lamellar structures L2 and L3 observed for amphipatic saccharide peptide block copolymers, 0βEb, with the molecular weight of the

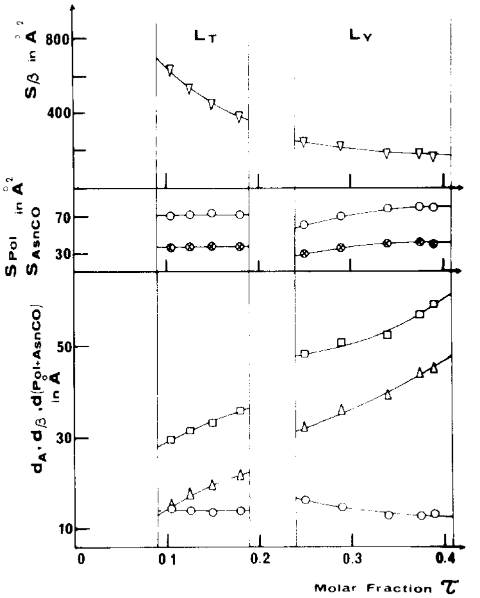


Fig. 11. Variation with τ of the geometrical parameters of the hydrated lamellae in the LT and LY structures for $C_{H_2O} = 15\%$; □, d_A; ○, $d_{(pol+AsnCO)}$; △, d_β; ○, S_{pol}; ⊗, S_{AsnCO}; ▽, S_β.

hydrophobic polypeptide block higher than 4000 [2]. First, the thickness, d_β, of the saccharide layer of the lamellar structure LT (from 13 to 22 Å) is similar to the thickness of the saccharide layers of the lamellar structures L3 and L2′ of 0βEb copolymers (from 17 to 20 Å) [2]. Then, for a value of τ coinciding with the disappearance of the lamellar structure, LT, the surface S_β attains a value of 370 Å^2, similar to that obtained for the lamellar structure L3 exhibited by amphipatic copolymers 0βEb [2]. From such similarities it is inferred that in the lamellar structure, LT, exhibited by the ternary systems β16/DPPC/H_2O for τ values smaller than about 0.19, the antennae of the saccharide part of the liposaccharide take up a T conformation and so would be nearly parallel to the interface (Fig. 9).

The increase in d_β with τ (Fig. 11) suggests that the saccharide chains in a single layer for the smaller values of τ form progressively a double layer as their specific surface decreases from 700 to 370 Å^2.

At the transition between the two lamellar structures (see Fig. 11) $d_{(\mathrm{pol+AsnCO})}$ remains roughly constant while d_β increases suddenly from 22 to 32 Å and S_β decreases from 370 to 250 Å^2. The increase in d_β (similar to the increase of the thickness of the saccharide layer in 0βEb copolymers at the transition L2 → L1) is attributed to a change in the average orientation of the antennae of the saccharide chains: the angle between the average direction of the antennae and the interface increases suddenly and the saccharide chains exhibit a Y conformation (Fig. 10).

As τ increases from about 0.24 to about 0.41:
$d_{(\mathrm{pol+AsnCO})}$ remains roughly constant;
d_β increases from 32 to 48 Å; S_β decreases slightly and reaches a value that is two-times smaller than that attained at the disappearance of the LT structure.

In the lamellar structure LY, the increase of d_β with τ suggests an increase with τ of the angle between the average direction of the antennae of the saccharide chains in a Y conformation and the interfaces.

(C) Influence of the water concentration

The domains of stability of the lamellar structures LT and LY are shifted with respect to the small values of τ as the water concentration increases (Fig. 3) and Table I).

As can be seen in Table I, the water concentration has a rather small effect on the values of the geometrical parameters of the two types of lamellar structure, except for the specific surface, S_β, of the saccharide chains.

TABLE I

INFLUENCE OF THE WATER CONCENTRATION ON THE GEOMETRICAL PARAMETERS OF THE LAMELLAR STRUCTURES LT AND LY

'% H_2O' = water concentration in wt%.

	LT				LY			
	15% H_2O		30% H_2O		15% H_2O		30% H_2O	
	$\tau_{\min}$ 0.09	$\tau_{\max}$ 0.19	$\tau_{\min}$ 0.04	$\tau_{\max}$ 0.12	$\tau_{\min}$ 0.24	$\tau_{\max}$ 0.41	$\tau_{\min}$ 0.18	$\tau_{\max}$ 0.27
d_{B} (Å)	25	20	30	26	25	21	26	22
$d_{(\mathrm{pol+AsnCO})}$ (Å)	14	14	23	20	17	13	20	17
d_β (Å)	13	22	14	27	32	48	35	48
S_{pol} (Å^2)	72	72	58	70	60	80	68	74
S_{Asn} (Å^2)	38	38	27	36	30	32	40	43
S_β (Å^2)	700	370	1350	550	250	180	365	270

Conclusions

The ternary system β16/DPPC/H_2O is shown to display three mesomorphic structures in terms of its β16 and DPPC content. At a molar fraction, τ, of β16 higher than about 0.5, the system displays a hexagonal structure similar to that exhibited by the system β16/H_2O.

As τ takes values smaller than about 0.5, the influence of the DPPC prevails and the system displays lamellar structures analogous to that exhibited by the system DPPC/H_2O. In these structures, the hydrated layer is formed by three superposed lamellae: the two lamellae adjacent to the hydrophobic layers contain the polar head groups of the DPPC and the AsnCO groups of the liposaccharide; they are separated by a layer containing the saccharide chains of the liposaccharide. As long as the liposaccharide β16 is in small quantities in the ternary system β16/DPPC/H_2O, the β saccharide chains take up a T conformation. As the number of the β16 moles relative to the number of the DPPC moles increases, the area, S_β, available to each saccharide chain at the interface of the hydrated lamellae decreases. When S_β reaches a value which is too small for the β saccharide chains to adopt a T conformation, the saccharide chains adopt a Y conformation.

The results presented here provide evidence for the occurrence of glycan conformational changes in model membranes. This property is of particular interest in regard to a better understanding of the biological role of glycans that are generally considered as being recognition signals. Now the question arises as to whether the conformation of glycans in membranes has to be related to the specificity of their interactions with biological effectors [14]. We will try to answer that question in further publications.

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