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Lyotropic Lipo-Amino-Acids: Synthesis and Structural Study

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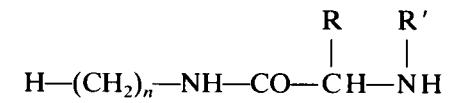
Lyotropic lipo-amino-acids $C_n(AA)$ where AA is one of the following amino-acids: glycine, alanine, sarcosine, serine, tyrosine, lysine, hydroxyethyl-glutamine, hydroxypropylglutamine, hydroxypentyl-glutamine and glutamic acid, and C_n is a paraffinic chain with 12 or 18 carbon atoms have been synthesized. The study by X-ray diffraction of the lipo-amino-acids in concentrated water solution and in the anhydrous state has shown the existence of two types of mesophases: lamellar and hexagonal. The respective influence of the water concentration, the nature of the amino-acid and the length of the paraffinic chain on the domain of stability of the mesophases and on the values of their structural parameters has been established.

In the framework of a general study of the properties and the relations between the structure and the properties of amphipatic lipopeptides, we have already described the synthesis,[1–3] the mesomorphic behavior[3–4] and the emulsifying properties[1,2,5] of lipopeptides $C_n(AA)_p$ with a hydrophobic paraffinic chain C_n containing from 12 to 18 carbon atoms and with a hydrophilic peptidic chain $(AA)_p$ of polysarcosine, polylysine or polyglutamic acid. We have shown that such lipopeptides exhibit mesophases in water solution[3,4] and good emulsifying properties.[5,6] The mesophases are of 3 types: lamellar, cylindrical hexagonal and body-centered cubic in the case of liposarcosine,[3] but only to two types: lamellar and cylindrical hexagonal in the case of lipolysine and lipo(glutamic acid).[4]

In order to establish with accuracy the influence of the nature of the amino-acid on the existence, the nature and the structural parameters of the mesophases, it was necessary to use products without any polydispersity so we undertook the synthesis and the study of lipo-amino-acids $C_n(AA)$ of general formula



with n = 12 or 18; R′ = H except for sarcosine where R′ = CH_3 and R is the side-chain of the amino-acid.

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In the present paper, we will describe the synthesis and the mesomorphic behavior of lipo-amino-acids C_n(AA) with n = 12 or 18 and prepared with amino-acids whose name, abbreviation and formula of the side chain R are given in Table I.

EXPERIMENTAL PART

Materials

Fatty amines (lauric and stearic), sarcosine, glycine, alanine, serine, tyrosine, glutamic acid, lysine, di-tert-butyl-dicarbonate, dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), amino ethanol, amino propanol and aminopentanol were all purchased from Fluka in the best grade of purity available.

Silica gel (Si 60 0.040–0.063 mm for column chromatography and Si 60 on aluminium plates for thin layer chromatography) were purchased from Merck.

Solvents were purified by classical methods.

Methods

Benzyl glutamate (Glu-OBzl) was obtained by action of benzyl alcohol on glutamic acid.[7]

N-tert-butoxycarbonylamino-acids (AA-BOC) were obtained by the action of di-tert-butyl-dicarbonate with the corresponding amino-acids.[8,9]

Synthesis of lipo-amino-acids C_n(AA). The synthesis of the lipo-amino-acids was performed in 3 steps and the products obtained at each step: C_n(AA)BOC, C_n(AA)HCl or C_n(AA)HBr, C_n(AA) were characterized by IR: 2920, 2840, 1470 (aliphatic chain); 1660, 1550 (amide), 1645, 1175, 1145 (BOC), 2420 cm^{-1} (> NH_2^+) and TLC in 3 eluant systems.

1) The synthesis of C_n(AA) with AA = Sar, Gly, Ala, Ser, Tyr, Lys, was performed as already described[3,5] and the results are illustrated in Table II for C_{12} Ser and C_{12} Lys.

2) The synthesis of C_nGlu(Na^+ or K^+)NH_2 was performed by reacting a methanol solution of C_nGlu(OBzl)HCl with 2.2 equivalent of NaOH or KOH in aqueous

TABLE I

Name, abbreviation, and chemical formula of the side chain R of the amino-acids.

Amino-acid	(AA)	Side chain: R
Glycine	Gly	H
Alanine	Ala	CH_3
Sarcosine	Sar	H
Serine	Ser	CH_2OH
Tyrosine	Tyr	CH_2—C_6H_4—OH
Glutamic acid	Glu	CH_2—CH_2—COOH
N-hydroxyethylglutamine	Gln(EtOH)	$(CH_2)_2$—CO—NH—$(CH_2)_2$—OH
N-hydroxypropylglutamine	Glu(PropOH)	$(CH_2)_2$—CO—NH—$(CH_2)_3$—OH
N-hydroxypentylglutamine	Gln(PentOH)	(CH_2)—CO—NH—$(CH_2)_5$—OH
Lysine		$(CH_2)_4$—NH_2

solution for 3 hrs at 37°C. Methanol was evaporated under vacuum. Acetone was added and the precipitate of lipo-amino-acid was filtrated, dried under vacuum, dispersed in water and lyophylised. Complete debenzylation was verified by U.V. at 258 nm (disappearance of benzene ring absorption) (Table II).

3) The synthesis of lipo(N^5-hydroxyalcooyle-L-glutamine) was performed by reacting C_nGlu(OBzl)BOC in solution in methanol for $n = 12$ and in chloroform for $n = 18$ with a large excess (10 equivalents) of amino-ethanol, amino-propanol or amino-pentanol until the absorption at 258 nm disappeared. Then the excess of amino-alcohol was extracted by water and the N protected amino-acid by diethyl ether.[5] At last the protecting group BOC was eliminated by the same method as for other lipo-amino-acids. Yields and characteristics of some products are given in Table II.

Purification of the lipo-amino-acids $C_n(AA)$. The lipo-amino-acids were purified by column chromatography on a silica gel column (150 cm × 2 cm) using as eluent a methanol solution containing 1 vol% of 34% aqueous ammonia. 0.5 g of lipo-amino-acid was purified in each run and detection was performed by UV spectroscopy at 210 nm.

The purity of the lipopeptides was checked by TLC on silicagel win 3 different types of eluents: ethanol containing 1% of acetic acid; methanol containing 2% of 37% aqueous ammonia; a mixture of 3 volumes of ethyl acetate and 2 volumes of the following mixture: acetic acid 6 V—pyridine 20 V—water 11 V; revelation was performed by ninhidrin and only one spot was observed for each lipo-amino-acid. Table II gives the R_F values for some lipo-amino-acids C_{12}(AA).

The composition and the purity of the lipo-amino-acids were also checked by ^{1}H-NMR spectroscopy using a Bruker WM 500 spectrometer operating at 500 MHz, at 22°C and with Fourier transform. Figure 1 gives an example of NMR spectra corresponding to C_{12}Ser.

Preparation of the mesomorphic gels. To prepare homogeneous samples of lipo-amino-acid/water systems, the same two methods as for lipopeptides were used.[3]

TABLE II

Yield of synthesis and R_F values in 3 eluent systems of some lipo-amino-acids.

Product	Yield	TLC: R_F		
		Eluent 1	Eluent 2	Eluent 3
C_{12}SerBOC	0.90	0.79	0.86	0.95
C_{12}SerHCl	0.92	0.45	0.67	0.52
C_{12}Ser	0.90	0.39	0.70	0.49
C_{12}Lys(BOC)BOC	0.85	0.82	0.47	0.63
C_{12}Lys(HBr)HBr	0.88	small	0.18	0.24
C_{12}Lys(NH_2)NH_2	0.90	0.40	small	0.15
C_{12}Glu(OBzl)BOC	0.8	0.86	0.56	0.40
C_{12}GLu(OBzl)HCl	0.92	0.73	0.79	0.52
C_{12}Glu(Na^+)NH_2	0.95	0.38	0.79	0.48
C_{12}Gln(EtOH)NH_2	0.75	0.40	0.11	0.43
C_{12}Gln(PropOH)NH_2	0.72	0.64	0.09	0.71
C_{12}Gln(PentOH)NH_2	0.70	0.62	0.09	0.62

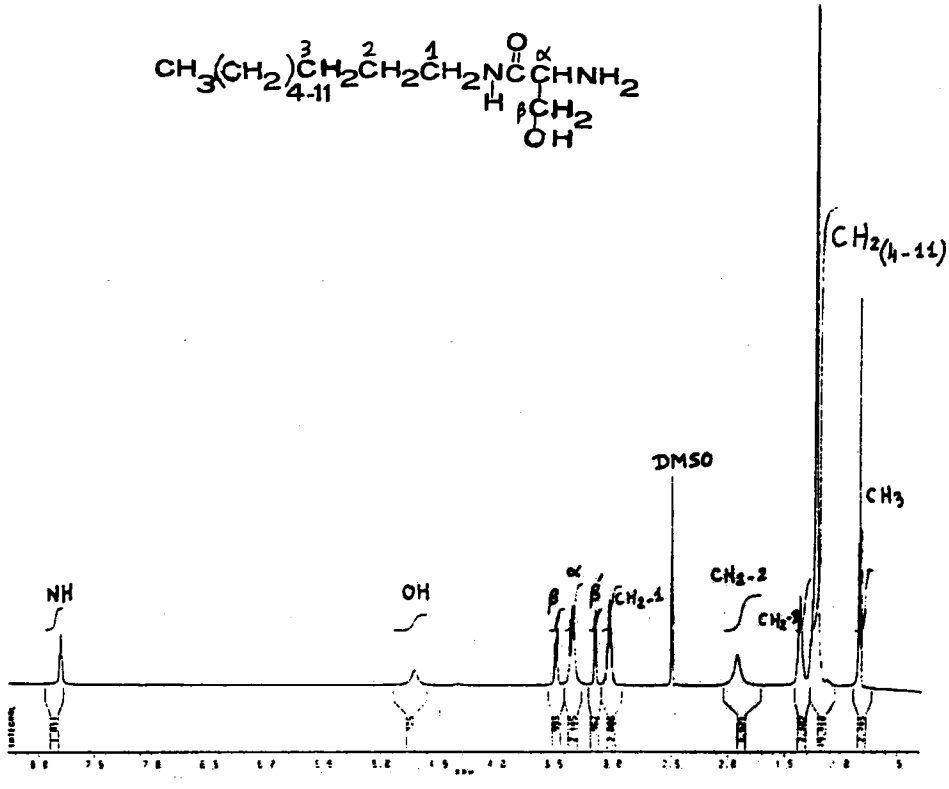


FIGURE 1 ^{1}H-NMR spectra at 500 MHz of liposerine C_{12}Ser.

The choice between the two methods was determined by the length of the paraffinic chain and the nature of the amino-acid.

X-ray diffraction studies. X-ray diffraction studies were performed under vacuum with a Guinier type focussing camera equipped with a bent quartz monochromator giving a linear collimation and a device recording the diffraction patterns from samples held at various temperatures with an accuracy of ± 1°C.

RESULTS

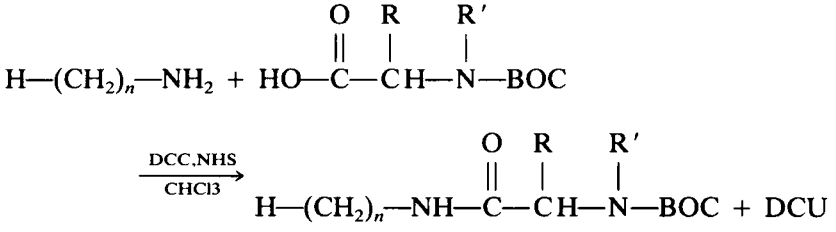
I. Synthesis of lipo-amino-acids

The synthesis of the lipo-amino-acids is generally performed in 3 steps: protection of the α-amino function of the amino-acid, coupling between the amine function of the fatty amine and the α carboxylic acid function of the N-protected amino-acid, elimination of the protecting groups. But, when the amino-acid is glutamic acid, the side chain carboxylic function has to be protected by a benzyl group[7] before performing the protection of the α-amine function.

A. Protection of the α-amine function. The α-amine function is protected by a tertiobutyloxycarbonyl group (BOC) by action of the ditertiobutyldicarbonate.[8,9] The reaction is performed in a mixture dioxane/water for glycine, sarcosine, alanine, benzylglutamate and lysine and in a mixture water/tertbutylalcohol for serine and tyrosine, in the presence of sodium hydroxide for all amino-acids except benzyl glutamate for which NaOH is replaced by $NaHCO_3$ to avoid the hydrolysis of the benzylester. In the case of lysine the two amine functions are protected by BOC groups. The yield of the reaction varies between 75 and 85% depending upon the nature of the amino-acid.[5]

B. Coupling between the fatty amine and the N-protected amino-acid. The coupling reaction between the N-protected amino-acid and the fatty amine is performed in chloroform solution, at 0°C, in the presence of a coupling agent the dicyclohexylcarbodiimide (DCC) and of a nucleophilic agent the N-hydroxysuccinimide (NHS) to avoid secondary reactions specially with the free hydroxyl group of serine and tyrosine.[10]

After elimination of the dicyclohexylurea (DCU) formed, the N-protected lipo-amino-acid C_n(AA)BOC is recovered by precipitation with water



The yield of the coupling reaction varies from 75 to 95%.[5]

C. Liberation of the amine functions. The lipo-amino-acid chlorhydrates C_n(AA)HCl and the lipo-amino-acid bromhydrates C_n(AA)HBr are obtained by action of HCl in diethylether solution or of HBr in acetic acid solution on the N-protected lipo-amino-acid in tetrahydrofuran and acetic acid solution respectively.[5]

The lipo-amino-acids under the free amine form C_n(AA) are obtained by action of a small excess of sodium hydroxide on the corresponding lipo-amino-acid chlorhydrate or bromhydrate in methanol solution.

II. STRUCTURAL STUDY OF LIPO-AMINO-ACIDS

Lipo-amino-acids have been studied in the anhydrous state and in concentrated water solution (less than 60% of water) by X-ray diffraction as a function of temperature.

As in the case of soaps,[11] two regions can be distinguished on the X-ray patterns:

—the central region (low angles) that presents a set of sharp reflexions whose Bragg distance ratios allow the determination of the structure: lamellar, hexagonal or cubic.[3,11]

—the external region (wide angles) that exhibits a set of sharp lines if the

paraffinic chains are crystallized and a diffuse band if the paraffinic chains are desorganized and nearly liquid.

The study of X-ray patterns has shown that lipo-amino-acids exhibit, as a function of water concentration and temperature, both crystalline and liquid-crystalline structures.

A. Stability domain of mesophases. The study of X-ray patterns showed that, at room temperature, all the lipo-amino-acids studied exhibit a lamellar crystalline structure and to obtain mesophases one has to heat the samples at a temperature higher than the melting temperature of the paraffinic chains. Nevertheless, lipoglycine, lipoalanine, lipotyrosine, lipobenzylglutamate, lipopropylglutamine and lipopentylglutamine go directly from the crystalline state to the liquid state without exhibiting mesophases in the anhydrous state and in concentrated solution as well. Anhydrous liposerine, liposarcosine, lipolysine and lipohydroxyethylglutamine also go directly from the crystalline state to the liquid state and necessitate the presence of a minimum amount of water (from 4 to 10%) to exhibit mesophases by heating at a temperature higher than the melting temperature of the paraffinic chains. On the contrary, lipo-amino-acid chlorhydrates or bromhydrates exhibit mesophases in concentrated solution and in the anhydrous state at temperatures higher than the melting temperatures of the paraffinic chains.

B. Structure of mesophases. As several lipo-amino-acids C_{18}(AA) give mesophases at temperatures higher than 100°C, we performed our structural studies mainly on lipo-amino-acids C_{12}(AA).

X-ray diffraction showed that all lipo-amino-acids giving mesophases exhibit a liquid-crystalline lamellar structure and that some lipo-amino-acids also exhibit a hexaogonal structure (Table III).

The lamellar structure (L) consists of plane parallel equidistant sheets; each sheet of thickness d results from the superposition of two layers: one of thickness d_B contains the hydrophobic paraffinic chains, while the other of thickness d_B contains the hydrophobic paraffinic chains, while the other of thickness d_A contains the hydrophilic part of the molecules (NH-CO-CHR-NHR′ or NH-CO-CHR-NHR′, HCl) and the water.

TABLE III

Structure and specific volumes of lipo-amino-acids.

Lipo-amino-acids	Structure	V $cm^3 \cdot g^{-1}$	V_A $cm^3 \cdot g^{-1}$
C_{12}Sar	L	1.14	0.910
C_{12}SarHCl	L	1.05	0.763
C_{12}GlyHCl	L	1.04	0.699
C_{12}AlaHCl	L	1.04	0.746
C_{12}Ser	L	1.08	0.781
C_{12}SerHCl	L + H	1.00	0.685
C_{12}Gln(EtOH)NH_2	L	1.02	0.799
C_{12}Lys(HBr)HBr	L + H	0.805	0.555
C_{12}Lys(NH_2)NH_2	L + H	1.100	0.910
C_{18}Lys(HBr)HBr	L + H	0.86	0.555

The hexagonal structure (H) consists of long and parallel cylinders of diameter 2R, filled with the hydrophobic paraffinic chains, and assembled in a hexagonal array of parameter D, while the space between the cylinders is occupied by the hydrophilic part of the molecules and the water.

The lattice parameters d and D were directly obtained from the X-ray patterns. The other parameters: d_A, d_B, 2R and S (average surface occupied by a molecule at the interface between the hydrophobic and hydrophilic domains) were calculated by the following formulae based on simple geometrical considerations.

$$d_B = d\left[1 + \frac{c\chi_A\, v_A + (1 - c)v_S}{c\chi_B\, v_B}\right]^{-1}$$

$$S_L = \frac{2M_B v_B}{\chi d_B}$$

$$R^2 = \frac{D^2\sqrt{3}}{2\pi}\left[1 + \frac{c\chi_A v_A + (1 - c)v_S}{c\chi_B\, v_B}\right]^{-1}$$

$$S_H = \frac{2M_B v_B}{\chi R}$$

with:
c: lipo-amino-acid content in solution (in mass)
χ_A and χ_B: weight fraction of the hydrophilic and hydrophobic parts of the molecules
v_A: specific volume of the hydrophilic moiety (Table III)[12]
v_B: specific volume of the hydrophobic paraffinic chains[3,13]
v_S: specific volume of the solvent
M_B: molecular weight of the paraffinic chains
χ: Avogadro's number.

C. Factors governing the structure of the mesophases. The structural study of the mesophases has been performed at temperatures higher than the melting temperature of the paraffinic chains and when possible at 60°C. Furthermore we have verified that in the domain of temperature used both the type of mesophase and the value of their structural parameters were independent of the temperature.

The main factors governing the existence, the nature and the structural parameters of the mesophases are: the water concentration, the "electrical state" of the amino-acids, the nature of the side chain of the amino-acids and the length of the paraffinic chains.

1. Influence of the water concentration

For some lipo-amino-acids in the free amine form, namely liposarcosine, liposerine, lipohydroxyethylglutamine and lipolysine, the presence of a minimum amount of water (from 4 to 10%) is necessary to obtain mesophases and for 3 types of lipo-amino-acids: liposerine chlorhydrates, lipolysine and lipolysinebromhydrates the

addition of a sufficient amount of water is able to transform a lamellar structure into a cylindrical hexagonal structure.

As illustrated by Figure 2 in the case of the lipo-amino-acid C_{12}SerHCl when the water concentration increases:

—for the lamellar structure: the intersheet spacing d, the thickness d_A of the hydrophilic layer and the average surface at the interface S_L all increase, while the thickness d_B of the hydrophobic layer decreases.

—for the hexagonal structure: the distance D between the cylinders and the average surface S_H both increase, while the diameter 2R of the hydrophobic cylinders decreases.

2. Influence of the "electric state" of the amino-acid

For amino-acids such as glycine and alanine that are too hydrophobic to give mesophases the transformation of the α-amine into a chlorhydrate increases the hydrophilicity of the amino-acids and allows the formation of mesophases.

For amino-acids such as sarcosine, serine, hydroxyethylglutamine and lysine that are hydrophilic enough to allow the formation of mesophases both in the amine state and in the chlorhydrate or bromhydrate state, the transformation of the amine form into the chlorhydrate or bromhydrate form involves an anisotropic swelling of the hydrophilic domains.

The Figures 3 and 4 illustrate the effect of the transformation of the amine function into chlorhydrate or bromhydrate for the lamellar structure of liposerines and the lamellar and hexagonal structures of lipolysines whose parameters are plotted as a function of the water content C_1 of the hydrophilic domains.

One can see that the transformation of the amine functions into chlorhydrates or bromhydrates involves a decrease of the characteristic parameters of the hydrophobic domains (d_B for the lamellar structure and 2R for the hexagonal structure) but an increase of all the other parameters: d, d_A, D and S. Furthermore the swelling of the hydrophilic domains is anisotropic: the dilatation in the direction perpendicular to the interface between the hydrophilic and hydrophobic domains is much higher than the dilatation in the plane of the interface; for the lamellar structure d_A increases much more than S. For instance, in the case of liposerines, for $C_1 = 0.4$, d_A increases of 3 Å and S only of 1.6 Å.[2] An isotropic swelling would correspond to $\Delta(d_A) = \sqrt{\Delta S}$, that is to say to an increase of 1.26 Å instead of 3 Å for d_A.

3. Influence of the nature of the side-chain

We have studied the influence of the nature of the amino-acid side chain on the lamellar structure of two sets of amino-acids: the first one on the free amine form, the second one on the chlorhydrate form.

a) The first set consists of two lipo-amino-acids whose amino-acid side chain is terminated by an OH group but differs by its length: CH_2OH for serine and $(CH_2)_2$-CO-NH-$(CH_2)_2$-OH for hydroxyethylglutamine.

On the Figures 5 and 6 are plotted the variation of the structural parameters of the two lipo-amino-acids versus the water content C_1 of the hydrophilic domains.

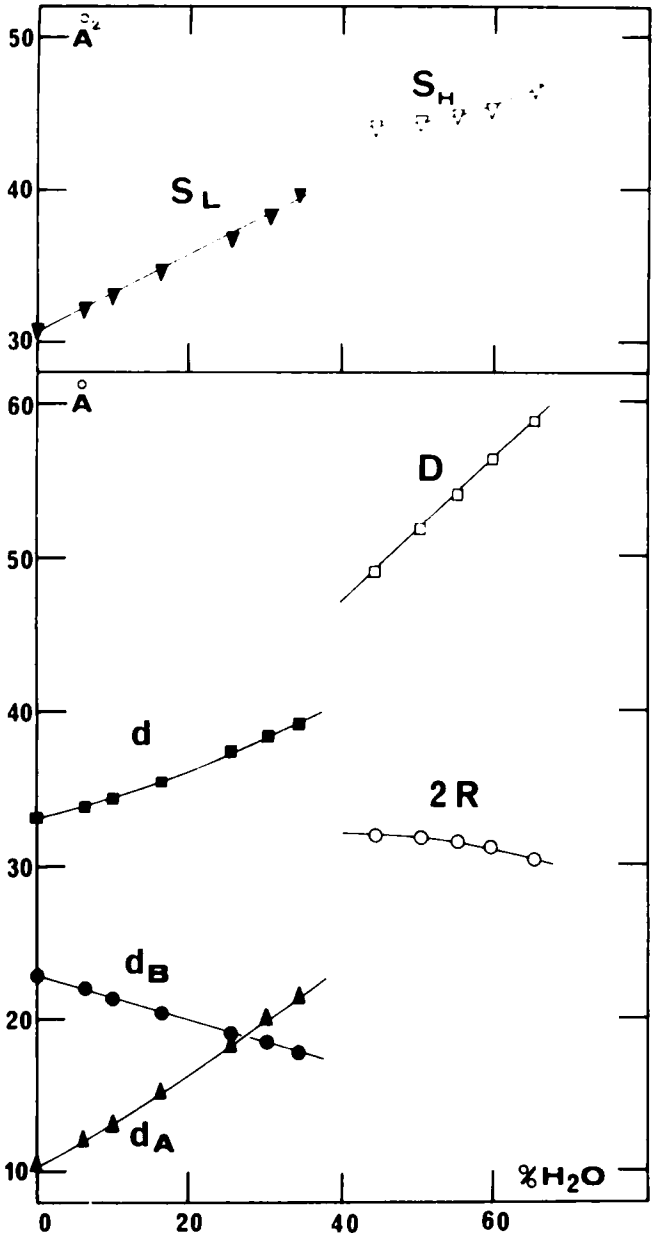


FIGURE 2 Variation of the structural parameters of the lamellar and hexagonal structure of the liposerinechlorhydrate C_{12}SerHCl versus water concentration: ■ d = intersheet spacing; ● d_B = thickness of the hydrophobic layer; ▲ d_A = thickness of the hydrophilic layer; ▼ S_L = average surface area per molecule in the lamellar structure; □ D = distance between neighboring cylinders; ○ 2R = diameter of the hydrophobic cylinders; ▽ S_H = average surface area per molecule in the hexagonal structure.

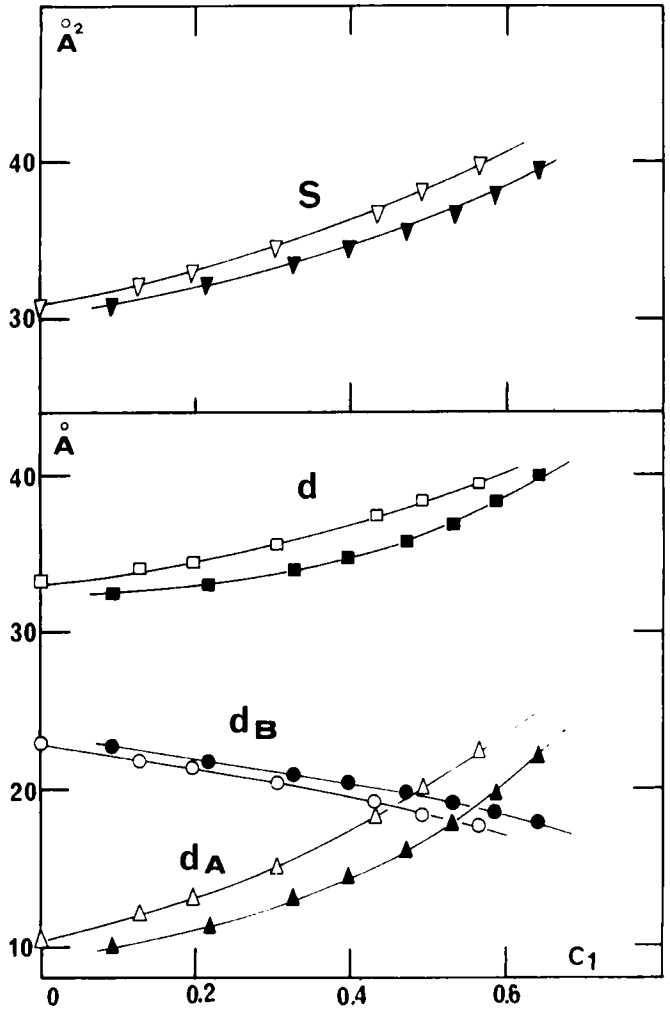


FIGURE 3 Variation of the structural parameters of the lamellar structure of liposerine C_{12}Ser and liposerine chlorhydrate C_{12}SerHCl versus the water content C_1 of the hydrophilic domains: C_{12}Ser: ■ d; ▲ d_A; ● d_B; ▼ S_L, C_{12}SerHCl: □ d; △ d_A; ○ d_B; ▽ S_L.

One can see that going from liposerine to lipohydroxyethylglutamine induces an increase of S but a decrease of d_B as the density of the paraffinic chains has to remain constant. Furthermore, when C_1 increases S and d_B tend towards common limits for the two lipo-amino-acids. On the contrary, one observes a large increase of d_A going from liposerine to lipohydroxyethylglutamine. So the swelling of the hydrophilic layer is anisotropic and the anisotropy increases with C_1 as illustrated by the Table IV. For an isotropic swelling d_A would increase as the square root of S, therefore of 2.1 Å instead of 6.8 Å for $C_1 = 0.2$ and of 1.3 Å instead of 12.8 Å for $C_1 = 0.5$.

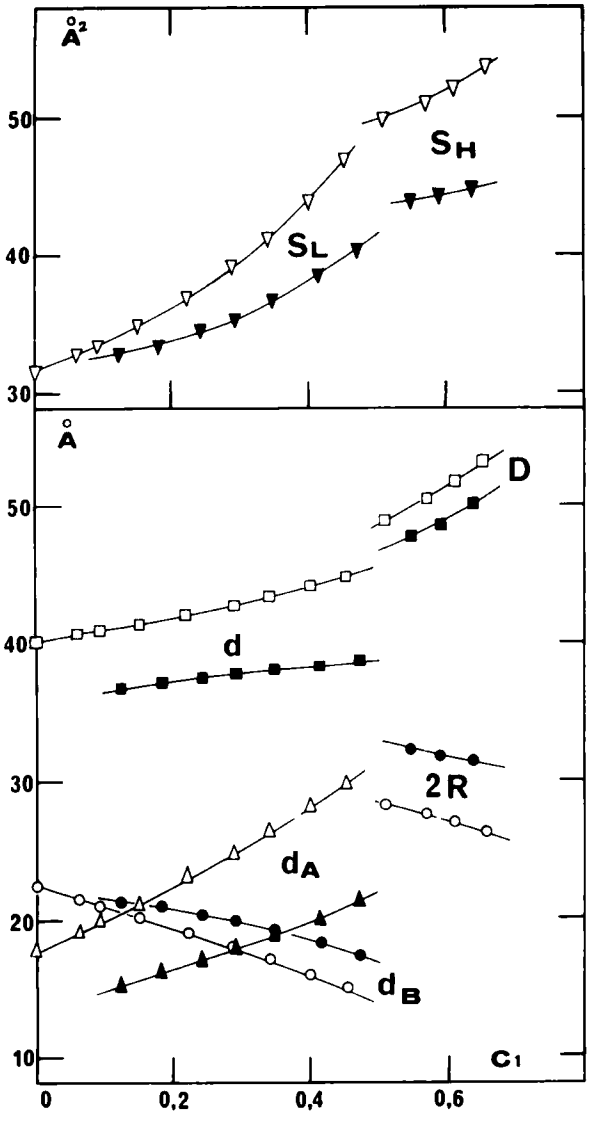


FIGURE 4 Variation of the structural parameters of the lamellar and hexagonal structure of lipolysine $C_{12}Lys(NH_2)NH_2$ and lipolysinebromhydrate $C_{12}Lys(HBr)HBr$ versus the water content C_1 of the hydrophilic domains: $C_{12}Lys(HBr)HBr$: □ d; △ d_A; ○ d_B; ▽ S_L; □ D; ○ 2R; ▽ S_H, $C_{12}Lys(NH_2)NH_2$: ■ d; ▲ d_A; ● d_B; ▼ S_L; ■ D; ● 2R; ▼ S_H.

Such a behavior suggests that the side chain of hydroxyethylgutamine is more or less perpendicular to the interface.

b) The second set consists of 4 lipo-amino-acids: lipoglycine, lipoalanine, liposarcosine and liposerine on their chlorhydrate form.

On the Figures 7 to 9 are plotted the variations of the structural parameters of the lamellar structure of the 4 lipo-amino-acid chlorhydrates as a function of the water content C_1 of the hydrophilic layers.

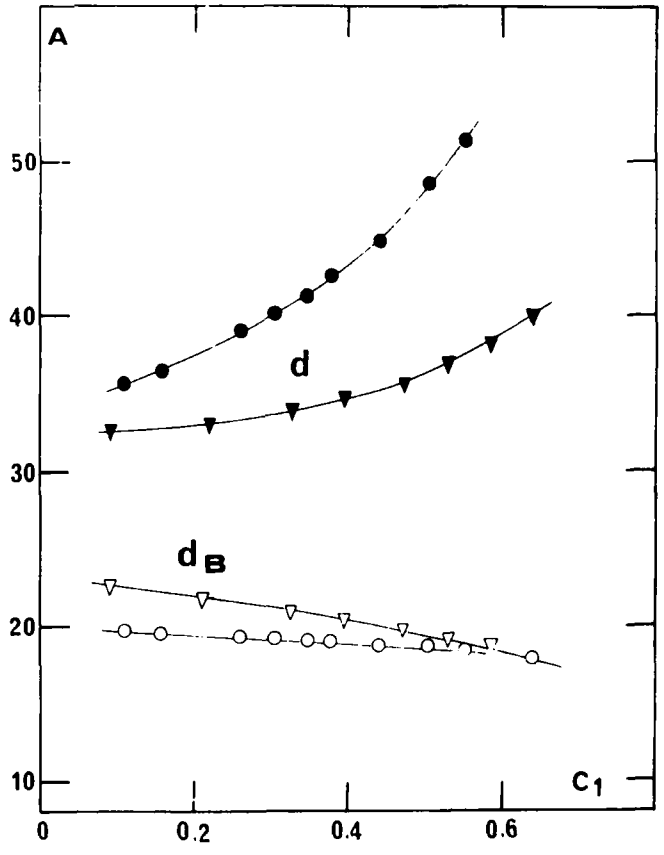


FIGURE 5 Variation of the intersheet spacings d and of the thickness d_B of the hydrophobic layer of the lamellar structure of the liposerine C_{12}Ser and of the lipohydroxyethylglutamine C_{12}Gln(EtOH) versus the water content c_1 of the hydrophilic domains: C_{12}Ser: d ▼; d_B ▽, C_{12}Gln(RtOH): d ●; d_B ○.

One can see that for any water content C_1, d_A is nearly the same for the 4 amino-acid chlorhydrates (Figure 7) while S decreases (Figure 8) from C_{12}SerHCl to C_{12}AlaHCl to C_{12}SarHCl and to C_{12}GlyHCl.

The size of the amino-acid side chain decreases in the same order (CH_2OH > CH_3 > H). So we can think that the side-chain takes an orientation more or less parallel to the interface between the hydrophilic and hydrophobic domains. This interpretation involves that when the size of the side chain increases the distance between the lipo-amino-acid molecules increases and the average area S increases. This behavior is in agreement with experimental results (Figure 8). For C_{12}SarHCl, the methyl group is linked to the terminal nitrogen atom of the amino-acid and is situated at a distance from the interface higher than in the case of the methyl group of alanine (linked to the α-carbon atom), so its influence on the average area S is smaller and can explain why S is smaller for C_{12}SarHCl than for C_{12}AlaHCl.

The increase of S with the volume of the side chain involves a decrease of d_B as the paraffinic chains have to keep a constant density.

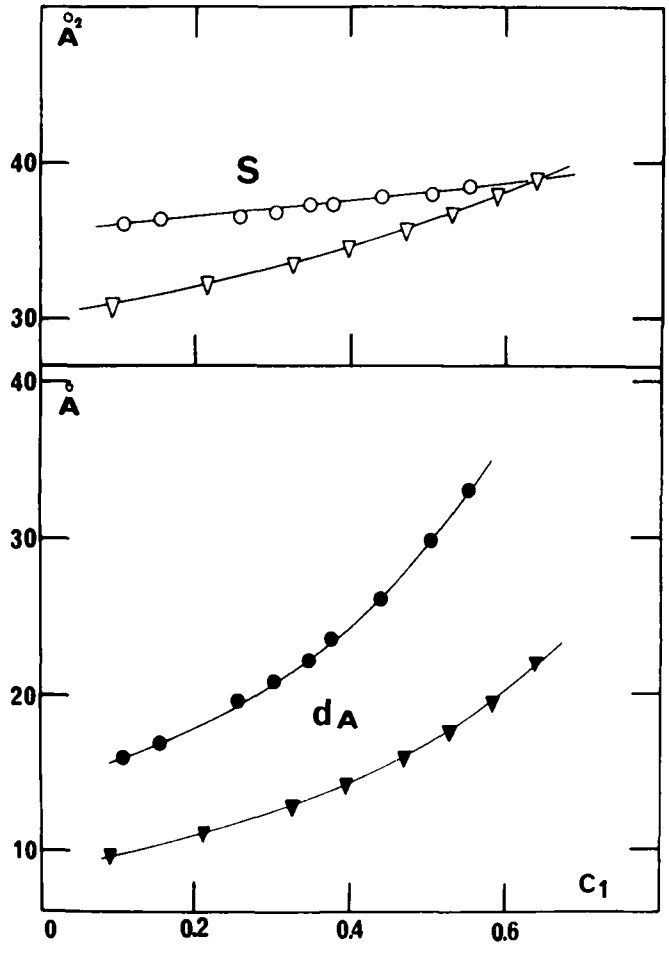


FIGURE 6 Variation of the thickness d_A of the hydrophilic layer and of the average surface per molecule S_L of the lamellar structure of the liposerine C_{12}Ser and of the lipohydroxyethylglutamine C_{12}Gln(EtOH) versus the water content C_1 of the hydrophilic domains: C_{12}Ser: d_A ▼; S_L ▽, C_{12}Gln(EtOH): d_A ●; S_L ○.

TABLE IV

d_A, Δd_A and $\sqrt{\Delta S}$ in Å. S and ΔS in Å².

	C_1 = 0.2					C_1 = 0.5				
Lipo-amino-acid	d_A	Δd_A	S	ΔS	$\sqrt{\Delta S}$	d_A	Δd_A	S	ΔS	$\sqrt{\Delta S}$
C_{12}Ser	11		32			16.8		36.2		
		6.8		4.5	2.1		12.8		1.8	1.3
C_{12}Gln(EtOH)NH_2	17.8		36.5			29.6		38.0		

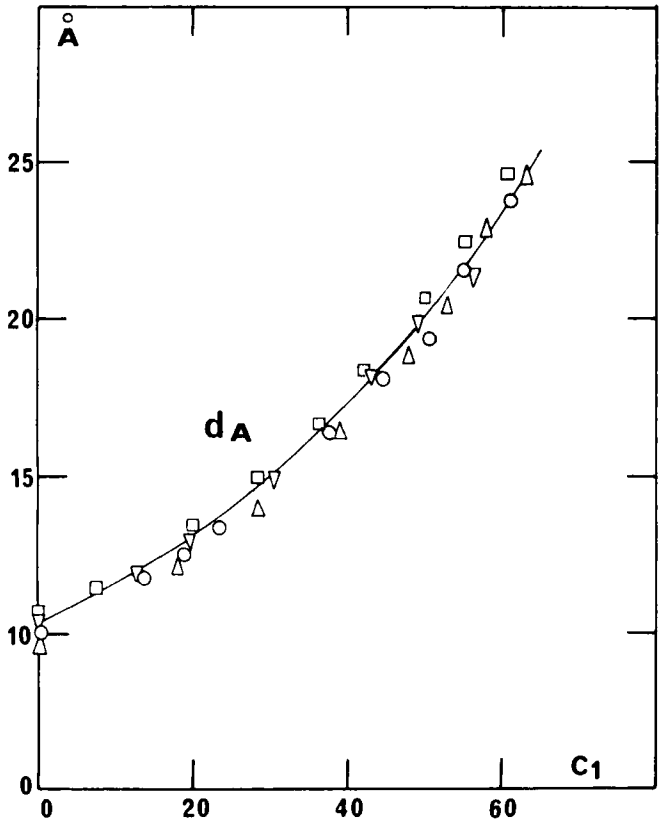
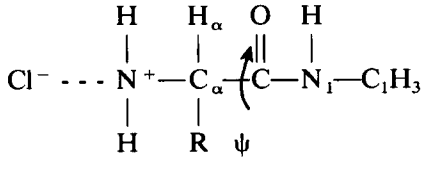


FIGURE 7 Variation of the thickness d_A of the lamellar structure of lipoglycine-chlorhydrate C_{12}GlyHCl (△), liposarcosinechlorhydrate C_{12}SarHCl (□), lipoalaninechlorhydrate C_{12}AlaHCl (○) and liposerinechlorhydrate C_{12}SerHCl (▽) versus the water content C_1 of the hydrophilic domains.

At last as d_A remains nearly constant and as d_B decreases when the size of the side chain increases d decreases in a way parallel to d_B with the nature of the amino-acid (Figure 9).

We have tried to calculate S and d_A for anhydrous lipo-amino-acid chlorhydrates using simple geometrical models of the amino-acid residues and a program of molecular graphism[14] without taking into account possible interactions.

The model is based on the standard geometry of the amino-acids: the distance N-Cl is taken equal to 2.5 Å and ion chloride is placed on the axis C_α-N and the volume of the paraffinic chain is simulated by a methyl group C_1H_3. The volume of the amino-acid residue depends upon its configuration therefore of the value of the angle of rotation around NC_α-CN_1



α) Calculation of the surface S

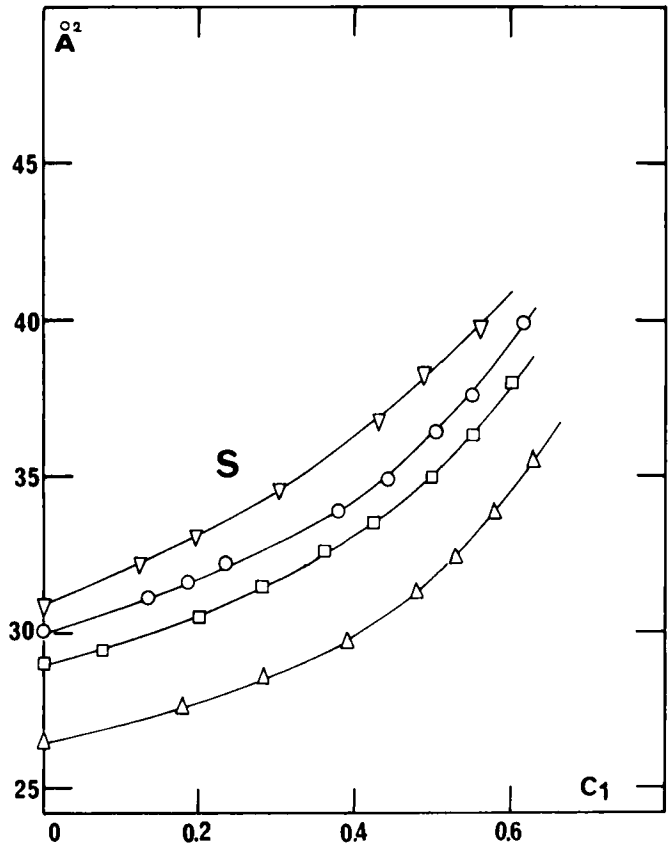


FIGURE 8 Variation of the average area per molecule S_L for the lamellar structure of lipoglycinechlorhydrate C_{12}GlyHCl (△), liposarcosinechlorhydrate C_{12}SarHCl (□), lipoalaninechlorhydrate C_{12}AlaHCl (○) and liposerinechlorhydrate C_{12}SerHCl (▽) versus the water content C_1 of the hydrophilic domains.

In order to calculate S the direction $C_\alpha \rightarrow C_1$ was taken as the Z axis; the volumic envelope of the molecule was drawn and sections perpendicular to Z axis were made at the level of C_1, N_1 and C_α using as values of the Van der Vaals radius: H = 1.2 Å, O = 1.52 Å, C = 1.70 Å, N = 1.55 Å, Cl = 1.81 Å[15] and giving to ψ the 3 following values 0°, 120° and 240°. In the case of C_{12}SerHCl two other parameters were considered the rotation angles χ_1 around the C_α—C_β bond and χ_2 around the C_β—O_γ bond; a lot of pairs of values were given to χ_1 and χ_2 and it was found that if $\chi_1 = 180°$, for any values of ψ and χ_2, S varies only between 30.5 and 31.1 Å^2 in good agreement with the experimental result (31 Å^2); so $\chi_1 = 180°$ was adopted and the results of the calculations were summed up in Table V and compared with the experimental values.

The examination of Table V shows that the calculated values are in good agreement with the experimental ones except in the case of C_{12}SarHCl for which only the value calculated with $\psi = 0°$ is similar to the experimental value. This fact may be attributed to the presence of the bulky methyl group on the terminal nitrogen atom that would be able to favor the conformation with $\psi = 0°$.

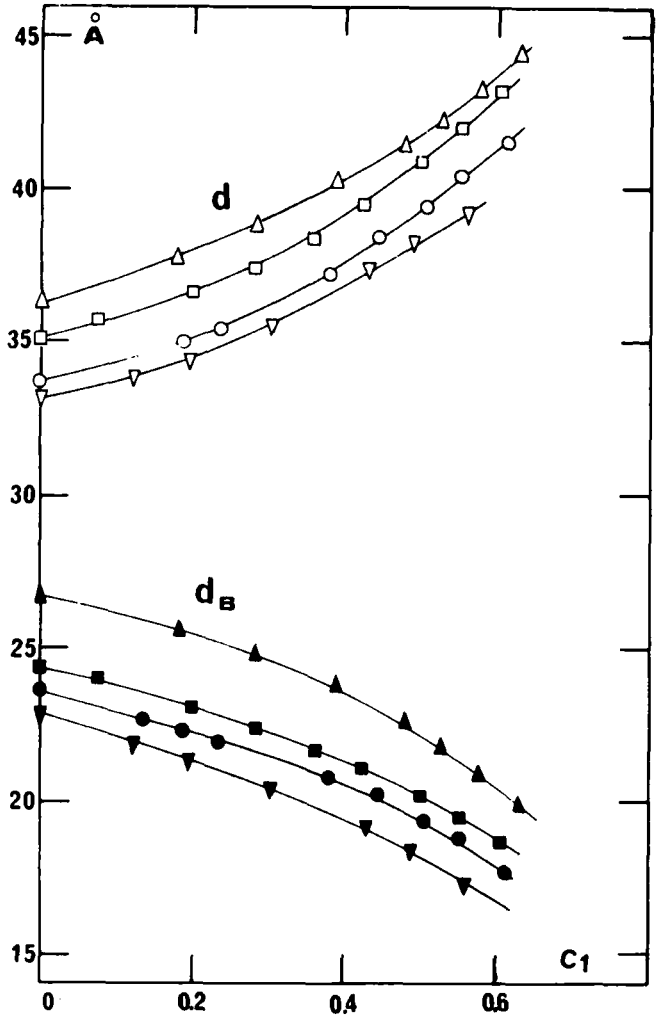


FIGURE 9 Variation of the intersheet spacing d and of the thickness d_B of the hydrophobic layer of the lamellar structure of 4 lipoamino-acid chlorhydrates versus the water content c_1 of the hydrophilic domains: C_{12}GlyHCl: d △; d_B ▲, C_{12}SarHCl: d □; d_B ■, C_{12}AlaHCl: d ○; d_B ●, C_{12}SerHCl: d ▽; d_B ▼.

β) Calculation of the thickness d_A

Calculations were performed for 3 values of ψ(0°, +120° and −120°) taking in account that the distance Cl^-—N^+ is equal to 2.5 Å, the ion Cl^- is on the axis C_α—N, the distance C_α—C_1 is constant and equal to 3.75 Å as generally admitted and that the interface has been placed in the middle of the bond C_1—N_1 what implies that half the length of the distance C_1N_1 (1.47 Å) has to be subtracted from the distance C_1—Cl^- to calculate d_A. Furthermore, in order to minimize the energy and stabilize the structure, one can position the amino-acid residues in such a way

TABLE V

Calculated and experimental values of S in Å^2.

	Section	C_{12}GlyHCl	C_{12}AlaHCl	C_{12}SerHCl	C_{12}SarHCl
0°	C_1	23.086	23.560	24.941	24.154
	N_1	26.144	27.213	27.956	26.020
	C	32.064	41.072	43.167	34.145
	average value	27.098	30.613	32.021	28.106
120°	C_1	24.189	24.967	25.155	23.830
	N_1	25.544	25.080	31.074	25.470
	C	30.132	35.586	36.869	29.232
	average value	26.621	28.544	31.032	26.177
240°	C_1	24.189	25.382	24.587	24.750
	N_1	25.752	26.961	30.076	24.373
	C	27.957	34.855	36.332	29.267
	average value	25.966	29.066	30.331	26.136
General average value		26.560	29.408	31.128	26.80
Experimental value		26.5	30.0	31.0	29.0

that a Cl^- interacts with a NH_3^+ of a neighboring molecule as indicated on the scheme:

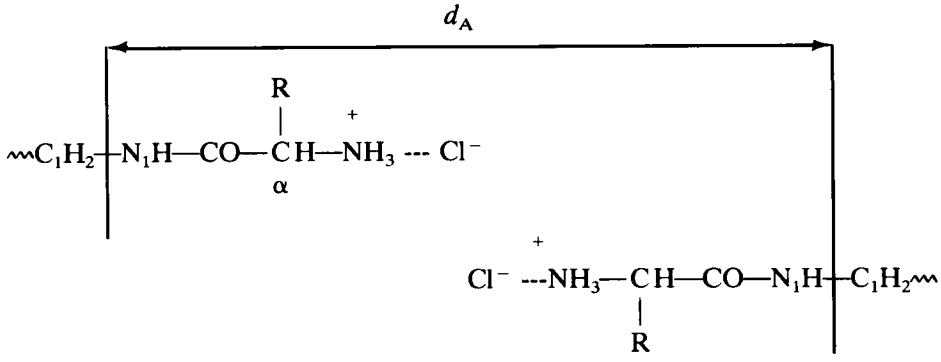


TABLE VI

Calculated and experimental values of the thickness d_A of the hydrophilic layer.

	Distances en Å			
Ψ (°)	N—C_1	C_1—Cl^-	d_A (cal.)	d_A (exp)
0	4.50	6.38	9.79	
+120	4.87	7.10	10.23	10.2 ± 0.5
−120	4.87	7.10	10.23	

As can be seen in Table VI, the calculated values of d_A vary between 9.79 and 10.23 Å and are in good agreement with the experimental value. So the calculations support the proposed model and allow to define the respective positions of the amino-acid residues in the hydrophilic layer.

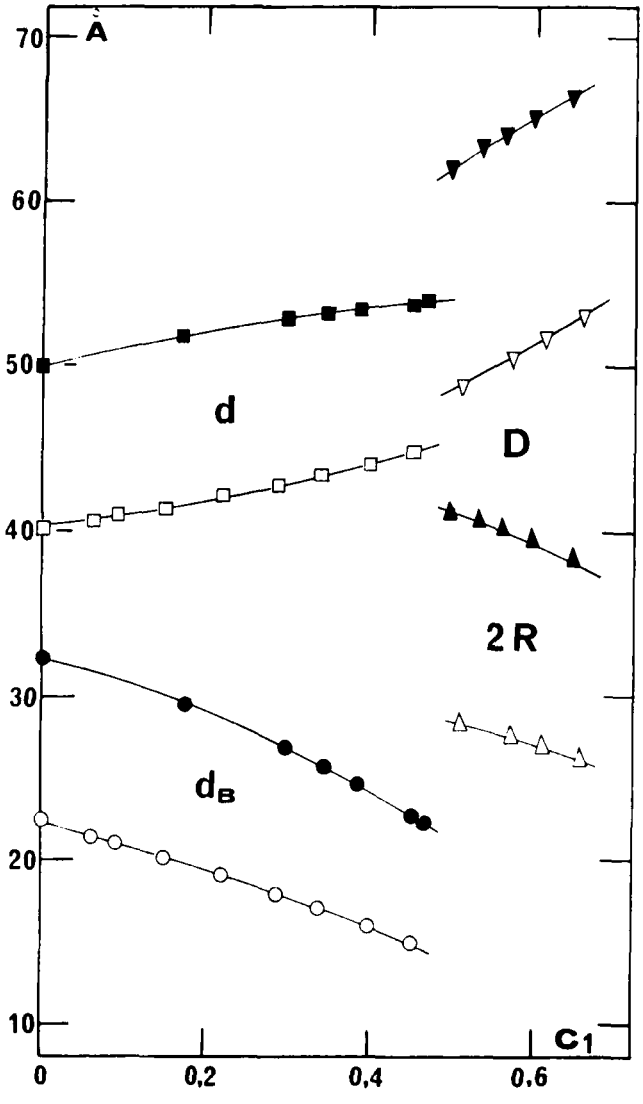


FIGURE 10 Variation of the geometrical parameters; intersheet spacing d, thickness of the hydrophobic layer d_B, distance between neighboring cylinders D and diameter of the hydrophobic cylinders 2R for two lipolysinebromhydrates C_{12}Lys(HBr)HBr and C_{18}Lys(HBr)HBr versus the water content C_1 of the hydrophilic domains: C_{12}Lys(HBr)HBr: d □; d_B ○; D ▽; 2R △, C_{18}Lys(HBr)HBr: d ■; d_B ●; D ▼; 2R ▲.

5. Influence of the length of the lipidic chain

In order to establish the influence of the length of the lipidic chains on the geometrical parameters of the liquid-crystalline structures we have performed a comparative study of the lipolysinebromhydrates C_{12}Lys(HBr)HBr and C_{18}Lys(HBr)HBr that both exhibit lamellar and hexagonal structures.

On the Figures 10 and 11 the variation of the geometrical parameters of the lamellar and hexagonal structures of the two lipolysinebromhydrates are plotted as a function of the water content C_1 of the hydrophilic domains.

One can see that going from the lipo-amino-acid with a paraffinic chain containing 12 carbon atoms to the lipo-amino-acid with a paraffinic chain containing 18 carbon atoms d, d_B, D and 2R increase (Figure 10), while d_A, S_L and S_H remain constant (Figure 11).

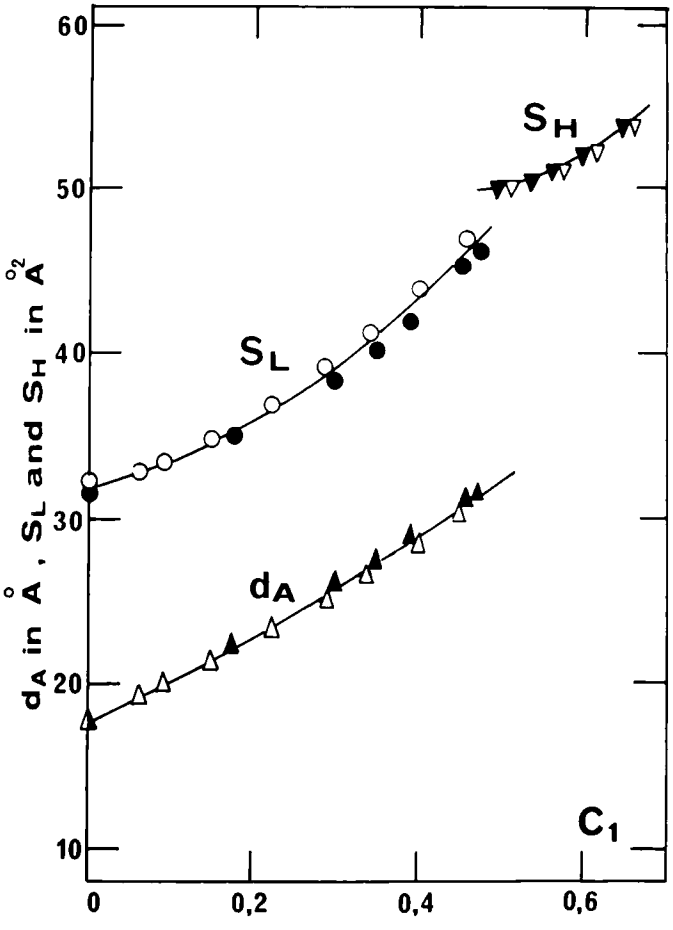


FIGURE 11 Variation of the geometrical parameters: thickness d_A (Å) of the hydrophilic layer, area per molecule S_L (Å^2) and S_H (Å^2) for two lipolysinebromhydrates C_{12}Lys(HBr)HBr and C_{18}Lys(HBr)HBr versus the water content C_1 of the hydrophilic domains: C_{12}Lys(HBr)HBr: d_A △; S_L ○; S_H ▽, C_{18}Lys(HBr)HBr: d_A ▲; S_L ●; S_H ▼.

It is not surprising that when the length of the paraffinic chain increases the characteristic parameters of the paraffinic domains: d_B for the lamellar structure and 2R for the hexagonal structure increase. Furthermore, as the nature of the amino-acid residue is the same for the two lipo-amino-acids one can understand that d_A, S_L and S_H remain constant.

The characteristic parameters of the hydrophilic domains of the lamellar and hexagonal structures of lipolysinebromhydrates are independent of the length of the paraffinic chains as already observed for the lamellar structure of lipo-amino-sarcosine in the free amine form.[3]

CONCLUDING REMARKS

The synthesis of lipo-amino-acids by coupling between fatty amines and N-protected amino-acids has allowed the study by X-ray diffraction of the mesomorphic behavior of amphipatic lipo-amino-acids. The nature of the mesophases, their domain of stability and the values of their geometrical parameters have been related with the water concentration, the nature of the amino-acids and the length of the paraffinic chains. It has been shown that the existence of mesophases requires a minimum of hydrophilicity for the amino-acids. When the side-chain of the amino-acid is not hydrophilic enough, the transformation of the α-amino function of the amino-acid into chlorhydrate or bromhydrate can provide the increase of hydrophilicity necessary to obtain the formation of mesophases. When the side-chain of the amino-acid is hydrophilic enough to give raise to mesophases the transformation of the α-amine function of the amino-acid into chlorhydrate or bromhydrate leads to an anisotropic swelling of the hydrophilic domains characterized by a higher dilatation in the direction perpendicular to the interface than in the plane of the interface.

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