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SYNTHESIS AND STRUCTURAL STUDY BY X-RAY DIFFRACTION OF LYOTROPIC BLOCK LIPOPEPTIDIC POLYMERS WITH POLYLYSINE AND POLY(GLUTAMIC ACID) PEPTIDIC CHAINS

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Abstract Amphipatic lipopeptides with polylysine or poly(glutamic acid) peptidic chains have been synthesized. Their structural study, in the dry state and in water solution, by X-ray diffraction, has shown the existence of two types of mesophases : lamellar and hexagonal. The influence of the nature and the degree of polymerization of the peptidic chains and of the water concentration on the type and the structural parameters of the mesophases has been established.

INTRODUCTION

Recently we have undertaken the synthesis and the study of amphipatic lipopeptides because they are both lyotropic liquid crystals and powerful emulsifiers[1,2]. We have synthesized a lot of lipopeptides $C_n(AA)_p$ differing by the number of carbon atoms n of their lipidic blocks (C_n) and the nature AA and the degree of polymerization p of their polypeptidic blocks $(AA)_p$.

In a first paper[3], we have reported on the mesomorphic behaviour of non ionic liposarcosine $C_n(Sar)_p$ with n between 12 and 18 and p between 1 and 61.

The present paper reports on two families of ionic lipopeptides : a cationic one lipolysine in the bromhydrate form C_nK_p and an anionic one lipoglutamic acid in the sodium or potassium salt form C_nE_p. Taking into account the

results of the study of liposarcosine[3], only lipopeptides with paraffinic chains containing 18 carbon atoms have been synthesized and studied.

SYNTHESIS OF LIPOPEPTIDES

Lipopeptides $C_{18}K$, $C_{18}K_2$ and $C_{18}E$ were prepared by a step by step method starting from octadecylamine and lysine or glutamic acid protected on their two amine functions or on their amine function and their side chain carboxylic function[4].

Lipopeptides $C_{18}K_p$ with $p > 2$ were prepared by polymerization of the N-carboxyanhydride of N^{ϵ}-benzyloxycarbonyl-L-lysine at the end of octadecylamine in chloroform solution followed by the elimination of the benzyloxycarbonyl protecting group by action of an excess of HBr in acetic acid solution[4].

Lipopeptides $C_{18}E_p$ were prepared by polymerization of the N-carboxyanhydride of γ-benzyl-L-glutamate at the end of octadecylamine in THF solution followed by elimination of the protecting benzyl ester group by an excess of NaOH or KOH in methanol solution[4].

Number average degrees of polymerization p of the lipopeptides were determined by titration of the terminal amine function of the protected lipopeptides and by NMR spectroscopy of deprotected lipopeptides[4].

STRUCTURAL STUDY OF LIPOPEPTIDES

Mesomorphic gel preparations and X-ray diffraction studies were performed as already described[3].

A) Structure of the mesophases

We have studied by X-ray diffraction in the anhydrous state and in water solution two sets of lipopeptides : a set of

lipolysine bromhydrates $C_{18}K_p$ with p = 1, 2, 5, 9, 14 and 18 and a set of lipoglutamic acid Na and K salts $C_{18}E_p$ with p = 1, 2, 5, 8, 10 and 20.

As in the case of classical amphiphiles[5] two regions can be distinguished on the X-ray patterns exhibited by the lipopeptides :

- the low angle region contains a set of 3 to 5 sharp lines whose Bragg spacings are in the ratio 1, 2, 3... for a lamellar structure and 1, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{7}$... for a hexagonal structure,

- the wide angle region contains a set of sharp lines when the lipidic chains are crystallized and a diffuse band when the lipidic chains are disorganized or liquid.

The examination of the wide angle region of X-ray patterns shows that for p = 2, 5 and 9 in the case of $C_{18}K_p$ and for p = 5 and 8 in the case of $C_{18}E_p$ lipopeptides exhibit mesophases at room temperature, whereas for $C_{18}K$, $C_{18}E$ and $C_{18}E_2$ the lipidic chains are crystallized at room temperature and to obtain mesophases lipopeptides have to be heated until 70°C, 120°C and 50°C respectively.

The examination of the low angle region of X-ray patterns shows that

- for p = 1 and 2, lipopeptides $C_{18}K_p$ and $C_{18}E_p$ exhibit mesophases with lamellar and hexagonal structures,

- for p = 5 and 9 in the case of $C_{18}K_p$ and p = 5 and 8 in the case of $C_{18}E_p$ lipopeptides exhibit mesophases with hexagonal structures only,

- for p = 14 and 18 in the case of $C_{18}K_p$ and for p = 10 and 20 in the case of $C_{18}E_p$ lipopeptides do not exhibit mesophases with periodic structures.

The lamellar structure 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 of the lipopeptides, while the other of thickness d_A contains the hydrophilic peptidic chains K_p or E_p and the water.

The hexagonal structure consists of long and parallel cylinders ; the cylinders of diameter $2R_H$ are filled with the hydrophobic paraffinic chains of the lipopeptides and are assembled on a hexagonal array of parameter D while the space between the cylinders is occupied by the hydrophilic peptidic chains K_p or E_p and the water.

The lattice parameters : intersheet spacing d for the lamellar structure and distance D between the cylinders for the hexagonal structure were directly obtained from the X-ray patterns. The other parameters : thickness d_A and d_B of the hydrophilic and hydrophobic layers in the lamellar structure, diameter $2R_H$ of the cylinders in the hexagonal structure and the average surface S occupied by a chain at the interface for the two structures were calculated by formulae based on simple geometrical considerations[3].

B) Influence of the water concentration

Depending upon their degree of polymerization, lipopeptides exhibit one or two mesophases as a function of water concentration.

Lipopeptides $C_{18}K_p$ and $C_{18}E_p$ with p = 1 and 2 exhibit successively two mesophases as the water concentration increases : a lamellar one and a hexagonal one.

Lipopeptides $C_{18}K_p$ with p = 5 and 9 and lipopeptides $C_{18}E_p$ with p = 5 and 8 exhibit only hexagonal structures.

Figures 1 to 4 illustrate the variation of the structural parameters of the mesophases for 2 lipopeptides $C_{18}K_p$ and 2 lipopeptides $C_{18}E_p$.

Inside the domain of stability of one type of meso-

phase the structural parameters vary on a monotonous way with water concentration. On the contrary one observes a discontinuity in the variation of the structural parameters at the transition between the lamellar and the hexagonal structures (Figs 1 and 3).

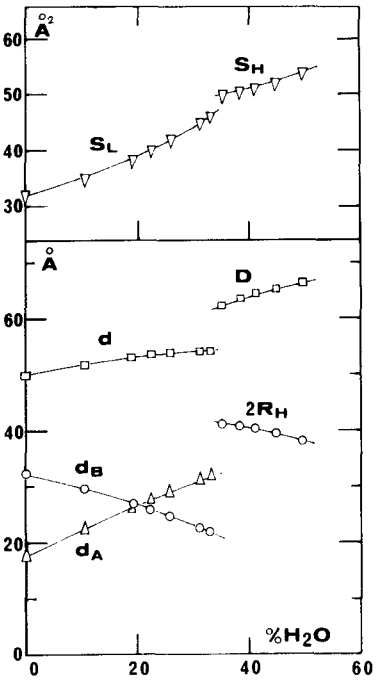

Fig. 1 - $C_{18}K_1$
Influence of the water concentration on the structural parameters of the mesophases.

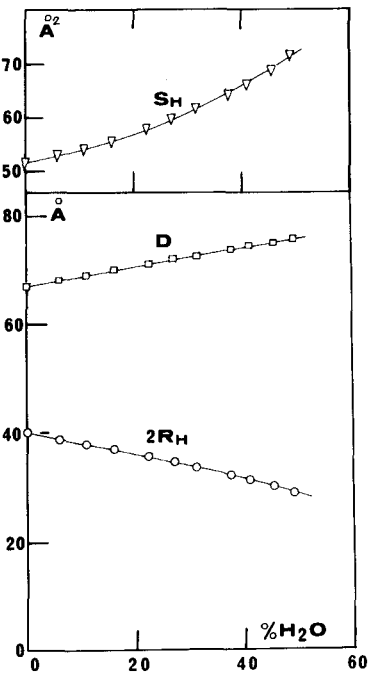

Fig. 2 - $C_{18}K_5$

a) Lamellar structure. When the water concentration increases : the total thickness d of a sheet, the thickness d_A of the layer containing the hydrophilic peptidic chains and the water and the specific surface S_L all increase, while the thickness d_B of the layer containing the hydro-

phobic paraffinic chains decreases.

b) Hexagonal structure. When the water concentration increases : the distance D between two neighbouring cylinders and the specific surface S_H both increase, while the diameter $2R_H$ of the cylinders filled with the hydrophobic paraffinic chains decreases.

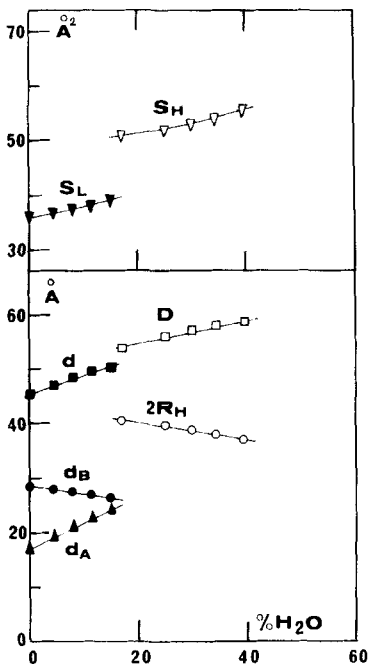

Fig. 3 - $C_{18}E_2$

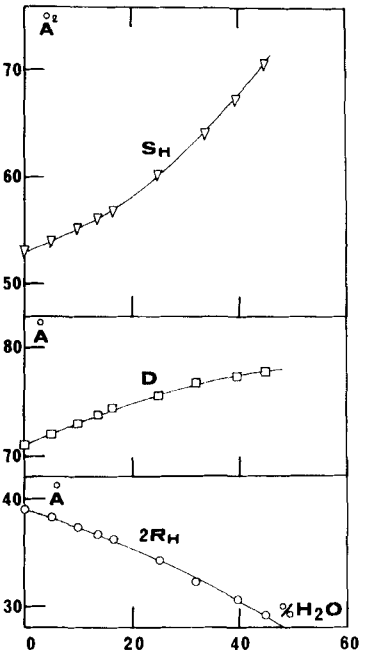

Fig. 4 - $C_{18}E_8$

Influence of the water concentration on the structural parameters of the mesophases.

The decrease of d_B for the lamellar structure and of $2R_H$ for the hexagonal structure when the water concentration increases may seem surprising but can be explained easily. The addition of water to lipopeptides swells the

peptidic chains and the surface S occupied by a molecule at the interface between the hydrophilic and hydrophobic domains increases. The increase of S involves a decrease of the characteristic parameter of the hydrophobic domains (d_B for the lamellar structure and $2R_H$ for the hexagonal structure) as the hydrophobic lipidic chains have to keep a constant density.

C) Influence of the degree of polymerization

In order to show the influence of the degree of polymerization p, we have plotted the variation of the structural parameters as a function of the water content C_1^3 of the domains filled with the hydrophilic polypeptidic chains and the water. Then, for a constant value of C_1 (C_1 = 0,5 for $C_{18}K_p$ and C_1 = 0,4 for $C_{18}E_p$) we have plotted the variation of the parameters of the hexagonal structure as a function of p. Fig. 5 for $C_{18}K_p$ and Fig. 6 for $C_{18}E_p$ show that for the two types of lipopeptides, when p increases, the distance D between neighbouring cylinders and the specific surface S_H both increase while the diameter $2R_H$ of the cylinders filled with the hydrophobic paraffinic chains decreases.

Such variations of the structural parameters is the result of the increase of the volume of the peptidic chains when p increases. This volume increase of the peptidic chains involves an increase of both the distance between the cylinders and of the surface S_H available for a chain at the interface. So the increase of S involves a decrease of the diameter $2R_H$ of the hydrophobic cylinders as the density of the paraffinic chains has to remain constant.

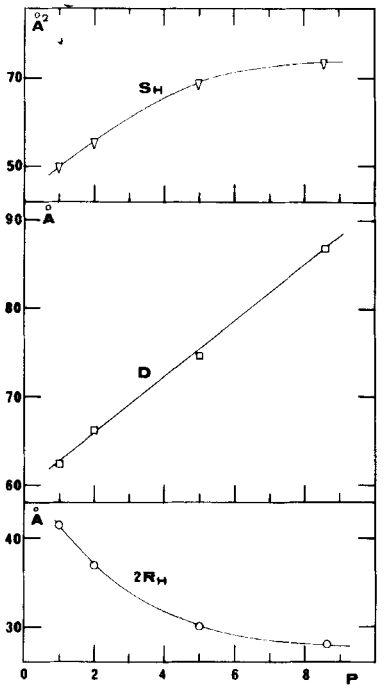

Fig. 5 - $C_{18}K_p$

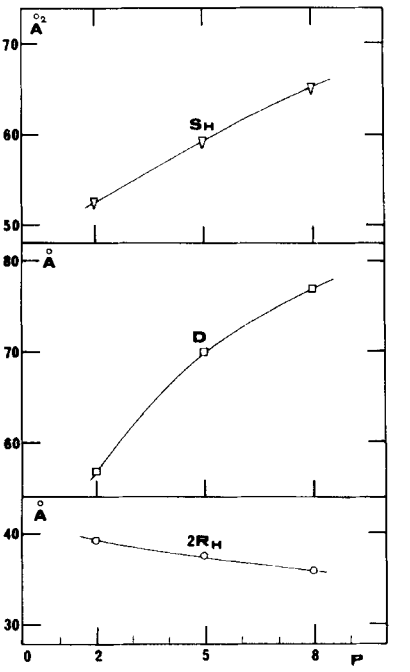

Fig. 6 - $C_{18}E_p$

Influence of the degree of polymerization p on the parameters of the hexagonal structure of lipopeptides.

CONCLUDING REMARKS

The comparison of the ionic lipolysine bromhydrate $C_{18}K_p$ and lipoglutamic acid sodium and potassium salts $C_{18}E_p$ with non ionic liposarcosine $C_n(Sar)_p$ previously studied[3] shows two striking differences : $C_n(Sar)_p$ exhibits mesophases until high degrees of polymerization ($p > 60$) while $C_{18}K_p$ and $C_{18}E_p$ exhibit mesophases for p smaller than 10 ; second when p increases $C_n(Sar)_p$ exhibits successively 3 types of mesophases : lamellar, hexagonal and body-centred cubic, while $C_{18}K_p$ and $C_{18}E_p$ exhibit only lamellar and

hexagonal structures. Such differences may find their explanation in the fact that for a given value of p the specific surface S is much higher for lipopeptides $C_{18}K_p$ and $C_{18}E_p$ than for lipopeptides $C_n(Sar)_p$ as illustrated in Fig. 7 for the hexagonal structure. So for C_nK_p and C_nE_p, when p increases S becomes rapidly too high to be compatible with the surface occupied by a "liquid" paraffinic chain and prevents the formation of the body-centred cubic structure.

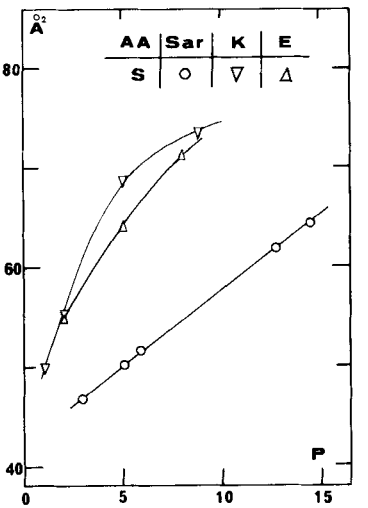

Fig. 7 - Influence of the nature of the peptidic chains on the variation with the degree of polymerization p of the average surface S per molecule in the case of the hexagonal structure.

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