A Lamellar Liquid Crystal with Fosinopril Sodium

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Received March 24, 1993, from the *Center for Advanced Materials Processing and Department of Chemistry, Clarkson University, Potsdam, NY 13699-5814, and the *Bristol-Myers Squibb Co., New Brunswick, NJ 08903-0191*
Accepted for publication August 5, 1993[⊗].

Abstract □ The location and conformation of fosinopril sodium (FS) in a lamellar liquid crystal of water, sodium dodecyl sulfate, and decanol was studied by low-angle X-ray diffraction. The result showed the FS molecule to be located within the amphiphilic part of the liquid crystalline structure with the polar parts anchored at the water/polar part interface. An area per molecule in the range of 95–100 Å^2 showed not only the polar groups but also the benzene ring to be located at this interface.

Introduction

The structure of lyotropic liquid crystals has been thoroughly investigated after Ekwall's pioneering research[1] in the area. Both the structure and the dynamics are known to a satisfactory degree for aqueous systems[2–10] as well as nonaqueous combinations in which water is replaced by polar organic substances.[11–18]

One structural feature that has not attracted the attention it deserves is the use of the lamellar liquid crystal to determine the location and conformation of a large and complex amphiphilic molecule at an interface.[19,20] This method has special appeal for biologically active molecules, because of the prevalence of lamellar structures in biological systems such as biomembranes, mitochondria, and chloroplast.

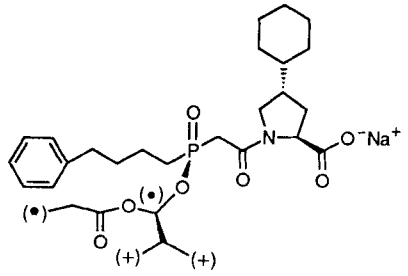
In fact, determining the location of drug molecules in membranes may help elucidate the mechanism of action. This has been elegantly demonstrated by Herbette, who had addressed the effect of membrane location on the activity of some cardiovascular drugs.[21–24] The same rationale should apply to ACE (angiotensin converting enzyme) inhibitors, which are typically amphophilic molecules. The use of the model liquid crystalline membrane system will hopefully serve to factor the contribution of the various membrane components in orienting the molecules.

We found a preliminary investigation of a cardiovascular drug of interest and chose fosinopril sodium (FS) as the model compound. FS (Monopril by Bristol-Myers Squibb) is the prodrug of a novel angiotensin converting enzyme inhibitor for the treatment of hypertension and congestive heart failure. Its structure is given in Figure 1 and, as the structure suggests, it is surface active in aqueous solutions and has been shown to begin aggregation at low concentrations (2–4 mg/mL) in aqueous solutions.[25] The structure and aggregation behavior made this an ideal model compound for our investigation.

Experimental Section

Materials—n-Decyl Alcohol ($C_{10}OH$) (98%) and sodium dodecyl sulfate (SDS) (lauryl sulfate, 99%) were purchased from Sigma Chemical Co., St. Louis, MO. The water used in the study was distilled twice. Fosinopril sodium was received from Bristol-Myers Squibb, Priceton, NJ.

Preparation of Samples—The solutions were prepared by adding the FS in weight percentages of 3, 7, 15, 25, 30, and 45 to a mixture of $C_{10}OH$/SDS, which latter were maintained at a weight ratio of 2/3, to which water was added in weight percents ranging from 17 to 42.



fosinopril sodium
$C_{30}H_{44}PNO_7Na$ MW 585.7

Figure 1—The structure of fosinopril sodium.

Low-Angle X-ray Diffraction—The interlayer spacings of the liquid crystalline phase were determined by low-angle X-ray, diffraction using a Siemens Crystalloflex 4, with a Tennelec detector system (PSD 100). A path length of 50 cm and 0.5-mm capillaries with 0.01-mm walls were used. The measurements were done at 40 kV, 30 mA and room temperature (22.5 °C).

Results and Discussion

The drug was accepted into the lamellar liquid crystal to a high degree (i.e., 45% by weight). The interlayer spacings as calculated from the low-angle X-ray diffraction patterns are shown in Figure 2. A linear increase in the interlayer spacing of the liquiid crystal was observed with the addition of water. Extrapolation of these data to zero water concentration gives the characteristic spacing (d_0) for the lipid part of the layered structure (B + C, Figure 3). The trends in the change of interlayer spacing with water content varied systematically with the content of the drug as follows (Table 1). Addition of FS up to approximately 7% (w/w) produced an increase in d_0. This change was accompanied by a decrease in the slope of the interlayer spacing versus water content curves (Figure 2). For drug content in excess of this value, the d_0 was reduced and the slope slightly increased. The consequence is that the d_0 value, which reflects the conditions in the amphiphile part of the structure (B and C, Figure 3) displayed a complicated dependence on drug content. This takes the form of a rapid increase to a maximum d_0 value (≈7%) followed by a steep decline (Figure 4).

The explanation for this behavior lies with the composition of the liquid crystalline phase. With no drugs present, the interlayer spacing reflects the chain length of the two amphiphiles as follows. Extended hydrocarbon chains have lengths of 1.5 + 1.265n Å, where n is the number of methylene groups.[26] With a molar ratio of $C_{10}OH$ to SDS of 1.4 and a 1.5 Å length value for the polar groups, the value of the interlayer spacing should be 30.9 Å. This is in good agreement with the experimental value of 30.3 Å, considering the disorder of the chains.

Initial addition of the drug caused an increase in d_0 to 33.7 Å (Table 1). This value agrees with the calculated length of close-packed SDS molecules (33.8 Å), indicating that the layer has been expanded to the limit of the longest chain present. With these values as a base, the following interpretation appears justified and reasonable. Before addition of the drug all the

* Abstract published in *Advance ACS Abstracts*, December 15, 1993.

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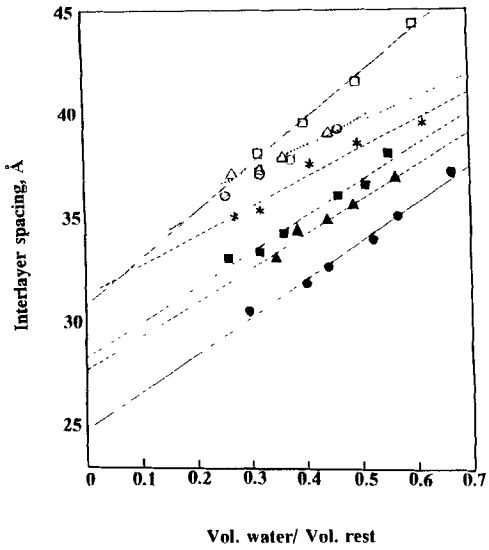

Figure 2—Interlayer spacings versus water volume ratio for different volume percents of fosinopril sodium in relation to the nonaqueous components of the structure: □, 0%; △, 2.4%; ○, 5.8%; *, 13.5%; ■, 24.8; ▲, 32.7%; ●, 62.5%.

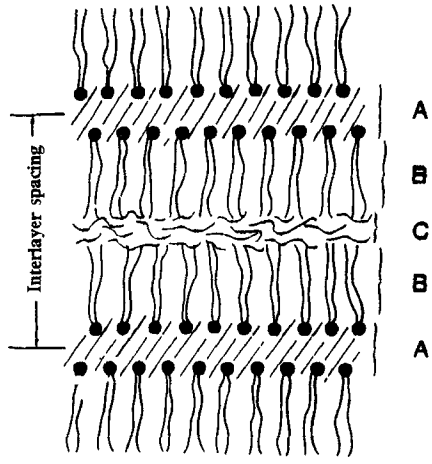

Figure 3—The lamellar liquid crystal is divided into three zones: (A) water layer, (B) amphiphiles, (C) space between the terminal methyl groups,

Table 1—Values of Penetration Coefficients (Data Corresponding to Figure 2)

Weight % Drug	Volume Ratio of Drug	Interlayer Spacing[a]	Penetration Value[b]	Sample SD[c]
0.0	0.000	30.76	0.210	0.020
3.0	0.024	32.04	0.445	0.016
7.0	0.058	33.65	0.580	0.022
15.0	0.135	31.26	0.469	0.043
24.8	0.248	28.22	0.309	0.028
30.0	0.327	27.62	0.327	0.026
45.0	0.625	24.77	0.202	0.024

[a] At zero water content. [b] Calculated average. [c] For penetration values (68% confidence interval).

alcohol and SDS molecules are anchored at the water/polar group interface (region A, Figure 3), and no alcohol molecules are found in region C (Figure 3). As mentioned, the d_0 value agrees exactly with the calculated average value for the extended hydrocarbon chains. Initial addition of the drug caused an increase in d_0, limiting its value to that of the extended chain of the SDS as described above.

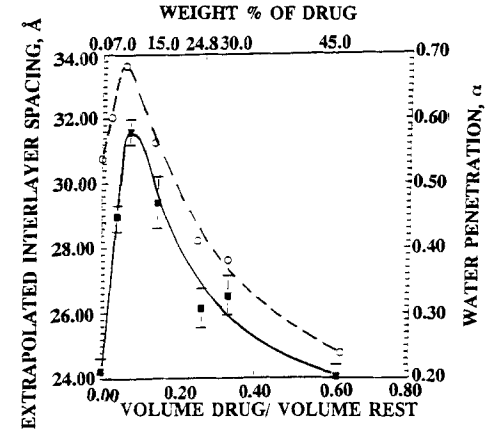

Figure 4—Interlayer spacing extrapolated to zero water content (○) and water penetration fraction (■) for different amounts of drug (in relation to decanol and sodium dodecyl sulfate).

This change may be achieved either by the drug molecule or the alcohol molecule occupying a position close to region C in zone B with the polar groups removed from the interface between B and A (Figure 3). The SDS molecules, with the ionic group, are firmly anchored at this interface as shown by a large number of investigations.[1] A further support for this assumption is the value of d_0 (≈34 Å). The agreement between twice the length of an extended SDS and ther interlayer spacing is rather compelling support for the suggested location of the SDS. The exact packing of the alcohol and drug molecules at low concentration cannot be determined from the present results, and is not essential for the main thrust of this investigation.

Larger addition of FS leads to a reduction of d_0 and it is, therefore, reasonable to assume that all the surfactant and drug molecules are now anchored at the interface between regions A and B. This hypothesis enables the calculation of the area per molecule for the drug based on the linear trend between added drug and decreasing d_0 (Figure 4).

On has

$$A = (2 \times 10^{24}\, M)/(N_A \rho d) \qquad (1)$$

in which A is the area per molecule, M is molecular weight in g, N_A is Avagadro's number, ρ is density in g/cm^3, and d is interlayer spacing in Å.

The calculation assumes a constant area for the alcohol and SDS attributing the reduction in d_0 to the drug per se. The slope of the curve for concentrations higher than that of the drug (>15%) (Figure 4) gave values in the range of 95–100 Å^2. Such a value requires the polar groups as well as the benzene ring of FS to be present at the polar group surface, a possibility that is supported by earlier investigations of benzene using NMR and calorimetry.[26,27]

These changes in location of the molecules are also supported by the slope of the curves in Figure 2. These are used to calculate the amount of water penetrating into layer B from layer A (Figure 3). The fraction of water located in B may be defined according to eq 2in which d is interlayer spacing, d_0 is its value extrapolated

$$d = d_0(1 + R)/(1 + \alpha R) \qquad (2)$$

to zero water content as before, α is the penetration fraction, and R is the volume ratio of water to amphiphile. This relationship factors the geometric changes due to water inclusion into the two components. The first (d_0R) describes the increase in d due to the water moving into region A (Figure 3). The second component ($d_0\alpha R$) describes the amount of decrease in interlayer spacing due to water penetrating into region B (Figure

3). Such a penetration will expand the region horizontally, causing a reduction in the thickness of region A.

Calculations of α using the results from Figure 2 (Table 1) are plotted in Figure 4 vs FS concentration and show an initially strong increase of α as the drug is added reaching a maximum value at approximately the maximum value of d_0, followed by a strong decrease.

These values are in good agreement with the interpretation of structural changes based on the d_0 values. Initial addition of FS causes a removal of polar groups from the surface, creating a structure in which the polar groups of the alcohol and/or Fs are located within zone B of the lamellar structure. Such an arrangement certainly facilitates water penetration. For greater amounts of drug, more polar groups become anchored at the water interface; the relative amounts of polar groups in region B are reduced and the water penetration reduced.

As mentioned in the introduction, lamellar structures are ubiquitous in biological systems. The absorption and activity of many drugs, and ACE inhibitors specifically, is in some measure a function of the drugs' interaction with these membranes.[25] It is reasonable to assume that the spatial relationship FS exhibits with the model system is a good starting point to define its interaction with biological lamella. With this in mind, work is in progress to study the interaction with more relevant biological systems.

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