Chiroptical Properties of Lecithin Reverse Micelles
and Organogels

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ABSTRACT The ultraviolet absorbance and circular dichroism (CD) spectra of lecithin reverse micelles and gels were investigated in order to establish whether the formation of these noncovalent macromolecular aggregates, which was induced by the addition of water to solutions of lecithin in organic solvents, was accompanied by specific spectroscopic changes. Systems containing the synthetic short-chain lecithins, 1,2-hexanoyl-, 1,2-dieheptanoyl-, 1,2-dioktanoyl-, and 1,2-dinonanoyl-sn-glycerol-3-phosphatidylycerolines were used as models for the long-chain lecithins, soybean phosphatidylcholine and palmityl-oleoyl phosphatidylcholine. All the molecules studied had asymmetric centres, formed reverse micelles under appropriate conditions, and, while both the long-chain lecithins also formed gels, none of the short-chain molecules did. As well as having CD spectra that were similar to simple, direct spectroscopic observations on solutions of the short-chain lecithins could be carried out over a large water content range. The ester chromophore of these compounds was shown to be highly sensitive to variation in both the solvent environment and the temperature, and components of both direct solvent effects and conformational change upon the addition of water were detected in the spectra.

The spectra of the longer chain lecithins were complicated by the presence of double bonds although, here again, it was found that significant changes occurred as the water content increased, as monitored by the ester chromophore. However, no specific effect that could be ascribed to gelation alone was detected.

The overall picture that emerged was that the ester chromophore of anhydrous micelles gave rise to a specific negative band in the CD spectrum (λmax ≈ 210 nm) whereas a positive CD signal (λmax ≈ 235 nm) was associated with the same chromophore in filled (i.e., hydrated) micelles. The two signals correspond to two different conformational states of the lecithin molecule, the hydrated state being not only more conformationally restricted but also providing a less polar environment for the ester groups, while the addition of water to the system shifts the conformational equilibrium. These observations have been interpreted as showing that only a limited range of lecithin conformation is compatible with the formation of the micellar structure and that it is this constraint, together with those introduced by the overall geometry of the aggregated state, that gives rise to the changes observed in the CD spectrum.

KEY WORDS: circular dichroism, spectroscopy, surfactant, optical activity, phosphatidylcholine

INTRODUCTION

In the last few years our interest in surfactant aggregation in organic solvents has shifted toward biosurfactants in general and lecithin in particular.1–6

There are several reasons for this. In addition to the biological importance of phospholipids, the use of biosurfactants, combined with biocompatible solvents wherever possible, allows the formulation of more suitable systems for biomedical applications. Furthermore, since proteins solubilized in reverse micelles are used as model systems that mimic biological membranes, the use of phospholipid surfactants, which are certainly closer to the natural systems than sulfosuccinates and similar synthetic compounds, would seem to be advantageous.

Systems involving lecithin dissolved in organic solvents are also able to form microemulsion gels, a property of growing interest (that has been reviewed recently7), as are the properties of organogels in general.2–8–14 The main component of an organogel is an organic solvent, whereas the prerequisite structure in solution for gelation in microemulsion gels is a water-in-oil microemulsion or reverse micelle.

In the case of lecithin gels, gelation is induced by the addition of water, usually in a stoichiometric amount with respect to the surfactant, to a solution of the lecithin in an organic solvent. The relative amount of water present is normally expressed as the quantity w0, which is given by the molar ratio, w0 = [H2O]/[lecithin]. For the 70 or so solvents investigated to

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date,\textsuperscript{2} \( n_0 \) has been in the range 2 to 10. Lecithin gels have been characterized by rheology and NMR measurements,\textsuperscript{3,5} and particular attention has been focused on the elucidation of solution structure, as it is far from obvious how lecithin, which is a low molecular weight compound, can form gels with viscosities of up to 10,000 poise. This problem has partially been solved by a combination of small angle neutron scattering (SANS) and light scattering techniques\textsuperscript{3} which have shown that addition of water to the small lecithin reverse micelles present in organic solvents induces monodimensional growth into long cylindrical aggregates above a critical volume fraction of lecithin, these rods become entangled with each other, forming a dynamic network of high viscosity. Bio-compatible lecithin gels are now under investigation as carriers for transdermal transport and also as novel microenvironments for proteins that may mimic the situation encountered by a protein when bound to a membrane.

Lecithin is a chiral compound, and this paper is devoted primarily to the chirurgical properties of lecithin reverse micelles. The main objective of this study is to assess whether, and to what extent, the formation of lecithin aggregates is accompanied by the appearance of specific spectroscopic signals such as novel absorbance bands and/or by the perturbation of existing chromophores. It is well known that the ordered association of chromophores in a macromolecular structure can give rise to novel absorbance features but, despite some interest devoted to chiral surfactants,\textsuperscript{15-16} no such phenomena have been reported for reverse micellar systems. We will show, however, that, in the case of lecithin, absorbance phenomena, connected with the formation of both the reverse micellar and the gel structure, can indeed be observed and that this appears to be a general property of lecithin.

The experimental approach taken has been to characterize the chirurgical properties of the synthetic short-chain lecithins 1,2-hexanoyl-, 1,2-dioleoyl-, 1,2-dioctanoyl-, and 1,2-dioleoyl-\( \omega \)-glycerol-3-phosphatidylcholine (abbreviated here as C\(_6\), C\(_8\), C\(_{10}\), and C\(_{12}\) lecithin, respectively) and the synthetic long-chain lecithin palmitoyl-\( \omega \)-oleoyl-phosphatidylcholine (POPC). The results of these experiments are then compared with those obtained on a chromatographically purified, naturally occurring soybean lecithin (PCCP).

Qualitatively similar observations have been made on chiral AOT analogues that have the property of forming reverse micelles (but do not gelate)\textsuperscript{19} and the spectroscopic behaviour of lecithin reverse micelles and gels containing guest molecules will be reported in a subsequent paper.

**MATERIALS AND METHODS**

The soybean lecithin Epikuron 200 was purchased from Lucas Meyer, Hamburg, Germany, and following chromatographic purification (see methods section) was designated PCCP (phosphatidylcholine chromatographically purified), 1-Palmityl-\( \omega \)-oleoyl-\( \omega \)-glycerol-3-phosphatidylcholine (POPC) was from Bachem AG, Bubendorf, Switzerland and the synthetic short-chain lecithins 1,2-hexanoyl-, 1,2-dioleoyl-, 1,2-dioctanoyl-, and 1,2-dioleoyl-\( \omega \)-glycerol-3-phosphatidylcholines were obtained from Avanti Polar Lipids, Inc., Pelham, AL.

All other materials and solvents were from Fluka and were of the highest purity available.

**Thin-Layer Chromatography (TLC)**

Lecithin purity and composition were checked by thin-layer chromatography on silica gel plates (Kieselgel 60 F 254; Merck, Darmstadt, FRG) developed with chloroform/methanol/water (65:25:4 v/v/v) and stained with iodine.

**Preparative Flash Chromatography**

In a typical purification procedure, 10 g of commercially supplied soybean lecithin was applied to a silica gel flash chromatography column (Kieselgel 60, 230–400 mesh, 50 × 4 cm ASTM; Merck) and eluted with chloroform/methanol/water (65:25:4 v/v/v) with a flow rate of 2 ml/min, 20 ml fractions being collected.

**Preparation of Microemulsion Lecithin Gels**

A 100–200 mM lecithin solution was prepared by dissolving the required amount of lecithin in the organic solvent, with stirring; at room temperature. Some of the solutions obtained were clear while others were cloudy, the latter, however, becoming clear upon heating at 45°C for 10–20 min. Gels were prepared from these solutions by adding (in one step) the appropriate amount of water (see Table 1) with vigorous mechanical stirring. The time required for gelation depended on the solvent used, and varied from a few seconds (in the case of octaeryl octanoate) to 30–40 min (in the case of cyclohexane or isooctane).

**Spectroscopic Measurements**

UV absorption measurements were carried out on either a Hewlett Packard 8452A UV/VIS diode-array or a Perkin-Elmer Lambda 9 spectrophotometer. Circular dichroism spectra were recorded with a Jasco J-600 spectropolarimeter interfaced with an IBM AT computer, using a cylindrical 0.05 cm pathlength quartz cell and a demountable quartz cell of 0.01 cm pathlength. Nine to 16 CD spectra in the 280 to 190 nm region were accumulated and, after subtraction of the solvent blank, data were stored at a resolution of 0.2 nm. The scan speed was 20 nm/min, time constant 1 sec, band width 1 nm, and the sensitivity range was 20 to −20 mdeg or 50 to −50 mdeg. All UV and CD measurements were performed at 22 ± 0.2°C, if not otherwise specified. In no case did the total absorbance of any sample exceed 1.5 absorbance units at any wavelength in the CD spectra reported. Error bars represent noise-to-noise limits at the relevant wavelength.

**RESULTS**

The spectroscopic observations reported here arise from two types of chromophore. All the compounds studied contain the ester chromophore which has an environmentally sensitive \( \pi \rightarrow \pi^* \) transition (\( \lambda_{\text{max}} \approx 204 \text{ nm} \) for model compounds in water\textsuperscript{20}). In addition POPC and PCCP have electric double bonds which contribute a \( \pi \rightarrow \pi^* \) transition to the spectrum at wavelengths of 200 nm and lower, depending on their number and position. The absorbance spectra of the latter two compounds will, therefore, be characterized by components of both transitions, the stronger being the \( \pi \rightarrow \pi^* \).

All these compounds are optically active and CD signals associated with the absorbance bands can be expected.\textsuperscript{21} The position of spectral bands in the CD spectrum and their inten-
The far UV CD spectrum of \( \beta \)-lecithin as a function of \( \Phi_a \) is shown in Figure 2 and it is characteristic of a compound containing an ester chromophore. As the water content is raised from \( \Phi_a = 0 \) to \( \Phi_a = 60 \), the negative band at 210 nm first decreases in negative intensity, then becomes positive, and the form and the intensity of the spectrum become practically the mirror image of those seen initially. There is a bathochromic shift of the spectral maximum of \( \approx 7 \) nm by \( \Phi_a = 60 \) and this, together with the change of sign of the CD signal, are typical for the transfer of the chromophore to a less polar environment. The reference spectrum in methanol is of smaller negative intensity, but similar form, to that of the empty micelles (i.e., \( \Phi_a = 0 \)), which suggests that, even in the absence of water, the formation of the micellar structure contributes to the CD spectrum. We have extended this type of study to other synthetic lecithins, and Figure 3 shows the dependence of the CD signal at 211 nm on \( \Phi_a \) for \( C_{16}, C_{18}, C_{16} \), and \( C_{18} \)-lecithins. Although \( \Phi_a \) values as high as 60 could not always be reached, the overall picture is clearly the same for each of the compounds.

Changes in temperature have a pronounced influence on the CD spectrum of \( \beta \)-lecithin at fixed \( \Phi_a (\approx 3) \), as illustrated in Figure 4. As the temperature falls the 211 nm minimum loses negative intensity and is apparently blue-shifted to \( \approx 207 \) nm. Concomitantly the signal at 233 nm, the contribution of which is barely discernible at room temperature, greatly increases in size. Results of this type are typical of the presence of a conformational equilibrium between two structural species that differ considerably in their CD properties. At all temperatures the net spectrum represents the weighted sum of the contribution of each of the two conformers. In this case, the species characterized by the positive band at 233 nm is presumably more conformationally restricted, as it is dominant at lower temperatures.

Table 1 lists the solvents that have been used to produce gels with the two longer chain lecithins, POCP and PCCP, and the corresponding \( \Phi_a \) values at which maximum gelation, determined viscometrically, has been observed. Cyclohexane was chosen as the solvent for the studies on POCP, as it allows investigations over a relatively large range, compared with other hydrocarbon solvents, prior to gel formation. Increasing the water content of the system brings about a significant increase in the UV absorbance at both 200 and 230 nm (Fig. 5). Artifacts due to light scattering can be excluded, on the grounds that none of the solutions showed any absorbance above 300 nm and, that in any case, scattering by the gel is generally very low. Figure 6 shows the corresponding CD spectra. Noticeable changes are again seen at 200 nm and between 220 and 230 nm. The changes in the latter spectral region are reminiscent of those seen in the \( \beta \)-lecithin water titration experiment, while those at 200 nm reflect environmental and/or conformational variation that affects the single double bond found in this compound. Pro rata the effects are of comparable magnitude at the two wavelengths. The spectra in Figure 6 have been normalized, in the usual manner, to the corresponding molar concentration but another way of presenting CD data is to normalize them to the same optical density, since solutions of the same molar concentration but having different UV absorbances (see Fig. 3) are expected to give different CD signals. In the insert to Figure 6 the changes in ellipticity as a function
Fig. 1. UV spectra of the commercial soybean lecithin Epikuron 200 at a concentration of 0.2 M in cetyl octanoate, 1 cm cell, before (1) and after (2) chromatographic purification.

Fig. 2. The CD spectrum of the short-chain C₈ lecithin as a function of the water content. Solutions were prepared by dissolving the lecithin (50 mM) in isooctane/hexanol 9:1 (v/v) and adding the quantity of water required to obtain the values of \( n_w \) indicated. A 50 mM solution of the C₈ lecithin in methanol was used as a reference. (0) \( n_w = 0; (1) n_w = 1; (5) n_w = 5; (10) n_w = 10; (15) n_w = 15; (30) n_w = 30; (60) n_w = 60; (Ref.) C₈ lecithin (50 mM) in methanol.

Fig. 3. Influence of \( n_w \) on the ellipticity at \( \lambda_{\text{max}} \) (211 nm) for various short chain lecithins. No appreciable variations were observed in the corresponding UV spectra. All spectra were recorded at a concentration of 50 mM in isooctane/hexanol 9:1 (v/v).

Fig. 4. The effect of temperature on the CD spectrum of C₈ lecithin at fixed \( n_w \). The spectra were recorded at a concentration of 50 mM in isooctane/hexanol 9:1 (v/v), \( n_w = 3.15 \). (1) -20°C; (2) -11.5°C; (3) 0°C; (4) 10°C; (5) 25°C; (6) 44°C.

of \( n_w \) are shown in both normalization modes. It is evident that the changes in the CD intensity are not trivially a function of the different absorbances of the various solutions.

The CD spectrum of POPC in different solvents is shown in Figure 7. As discussed above, reverse micelles form in all these solvents, except for methanol. Parallel changes to those described for POPC in cyclohexane occur in the CD spectrum upon the addition of water to POPC in isooctane and n-pentane. As can be seen, the spectral changes discussed above appear to reflect the formation of micellar structures directly, although there are small solvent-related differences between the spectra.

POPC CD spectra were also sensitive to other experimental parameters such as pH, which is perhaps not surprising in view of the zwitterionic nature of the surfactant. Interestingly enough, it was not possible to make gels by adding water of pH<1.6 to organic lecithin (conditions under which it is positively charged) although at pH 14 or above, where lecithin is negatively charged, gel formation still occurred.

TABLE 1. Gel formation in various solvent systems containing either POPC or P CCP

<table>
<thead>
<tr>
<th>Lecithin</th>
<th>Solvent</th>
<th>( W_{\text{gel}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>Cyclohexane</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Isooctane</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Isoparaffin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Cetyl octanoate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cetyl octanoate/butyl acetate (9:1 v/v)</td>
<td>3</td>
</tr>
<tr>
<td>P CCP</td>
<td>Cyclohexane</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Isooctane</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Isoparaffin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cetyl octanoate</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Cetyl octanoate/butyl acetate (9:1 v/v)</td>
<td>4</td>
</tr>
</tbody>
</table>

*The value of \( n_w \) at which the viscosity is at a maximum.
POPC may, in part, be due to conjugation, as this material is known to be both heterogeneous in terms of double bonds and to contain other unidentified species that absorb in the UV (see Fig. 1). However, in this case, this band seems to be insensitive to water concentration. On the other hand, the 220 nm band, arising from the ester linkages, shows impressively similar behaviour to that seen in the simpler lecithins.

**DISCUSSION**

The appearance of new spectroscopic signals and the perturbation of existing ones arise in polymers (or in supramolecular complexes) when individual chromophores interact with each other and/or the solvent, usually, but not always, as a result of geometric constraints introduced by the formation of specific, and ordered, structures. Whether this kind of phenomenon might also be observed in reverse micelles, which are relatively monodisperse and geometrically well defined, is an interesting question. Micelles and microemulsion gels are very flexible, dynamic entities and, in contrast with polymer chains, their components are not covalently linked. As far as we know, no significant spectroscopic perturbation resulting from the formation of micellar structures has ever been described. The present study provides first-hand evidence that novel spectroscopic features may indeed arise as a result of the generation of the organized reverse micelle and microemulsion gel structures.

Phospholipids are known to have environmentally sensitive CD and ORD spectra although there are considerable spectroscopic differences between the various members of this group of compounds. Phosphatidylcholine and sphingomyelin, in trifluoroethanol, both have a weakly positive CD spectrum above 210 nm which becomes intensely negative below this wavelength for sphingomyelin, but only slightly so in the case of phosphatidylcholine. The CD of both compounds is temperature dependent, the positive band above 210 nm becoming more positive with decreasing temperature in phosphatidylcholine but more negative for sphingomyelin under the same conditions. A temperature dependence of this type may indicate the “freezing-out” or particularly stable conformation at lower temperatures.

The electronic transitions underlying the CD spectra were discussed in the previous section and the question now arises as to how the experimental observations made can be accommodated in a consistent model of micelle and gel formation. It is probably easiest to start with the behaviour of the ester chromophore, which is present in all the compounds studied. In every case this chromophore exhibits a red shift during micelle and/or gel formation, indicating that the chromophore consistently experiences a less polar environment in the organized structures. Bearing in mind that micellar growth (and gel formation in the longer chain lecithins) is initiated by the addition of water, this, perversely, appears to suggest that the overall polarity of the system (as experienced by the chromophore)
Interacts with it more weakly. This can be explained by assuming that the packing density of the lecithin molecules increases as the spherical micelle grows in size, restricting the conformational space available to individual lecithin molecules, and that, at the same time, the ester bonds become increasingly shielded from the water pool in the centre of the micelle by the phosphate head groups. In this regard, it is useful to note that, concomitantly with the increase of viscosity seen during the formation of isooctane-lecithin gels, significant changes in the $^3$P NMR are observed\textsuperscript{5}: the line width of the phosphorus resonance first broadens and then sharpens, behaviour that has been taken to indicate a change of the conformation of the phosphatidyl head group upon the formation of the gel, the plane defining the phosphate moiety moving from a parallel to a perpendicular orientation with respect to the axis of the glycerol moiety. The change in the orientation of the head group may be part of the concerted rearrangement of the lecithin conformation that occurs in order to accommodate the tighter packing of molecules required by micellar growth, the same event that is reported by the changes in the CD spectrum.

The lower wavelength band in the CD spectrum of the longer chain lecithins is much less amenable to interpretation, particularly in the case of PC/P where its origins are far from certain. The signal probably arises either from conjugation (or hyper-conjugation) or from more general interchromophore interaction. If the latter were to prove the overriding mechanism then this spectral feature might also prove useful in monitoring both the lecithin conformation as well as overall structure of the micelle.

One thing that is apparent from these results is that the process of gelation itself has no effect on the CD spectrum. It has been demonstrated by SANS and light scattering techniques\textsuperscript{3} and also recently confirmed by high-resolution multi-

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**Figure 7.** Solvent effects on the CD spectrum of POPC at a concentration of 100 mM in (1) methanol, (2) x-pentane, (3) cyclohexane, and (4) isooctane, in the absence of water.

**Figure 8.** The effect of $\omega_0$ on the CD spectrum of the POPC core, with $\xi$ varying, in (5) isooctane/boehsol 9:1 (v/v): (1) $\omega_0 = 0$, (2) $\omega_0 = 5$, (3) $\omega_0 = 10$, (4) $\omega_0 = 15$, (5) $\omega_0 = 20$. In the inset the molar ellipticity at 222 nm is plotted as a function of $\omega_0$. 

nuclear NMR, that the addition of water induces a progressive change of the lecithin reverse micelle structure present in organic solution and that gel formation in lecithins is a continuous, rather than an all-or-nothing, process. In this study we have been able to assign the changes observed by CD to the micellization rather than gelation phase. The process is envisaged as a shift in an equilibrium between two conformers of the lecithin molecule caused by the addition of water. One of the two conformers has a structure which is similar to the conformation (or range of conformations) of the lecithin in the anhydrous micelle, while the second experiences severe conformational restrictions that are imposed by the packing requirements of the micellar structure. The current model of lecithin micellization and gelation is presented pictorially in Figure 9.

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LITERATURE CITED


