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THE OPTICAL ACTIVITY AND CIRCULAR DICHROIC SPECTRA OF DIACETYLENIC PHOSPHOLIPID POLYMERS

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Phospholipids (phosphatidylcholines) which contain a diacetylene group in a single acyl chain and within both acyl chains have been synthesized. Upon irradiation with ultraviolet light, both types of lipid crosslink via the diacetylene groups to produce coloured polymers. The colour arises form the conjugated double and triple bonds which make up the polymer backbone. These phospholipid polymers can exhibit optical activity, as shown by their circular dichroic spectra. The optical activity is thought to stem from asymmetric packing of the polydiacetylene chains, a packing of one particular screw sense being favoured by the chiral glycerol moiety of the lipid. The presence of an intrinsic membrane protein within the liposome structure affects the CD spectra of polymer produced by irradiation.

Introduction

A lipid bilayer is considered to be the basic structural matrix of biomembranes. We are presently exploring various methods for manipulating and modulating these bilayer structures. This includes a study of covalently linking the individual lipid molecules within model and natural biomembranes. We have shown that such cross-linking can be accomplished with monolayers, multilayers, liposomes and the biomembranes of certain microorganisms [1–5].

In the present report we show that phospholipids containing a diacetylene group in one acyl chain of the phospholipid molecule (phosphatidylcholine) will form polymers. We also show, by measurements of circular dichroism, that polymers formed by phosphatidylcholines with the diacetylene group in one or both acyl chains are optically active. The influence of an intrinsic membrane protein on the optical activity of polymerized bilayers has been studied.

Materials and Methods

Materials

The synthesis and purification of diacetylenic phospholipids, their polymerization by ultraviolet light and the isolation and purification of phospholipid polymer have been described previously [1]. In the present study, a 23-carbon acyl chain lipid was studied. This lipid is 1,2-bis(10,12-tricosadiynoyl-*sn*-glycero-3-phosphocholine. A mixed acetylenic-saturated chain lipid was synthesized by acylating Sigma L-1-lysophosphatidylcholine. Its optical purity was confirmed by treatment with phospholipase A_2 (hog pancreas). The L-1-lysophosphatidylcholine was reformed quantitatively. GLC analysis showed that the saturated acyl chain distribution in this material was 70% palmitoyl:30% stearoyl.

Phospholipid polymer was obtained by passing partially polymerized monomer from liposomes through a Sephadex LH60/chloroform column. Polymerizations were carried out under nitrogen

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and in the case of the lipid containing two acetylenic acyl chains, irradiation was limited (10% conversion) to minimize the formation of insoluble cross-linked polymer.

Liposomes containing polymer were formed in two ways: by irradiation of N_2-purged multilamellar liposomes in an ice bath; alternatively, pure phospholipid polymer and a lipid were dissolved in chloroform, the chloroform was evaporated and dispersion was achieved by sonication in a bath sonicator (Dawe 6441A) for 30 min. Two groups [6,7] have used electron microscopy to show that the lamellar structure of liposomes is retained after polymerization.

Ca^{2+}-ATPase was isolated from sarcoplasmic reticulum of rabbit back and leg muscle. The isolation procedure and the method used to replace the native lipids of the membrane with the synthetic diacetylenic phospholipid have been previously described in detail [8]. In the protein lipid recombinant used for these experiments there were 130 molecules of lipid for every molecule of protein. The molecular weight of the lipid is 914 and that of the protein, 105 000. The enzymatic activity was measured by a standard procedure [9]. After ultraviolet irradiation, the activity was reduced by 50%. Control experiments with native Ca^{2+}-ATPase demonstrated that the activity of the protein did not change with the irradiation required for the polymerization process. From the known extinction coefficient of the chain [1] we estimate that conversion lies between 2 and 5%. Considerably more cross-linking with the formation of short-linked units may nevertheless occur.

Methods

Visible spectra of solutions and lipid-water dispersions were recorded with a Cary 17 spectrophotometer. Circular dichroic spectra were recorded on a Jasco-J40CS instrument. Both instruments belong to London University's Intercollegiate Research Service and are equipped with facilities to control sample temperature. The turbidity of Ca^{2+}-ATPase recombinants precluded measurement of the visible absorption spectrum of the polymer with the Cary 17 instrument but the use of the Shimadzu MPS 50L spectrophotometer of London University's School of Ophthalmology enabled satisfactory spectra to be obtained. Calorimetric studies were made with a Perkin Elmer DSC2 instrument.

Results

Calorimetric heating curves of each type of phosphatidylcholine show a sharp endothermic phase transition. A typical curve is shown in Fig. 1 (A detailed analysis of the phase transitions of such lipids and the effect of polymerization on the phase transition will be given elsewhere.) Both types of phosphatidylcholine, those with a diacetylene group in one or both acyl chains, produce coloured polymers when irradiated with ultraviolet light. A typical spectrum obtained from a phospholipid polymer is shown in Fig. 2.

When lipid with diacetylene in both acyl chains polymerizes in the presence of the intrinsic membrane protein, Ca^{2+}-ATPase, the absorption maximum lies at 616 nm, nearly 90 nm to the red of its usual position. The position of the absorption maximum of a polydiacetylene is determined by the length of uninterrupted overlapping of *p*-orbitals [10]. Evidently, the membrane protein is able to influence this chain property. The polymer exhibits an irreversible thermochromism similar in nature to that of the diacetylenic fatty acid [11,12], the absorption maximum shifting from 616 nm to 540 nm up on heating.

Circular dichroism spectra of methanol solutions of phospholipid polymer containing two diacetylenic acyl chains are shown in Fig. 3. No optical activity is observed above 20°C. As solu-

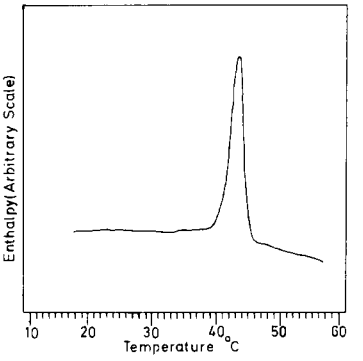

Fig. 1. Differential scanning calorimetric thermogram of phosphatidylcholine with two diacetylenic acyl chains.

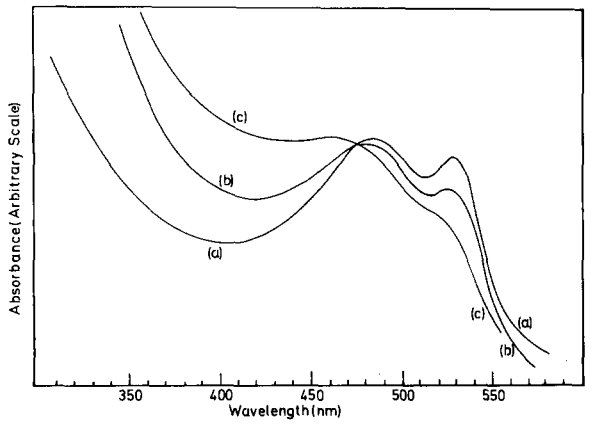

Fig. 2. Visible absorption spectra of polymer from phospholipid with diacetylene in both acyl chains. Polymer dissolved in methanol. (a) Polymer concentration = 1200 mg/l, cell path lenght = 1 cm, spectrophotometer sensitivity × 1.0 (b) 150 mg/l, 2.0 cm, ×5.0 (c) 75 mg/l, 2.0 cm, ×10.

tions are cooled below this temperature, optical activity becomes apparent, at first increasing rapidly in magnitude and then levelling off until no further change is observed below −15°C. The circular dichroism has the form of a positive-negative band associated with the total visible spectrum and an overlapping sharp negative band arising from the chromophore responsible for the visible absorption band at 530 nm. Solutions of the phospholipid polymer in chloroform show no optical activity.

Polymer in liposomes has a CD spectrum similar to the low temperature spectra observed with methanol solutions. However rather than a positive-negative band there is a single broad negative band.

The CD spectra of irradiated liposomes containing the intrinsic membrane protein Ca^{2+}-ATPase are shown in Fig. 4. At 0°C there is a negative band at 630 nm, superimposed on a negative-positive band that crosses the baseline at 640 nm. These bands correspond to the narrow and broad components in the visible spectrum of the 616 nm form of the polymer. As the temperature is increased, this part of the spectrum decreases in intensity while an equivalent system appears around 530 nm. At the same time, the CD bands corresponding to the 616 nm form of the polymer are shifted slightly to shorter wavelength. These shifts correspond roughly with changes in the visible spectra.

CD spectra obtained with the phospholipid containing one diacetylene acyl chain are shown in Fig. 5. Irradiation of liposomes at 0°C yields a polymer with a maximum ΔA at 540 nm. As seen from the visible spectra, the polymer structure that

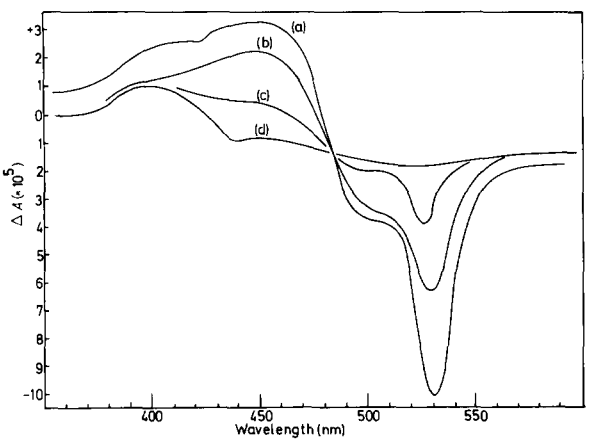

Fig. 3. Circular dichroism spectra of polymer from phospholipid with diacetylene in both acyl chains. Polymer dissolved in methanol at (a) −2°C, (b) 4°C, (c) 10°C, (d) 20°C. Absorbance of sample used for measurement 0.5 in a 1.0 cm cell.

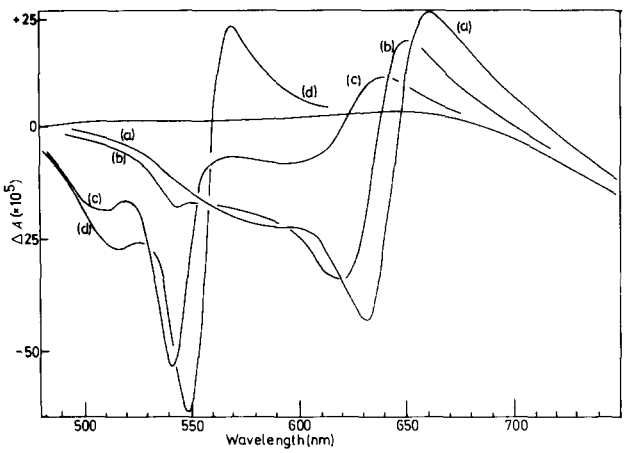

Fig. 4. Circular dichroism spectra of irradiated diacetylenic phospholipid liposomes containing the intrinsic membrane protein Ca^{2+}-ATPase. At (a) 0°C, (b) at 20°C, (c) 60°C, (d) 80°C. Absorbance of samples used for measurement = 0.1 in 1.0 mm cell. Phospholipid has diacetylene groups in both acyl chains. The zero line represents the instrument response to the cell filled with water.

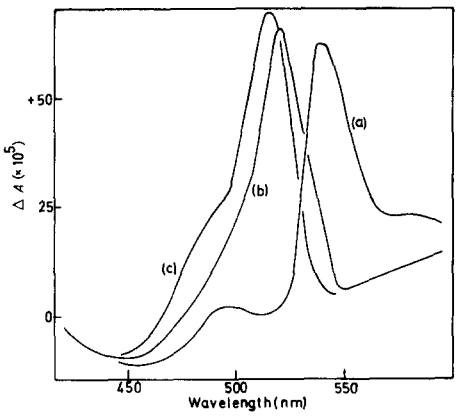

Fig. 5. Circular dichroism spectra of irradiated mixed acyl chain phospholipid liposomes. The phospholipid, a phosphatidylcholine, has a chain containing a diacetylenic group on the 2 position and a fully saturated acyl chain (either stearoyl or palmitoyl) on the 1 position of the glycerol moiety. Irradiation carried out at 0°C spectrum (a), and heated to 25°C (b), heated to 50°C (c). Absorbance 0.3 in 1 cm path-length cell.

gives rise to this spectrum is metastable. Upon heating, the CD spectrum shifts non-reversibly 30 nm to the blue. However, further heating to 80°C, has little effect on the circular dichroism of the dispersion. The optical activity associated with the

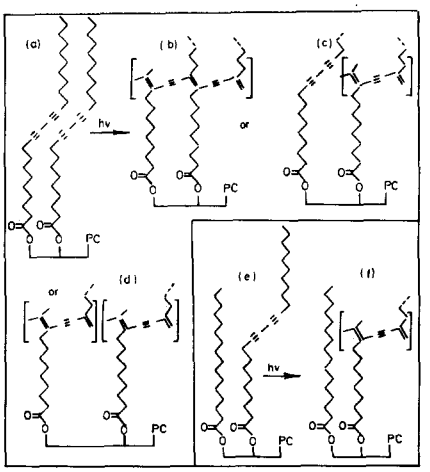

Fig. 6. Structures of diacetylenic phospholipid monomers and polymers. (a) Monomer, both acyl chains contain diacetylene groups; (b), (c) and (d), polymer from (a) intramolecularly cross-linked, one acyl chain intermolecularly cross-linked, both acyl chains intermolecularly cross-linked (in case (c) there are two isomers). (e) monomer with one saturated and one acyl chain containing a diacetylene group; (f) polymer from (e) intermolecularly cross-linked.

narrow long wavelength band in the visible spectrum is very strong. A comparatively weak negative-positive band lies between 420 and 520 nm.

Discussion

It is of interest to see that both types of phosphatidylcholine molecule, with diacetylene in one or both acyl chains, can crosslink to produce coloured polymers. In the case of the lipid containing one saturated acyl chain and one diacetylenic chain this indicates that below the lipid transition temperature in the ordered crystalline phase acyl chains containing the diacetylene group pack side by side. This is necessary condition for extensive polymerization to occur with diacetylene groups.

It can be seen from Fig. 6 that the phosphatidylcholine (with two unsaturated chains) can undergo intramolecular crosslinking, whilst the other lipid can only form intermolecular links. Polymer produced by both types of lipid are optically active.

The conjugated backbone of a diacetylene polymer is a symmetric chromophore and can have no intrinsic optical activity. Optical activity can arise in symmetric chromophores in two ways: configurationally and conformationally. In the first instance, a chromophore acquires optical activity because of its proximity to a chiral centre, in the second, the symmetric chromophores are arranged in a particular asymmetric fashion with respect to each other.

Polydiacetylene phospholipids have a chiral centre, the 2-carbon of the glycerol moiety. In the organized structure of liposomes, these chiral centres will be packed closely in the lamellar arrangement. Since absorption of radiation in polydiacetylenes involves the displacement of charge over a considerable distance, the transition dipole will come under the influence of many chiral centres. However, from knowledge of the structure of liposomes it is clear that the chiral centres must lie at least 15 Å from the chromophore. It is unlikely that optical activity of the strength recorded here (g factor $\frac{\Delta A}{A}$, 10^{-3}) could be induced over this distance.

For the manifestation of conformational optical activity, chromophores must occupy fixed positions with respect to each other. In fact, the results

do show that optical activity is apparent only under conditions likely to lead to restriction of movement of the polymer chain. This is best seen by a comparison of the spectra of phospholipid polymer in chloroform and methanol solution [1]. Chloroform is a very good solvent for both polymers. Such solutions absorb at relatively short wavelengths, indicating considerable free rotation about chain single bonds. Optical activity is not observed. Methanol is a much poorer solvent, only one polymer is soluble, and it absorbs at longer wavelength than when dissolved in chloroform, i.e., free rotation about the polymer chain single bonds is reduced. At low temperatures, methanol solutions of the polymer are optically active. The marked concentration dependence of the visible spectra found at low temperature [3] suggests that polymer chains aggregate. In aggregates, the chains will be tightly packed and occupy fixed positions with respect to each other.

Further information about the disposition of polymer chains in the low-temperature state can be gained from the form of the CD spectrum. The bisignate curve at the short wavelength end of the spectrum is indicative of an exciton interaction between chromophores. Such interactions are only possible when chromophores lie in a rigid close-packed array. The sign of the bisignate curve, positive lobe at short wavelength, negative lobe at long wavelength, suggests the chromophores are arranged in a left-handed screw sense. The existence of a specific asymmetric packing, left as opposed to right, must stem from the chirality of the glycerophosphocholine group.

Within the lamellae of a liposome the motion of polymer will be greatly restricted. It might be anticipated that optical activity would persist at higher temperatures in liposomes than with solutions. Such is indeed the case. In fact, optical activity is not lost even when the liposomes are heated to 80°C.

Conclusions

We have shown that phospholipids (phosphatidylcholines) which contain diacetylene in either one or both acyl chains polymerize upon ultraviolet irradiation. The polydiacetylene chain formed in each case absorbs in the visible region of the spectrum, is thermochromic and optically active. The visible and CD spectra of the two polymers differ, as does the response of the spectra to temperature variation. The most likely explanation for this lies in the possibility of intramolecular linking occurring with the phosphatidylcholine which contains diacetylene groups in both acyl chains. An asymmetric chromophore packing within the polymerized lipid where the packing of one screw sense is favoured by the chiral carbon at the glycerol moiety appears to cause the observed optical activity. The presence of other molecules in the lipid bilayer, such as Ca^{2+}-ATPase, can influence the CD spectrum of the polymer.

Acknowledgements

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References

1 Johnston, D.S., Sanghera, S., Pons, M. and Chapman, D. (1980) Biochim. Biophys. Acta 602, 57–69
2 Johnston, D.S., Sanghera, S., Manjon-Rubio, A. and Chapman, D. (1980) Biochim. Biophys. Acta 602, 213–216
3 Pons, M., Johnston, D.S. and Chapman, D. (1982) J. Polymer Sci. 20, 513–520
4 Albrecht, A., Johnston, D.S., Villaverde, C. and Chapman, D. (1982) Biochim. Biophys. Acta 687, 165–169
5 Alonso, A., Leaver, J., Johnston, D.S., Sanghera, S., Villaverde, C. and Chapman, D. (1982) Biochim. Biophys. Acta 712, 292–298
6 O'Brien, D.F., Whitesides, T.H. and Klingbiel, R.T. (1981) J. Polymer Sci. (Polymer Lett. Edn.) 19, 93–101
7 Hub, H.H., Hupfer, B., Koch, H. and Ringsdorf, H. (1980) Angew. Chem. (Int. Edn.) 19, 938–940
8 Gomez-Fernandez, J.C., Goni, F.M., Bach, D., Restall, C.J. and Chapman, D. (1980) Biochim. Biophys. Acta 598, 502–516
9 Restall, C.J., Arrondo, J.L.R., Elliot, D.A., Jaskowska, A., Weber, W.V. and Chapman, D. (1981) Biochim. Biophys. Acta 670, 433–440
10 Davydov, B.E. and Krentsel, B.A. (1977) Adv. Polymer Sci. 25, 1–47
11 Lieser, G., Tieke, B. and Wegner, G. (1980) Thin Solid Films 68, 77–90
12 Tieke, B. and Bloor, D. (1979) Makromol. Chem. 180, 2275–2278