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The phase behaviour of dispersions of Bis-Azo PC: photoregulation of bilayer dynamics via lipid photochromism

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A phospholipid, 1,2-bis(4-(*n*-butyl)phenylazo-4′-phenylbutyroyl)phosphatidylcholine (Bis-Azo PC), has been synthesised and shown to form stable bilayer vesicles. Light-scattering measurements and differential scanning calorimetry show that a dispersion of the lipid has a cooperative phase transition at a similar temperature to that of dipalmitoylphosphatidylcholine, which Bis-Azo PC resembles in overall size. The phase behaviour of Bis-Azo PC has been investigated by fluorescence spectroscopy and using a series of spin-labelled fatty acid probes. Fluorescence measurements using chlorophyll *a* as probe sense the onset of the cooperative phase transition, but this is not clearly revealed by any of the spin probes tested. Hysteresis in the phase transition is detected both by light scattering measurements and by fluorescence spectroscopy. No transition is observed for a lipid analogue having a palmitic acid chain and a single azo-containing substituent. Bis-Azo PC is reversibly photochromic, isomerising on exposure to ultraviolet light to a photostationary state mixture where *cis* isomer predominates. Electron microscopy shows that photoisomerisation decreases average vesicle size, and light scattering and calorimetry demonstrate that the cooperative phase transition is abolished. Illumination with visible light establishes a new photostationary state where *trans* isomer predominates, and the phase transition is restored. The ability to modulate bilayer phase behaviour reversibly has possible application to relaxation studies of bilayer membrane function, and to drug delivery research.

Introduction

Phospholipids are well known for their highly cooperative phase behaviour, which is strongly influenced by the length and degree of unsaturation of acyl chains, as well as by head group composition [1]. For a given head group and acyl chain length, *cis*-unsaturation reduces cooperativity of interactions because of packing effects due to the bond fixation, which results in a 'bent' acyl chain. Comparison between *cis* and *trans* unsaturated lipids of identical chain length and head group type shows that the *trans* compounds have phase transitions which are more cooperative and at higher temperature than the *cis* compounds [2]. Isomerisation of an unsaturated lipid molecule would thus offer a means of controlling bilayer 'fluidity', and would be useful in principle for the study of the effects of bilayer order on biochem-

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ically relevant areas such as ion transport and membrane-bound enzyme activity. Photoisomerisation would allow the fluidity of such lipids to be controlled non-invasively, and would allow time-resolved studies to be performed. However, simple unsaturated lipids are photoisomerised only by light of rather short wavelength, and photochemical decomposition competes in such cases. Ideally, one would wish for a molecule which could be photoisomerised with light of fairly long wavelength, so as to minimise photochemical effects. In addition, it is desirable that isomerisation be complete and preferably reversible under controlled conditions, and that isomers differ considerably in molecular shape to maximise effects of isomerisation on packing. In an attempt to achieve the goal of a lipid analogue, the properties of which can be conveniently photoregulated, an azobenzene-containing carboxylic acid has been synthesised and incorporated into phosphatidylcholine molecules.

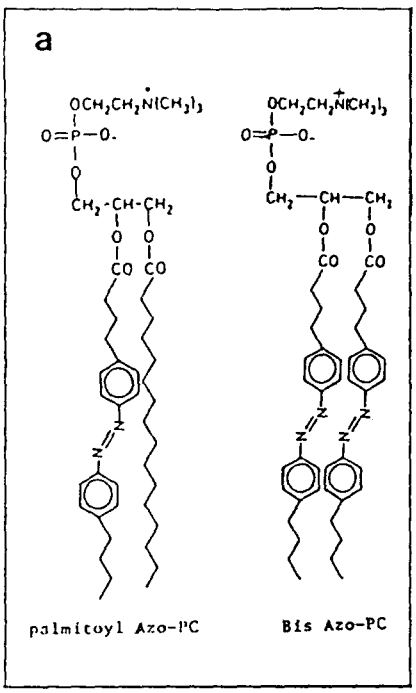
The azobenzene group is well known for its facile photoisomerisation [3]. Irradiation with long-wavelength ultraviolet light isomerises the molecule to a photostationary state where the *cis* form predominates, while visible light absorption gives a photostationary state which is mainly *trans*-azobenzene. The composition of the photostationary state in each case depends on the wavelength of illumination and on temperature, but substantial conversion (more than 60%) is easily achieved. Photochemical decomposition is not a significant problem with most simple azobenzene derivatives. The *cis* isomer of azobenzene has a dipole moment and has phenyl groups which are out of plane, and the long axes of the ring are oriented at an angle, while the *trans* isomer is virtually planar and linear. Isomerisation of 4,4′-substituted azobenzenes to the *cis* form consequently causes the substituent chains to lie at an angle, as they would if a carbon–carbon double bond were present. Fig. 1a shows the structures of two phospholipid derivatives which we have examined. These are γ-palmitoyl-β-(4-(4′-*n*-butylphenylazo)phenylbutyroyl)-L-α-phosphatidylcholine (palmitoyl Azo-PC) and β,γ-bis(4-(4′-*n*-butylphenylazo)phenylbutyroyl)-L-α-phosphatidylcholine (Bis-Azo PC). Space filling models of these molecules, showing the structures of the *cis*- and *trans* forms, are presented elsewhere [4].

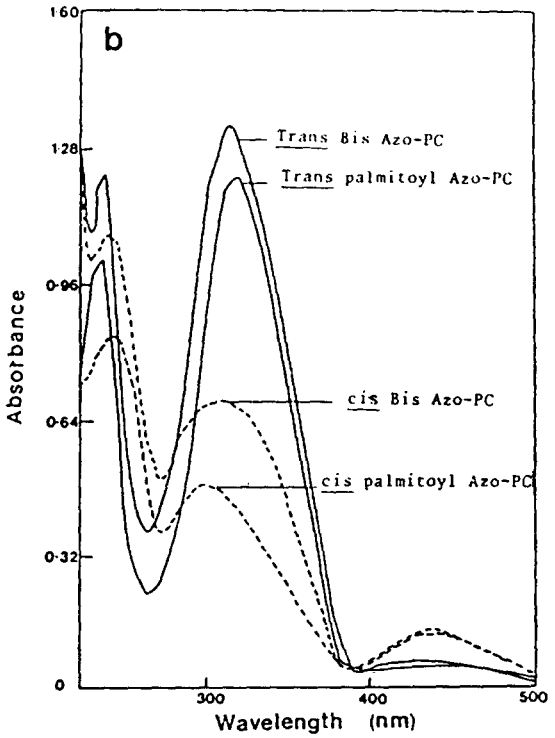
In a previous paper we have investigated the steric properties of palmitoyl Azo-PC and Bis-Azo PC, using the Langmuir trough to study surface monolayers of these lipids [4]. The effect of isomerisation of palmitoyl Azo-PC on permeability of a host lipid bilayer has also been studied [5]. In this paper we show that the azobenzene-containing phospholipids described are capable of forming small lipid vesicles, and that the presence of the azo linkage does not preclude cooperativity. It is shown that Bis-Azo PC has highly cooperative phase behaviour not exhibited by palmitoyl Azo-PC. In addition, it is shown that photolysis has clearly detectable effects on bilayer packing and vesicle size for Bis-Azo PC. These observations point the way to further experiments on the control of bilayer vesicle properties by light absorption.

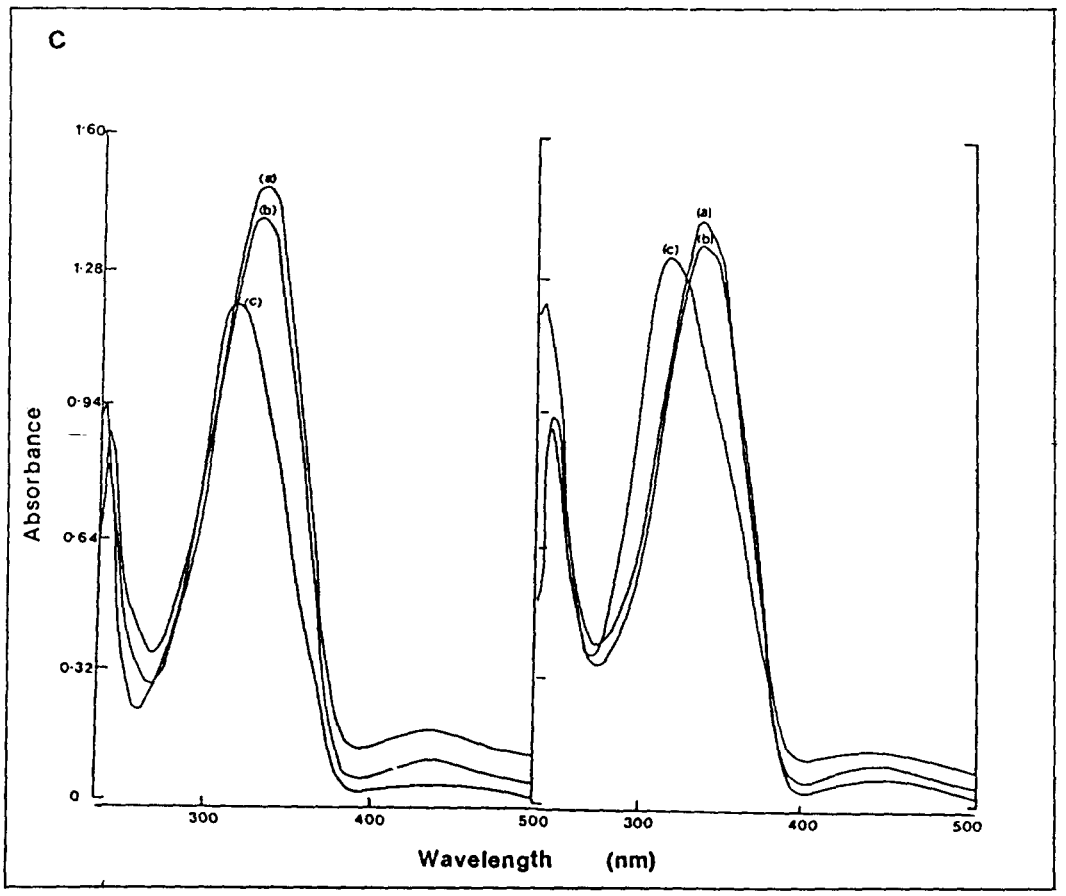
Materials and Methods

The synthesis of Bis-Azo PC involved esterification of L-α-glycerophosphorylcholine as the $CdCl_2$ complex using 4-(4′-*n*-butylphenylazo)-phenylbutyric acid, itself prepared by reaction of 4-nitroso-*n*-butylbenzene (I) with 4-aminophenylbutyric acid (II). Compound (I) was prepared by oxidation of *n*-butylaniline (Aldrich) with either peroxybenzoic acid or *m*-chloroperoxybenzoic acid, while compound (II) was prepared by catalytic hydrogenation of 4-nitrophenylbutyric acid (Aldrich). Details of the synthesis of the azobenzene containing acid and mixed phospholipid derivatives have been published [5], and the preparation and purification of Bis-Azo PC are described elsewhere [4]. The spin labels 5-, 7-, 12- and 16-doxylstearic acid were obtained from Aldrich, and

Fig. 1. (a) The structures of the photochromic lipids used in this study. (b) Absorption spectra for the photochromic lipids as sonicated aqueous dispersions in the *cis* and *trans* photostationary states as described in the text. (c) (i) Absorption spectra for *trans*-palmitoyl Azo PC at 20 °C in: (a) chloroform solution; (b) 2% w/w mixture with DPPC codispersed by sonication in 50 mM phosphate buffer (pH 7.5); (c) vesicular dispersion of pure palmitoyl Azo PC in the same buffer. (ii) Absorption spectra for *trans*-Bis-Azo PC under the same conditions as those in c) (i).


a
palmitoyl Azo-PC
Bis Azo-PC


b
Trans Bis Azo-PC
Trans palmitoyl Azo-PC
cis Bis Azo-PC
cis palmitoyl Azo-PC
Absorbance
1·60
1·28
0·96
0·64
0·32
0
300
400
500
Wavelength (nm)


c
Absorbance
1·60
1·28
0·94
0·64
0·32
0
(a)
(b)
(c)
300
400
500
Wavelength (nm)

9-doxylstearic acid from Molecular Probes. They were used without further purification.

ESR measurements were made on a Varian E-9 spectrometer using capillary cells maintained at fixed temperatures regulated to $\pm 0.1°$ C, at a microwave frequency of 9.2 GHz and incident power of 10 mW. The magnetic field was modulated at $1 \cdot 10^5$ Hz with an amplitude of 1 G. Data were accumulated in a Nicolet 1170 signal averager before transfer to a Hewlett-Packard HP 85 computer for analysis. The microwave cavity of the spectrometer was shielded from light to prevent photoisomerisation during measurements. Light scattering and fluorescence were measured in a Perkin Elmer 1000 fluorometer equipped with a thermostatted cell compartment and an electronic thermometer for continuous monitoring of temperature profiles. Scattering was measured at a wavelength (540 nm) not significantly absorbed by any isomer of Bis-Azo PC. A differential thermogram of Bis-Azo PC was recorded using a Perkin Elmer 7 Series Thermal Analyser (courtesy of Perkin Elmer Ltd., Beaconsfield, U.K.) in addition to thermograms recorded using a Mettler 300 thermal analysis system.

Phospholipid dispersions for ESR measurement were prepared by hydrating a mixture of lipid and spin-label with water or buffer at a temperature of 60° C and suspending by repeated uptake into a Pasteur pipette. Labelling ratios of 1 : 100 and 1 : 230 (mole : mole spin label : lipid) were used. The samples were allowed to stand at room temperature for 1–2 h before measurement. A similar technique was used to prepare samples for calorimetry. Samples were photolysed in the capillaries used for ESR measurement, using a long-pass ultraviolet filter (centred at 360 nm) with a medium pressure mercury arc source fitted with a heat filter. Control experiments showed no significant destruction of spin label by light under these conditions. After photolysis, samples were stored in the dark at room temperature, and were measured within 3 h of preparation. No significant thermal reversal of isomerisation was found under these conditions. Phospholipid vesicles for light scattering measurements were prepared by ethanol injection into vortexing buffer above 60° C, and were held at 40–45° C for 30 min to equilibrate trapped ethanol before measurement [6], or were prepared by brief sonication. Samples prepared by both methods gave similar results. Chlorophyll *a* (Sigma) was incorporated into vesicles of Bis-Azo PC by co-evaporation from ethanolic solution followed by brief sonication. Chlorophyll fluorescence was excited at 402 nm (which does not disturb the photostationary state composition of photolysed vesicles), and emission was monitored at 670 nm.

Results and Discussion

The absorption spectra of dispersions in phosphate buffer (pH 7.5, 50 mM) of palmitoyl Azo-PC and Bis-Azo PC are shown in Fig. 1b, both for the predominantly *trans* forms, which are stable in visible light at room temperature, and for the photostationary states produced on irradiation with ultraviolet light. The spectrum of the Bis-Azo PC in the *trans* form (i) shows a slight blue shift in absorption relative to the lipid containing the palmitoyl chain (ii), and both show blue shifts relative to solutions in chloroform (Fig. 1c), or dispersions in the host phospholipid β,γ-dipalmitoyl-L-α-phosphatidylcholine (DPPC). These shifts are to be expected for dispersions of the pure azo-lipids, where interchromophore interactions will be important, and similar absorption spectra have been reported for other amphipathic azobenzene molecules [7]. The close resemblance of the absorption spectrum of a dispersion of Bis-Azo PC in DPPC vesicles to that seen for solution in organic solvents suggests that the lipid does not show a very marked tendency to phase-separate into concentrated clusters within the host, at least at low molar ratios. Photolysis results in enhancement of the weak $n-\pi^*$ transition responsible for visible absorption, with an accompanying shift to shorter wavelength of the main $\pi-\pi^*$ transition.

Fig. 2a shows light-scattering data for dispersions of palmitoyl Azo-PC and for Bis-Azo PC in phosphate buffer in the *trans* photostationary state. While there is no evidence for any co-operative phase transition for the palmitoyl Azo-PC, Bis-Azo PC clearly shows a sharp phase transition. The light scattering data are supported by the scanning calorimeter trace shown in Fig. 2b, which clearly shows an endothermic transition with an

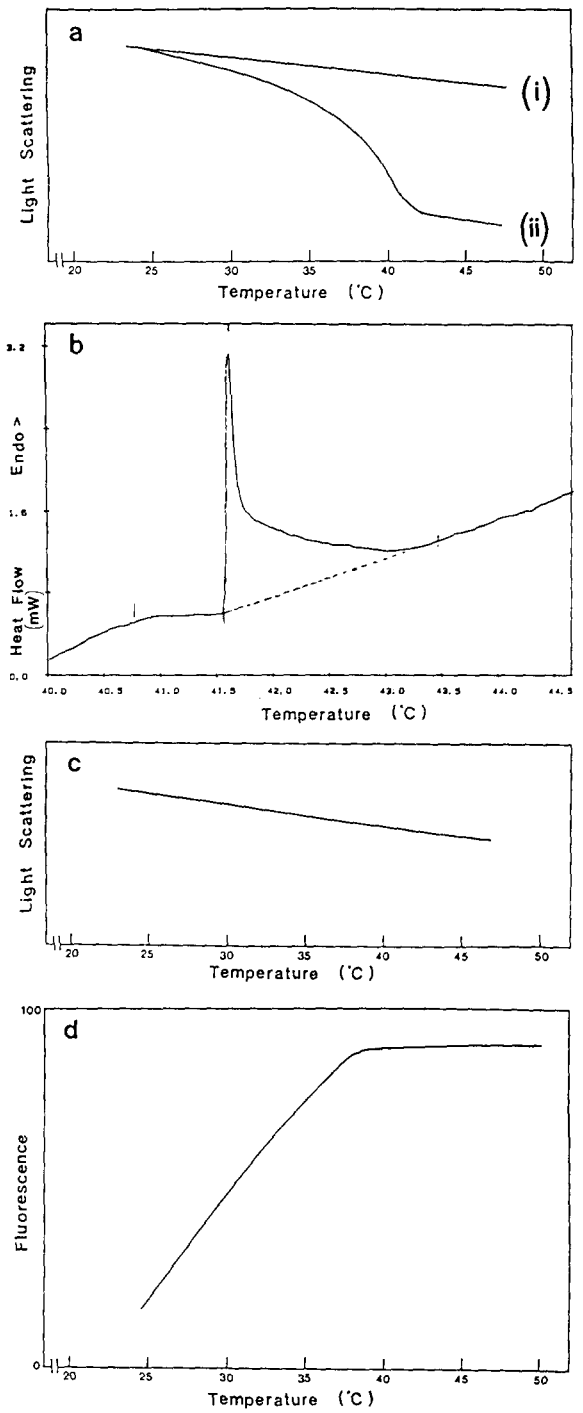


Fig. 2. (a) Light-scattering data at 540 nm as a function of temperature for dispersions of Bis-Azo PC in (i) the *cis* and (ii) the *trans* photostationary state. Data are normalised to the same initial value. Dispersions were 0.1 mg/ml lipid in 50 mM phosphate buffer (pH 7.5). The data were obtained on heating samples previously cooled to below 20 °C: results depended on sample history (see text). (b) Thermogram obtained courtesy of Perkin Elmer Ltd. for Bis-Azo PC in the *trans* photostationary state as a dispersion in distilled water. Sample weight was 4.7 mg before hydration. Scan rate was 1 K/min. (c) Light-scattering at 540 nm for a dispersion of *trans*-palmitoyl Azo PC (0.1 mg/ml in 50 mM phosphate buffer (pH 7.5)). Data were obtained on heating at approx. 2 K/min a sample previously kept below 20 °C. (d) Fluorescence emission for chlorophyll *a* in *trans* Bis-Azo PC 0.2 mg/ml (1:200 mol:mol) in 50 mM phosphate buffer (pH 7.5). Results were obtained on heating, and depended strongly on previous thermal history of the sample. Excitation was at 402 nm, emission at 700 nm. Heating rate was approx. 2 K/min.

enthalpy of approx. 9.1 kJ · mol^{-1}. There is reason to expect that the phase behaviour of Bis-Azo PC will not be simple, as light-scattering data show clear evidence of marked hysteresis [4], and the calorimetric data are influenced by thermal history of samples. Thus, it is likely that the samples are not truly at equilibrium under typical scan conditions. Not all calorimetric scans showed a peak as markedly asymmetric as the endotherm shown in the figure. Recently, a report has appeared in which a phospholipid containing two azobenzene chromophores was used in a circular dichroism investigation of lipid phase behaviour [8]. While the lipid used was somewhat different to the azo-lipids discussed here, it is interesting to note that hysteresis was also seen by these workers. Such hysteresis is analogous to 'supercooling' in organic compounds, and is a consequence of a metastable state in a system which is not at true equilibrium.

Fig. 2c shows a light scattering trace for a dispersion of Bis-Azo PC after ultraviolet photolysis to the photostationary state. The phase transition is abolished, and a continuous trace is seen, even if scans are taken to higher or lower temperatures than shown in the figure. Photoisomerisation of such a sample back to the *trans* photostationary state using visible light causes the phase transition to reappear. These results were confirmed using scanning calorimetry (not shown).

Electron microscopy shows that photolysis of Bis-Azo PC dispersions resulted in a reduction of apparent vesicle size, as shown in Fig. 3. It may be that isomerisation fragments large vesicles as a result of packing constraints on the bulky *cis* isomer. Alternatively, since the *cis* isomer is more

polar than the *trans* isomer, vesicles might be in equilibrium with monomer or micellar forms of the lipid.

Although scanning calorimetry is an excellent technique to quantitatively investigate phase behaviour, it gives no information on the motion of lipid chains in defined regions of the bilayer. Fluorescence methods based on polarisation of emission from fluorophores in well defined bilayer locations (e.g., the anthroyloxy-labelled fatty acid probes [9]) are often used to complement calorimetric investigations. Unfortunately, such methods are not easily applicable to dispersions of the azo-lipids. The absorption spectrum of the azo-lipids in the ultraviolet overlaps that of most useful fluorescence probes (including the anthroyloxy group), and the visible $n-\pi^*$ absorption efficiently quenches most fluorescence emission by energy transfer through dipolar and higher order interactions. The fluorescence of chlorophyll *a* has been used previously to monitor lipid phase equilibria [10]. For our present purposes, chlorophyll fluorescence has the advantage of emission in a spectral region remote from absorption by Bis-Azo PC. In addition, chlorophyll *a* has useful absorption at wavelengths not significantly absorbed by either isomer of Bis-Azo PC. Chlorophyll *a* was therefore used as a fluorescence probe for our study. In Fig. 2d are shown data obtained on heating a sample of Bis-Azo PC containing a low concentration (1 : 200 mol fluorophore : lipid) of probe. Results parallel those previously cited [10] for phase transitions in the DPPC system. In fact, the actual shape of the trace was variable, depending on the number of heating and cooling cycles undergone by the sample. All traces, however, showed a breakpoint at the same temperature on heating, and showed marked hysteresis on cooling. The likely origins of fluorescence changes seen using chlorophyll *a* include changes in probe stacking and orientation, and are discussed in Ref. 10.

In addition to fluorescence measurements, we used the ESR methods of spin labelling to study the phase behaviour of dispersions of azo-lipids. ESR probes are available as fatty acids containing the 'doxyl' group in defined positions on the acyl chain [11]. The amphiphilic spin labels partition into lipid bilayers, where they align with the surrounding lipid molecules. The polar carboxylic acid group, which localises in the bilayer/aqueous interface, is relatively immobilised. The lipophilic

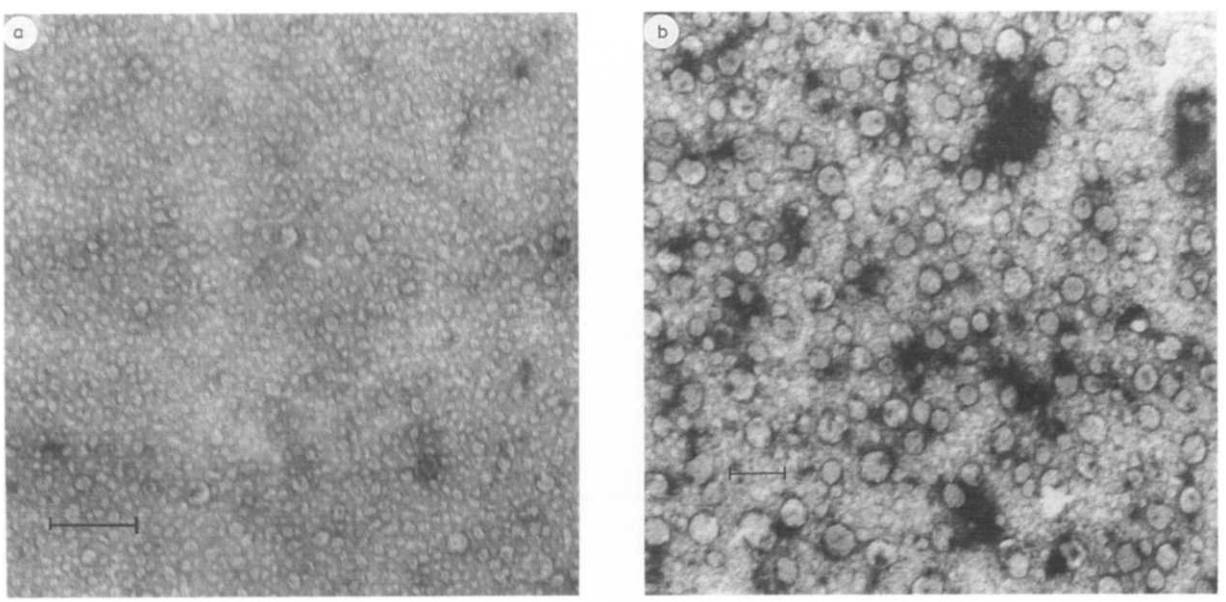


Fig. 3. Electron micrographs of Bis-Azo PC in the *cis* (a) and *trans* (b) photostationary states. Samples were prepared by sonication and negatively stained with 1% uranyl acetate. Bars represent 100 nm.

polymethylene chain extends into the bilayer, its mobility increasing with increasing carbon number. Axial rotation of the spin label is rapid in the bilayer. Spin labels are generally held to provide an efficient means to monitor the lipid environment at different depths within a bilayer.

Spin label measurements concentrated on Bis-Azo PC, since other techniques showed no evidence of a phase transition for palmitoyl Azo-PC. Dispersions of Bis-Azo PC were labelled with fatty acids containing the 'doxyl' group in the 5-, 7-, 9-, 12- and 16-positions, and samples were investigated both in the *cis* and in the *trans* photostationary states. Fig. 4 shows plots of the separation of the outer extrema of the ESR spectrum

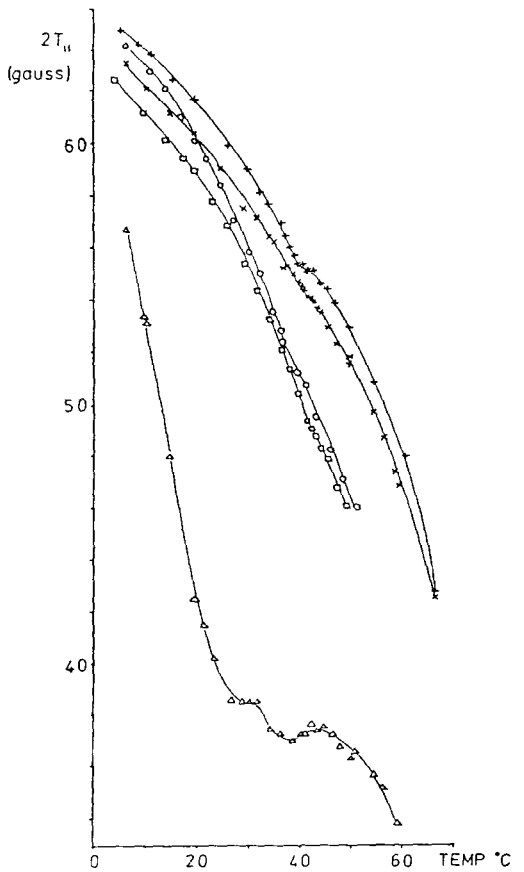


Fig. 4. Plots of $2T_{\parallel}$ as a function of temperature for a series of spin labels in dispersions of Bis-Azo PC in 50 mM phosphate buffer (pH 7.5). Data were obtained on heating. Sample preparation and conditions were as described in the text. Doxylstearic acids: ○, 5-; +, 7-; ×, 9-; □, 12-; △, 16-.

(the so-called $2T_{\parallel}$ value [11], measured as shown in the inset to Fig. 5a) versus temperature for the series of spin labels in *trans* photostationary state Bis-Azo PC dispersions. This presentation is preferred relative to the usual plot of order parameter versus reciprocal temperature, because it is not reasonable to ascribe precise physical meaning to apparent order parameters without complex, model-dependent, computer analysis of lineshapes. A decrease in $2T_{\parallel}$ value is broadly associated with an increased motional freedom for the spin probe. In labelled Bis-Azo PC dispersions, it can be seen that motional freedom generally increases, as one would expect, with depth in the bilayer and, at constant depth, with temperature. An anomaly is seen for the 5-doxyl-labelled stearic acid probe, which crosses over the plot for the 9-doxyl-labelled acid near 20 °C. Molecular models show that the 'doxyl' moiety in the 5-positions is close to the azo-nitrogens of the lipid, and thus might be expected to have an environment somewhat different from that of the remainder of the labels.

If 5-doxyl stearic acid is used as probe with vesicles prepared in distilled water, rather than buffer, a plot of $2T_{\parallel}$ vs. temperature is superimposable on that obtained for the 7-doxyl labelled material in buffered solution. It has previously been shown [12] that pH and ionic strength influence the spectrum of 5-doxylstearic acid. The study cited concludes that in distilled water the probe is un-ionised, and moves a distance equivalent to two methylene units into the bilayer interior relative to its position when ionised. This is in accord with our observation.

The most striking aspect of Fig. 4, is that none of the spin labels shows evidence of the phase transition clearly demonstrated both by light scattering and by calorimetry. Data for the 16-doxyl label show changes in slope at elevated temperature, but the shape of the plots is quite different to that seen for DPPC and similar well-characterised lipid systems.

The data in Fig. 5a shows the effect of photoisomerisation of Bis-Azo PC on the temperature profile of $2T_{\parallel}$ for the 5- and 16-labelled doxylstearic acid probes. In both cases, the $2T_{\parallel}$ value is decreased at constant temperature after isomerisation. Similar effects are seen with the

Figure (5a)

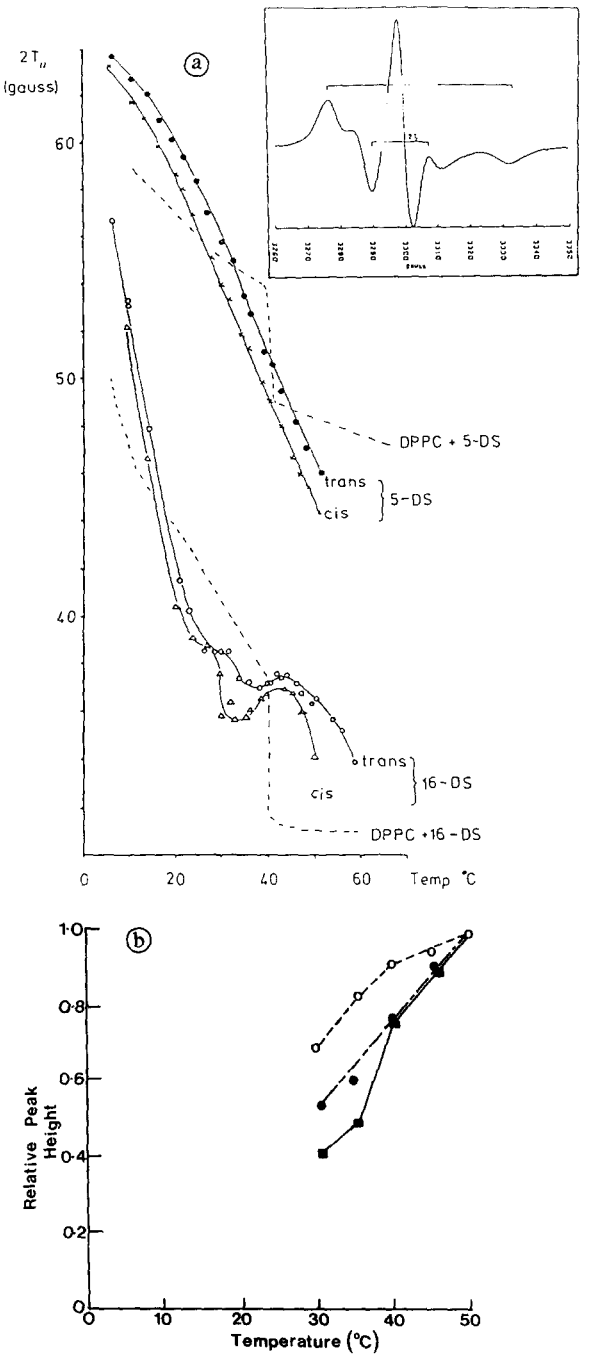


Fig. 5. (a) Plots of $2T_{\parallel}$ as a function of temperature for 5-doxyl stearate (5-DS) and 16-doxyl stearate (16-DS) spin labels in Bis-Azo PC dispersions in the *cis* and *trans* photostationary states. The dotted traces show for comparison results for DPPC at the spin label ratio used (1:100 mol:mole probe:lipid). Inset is a typical ESR spectrum showing how $2T_{\parallel}$ is measured. (b) A plot of the relative height of the central peak of the ESR spectrum for 5-doxyl stearate in dispersions of Bis-Azo PC as a function of temperature, state of isomerisation and spin label concentration. ○, *trans*-Bis-Azo PC, molar ratio of lipid:spin label, 400:1; ●, *cis*-Bis-Azo PC, lipid:spin label 100:1; ■, *trans*-Bis-Azo PC, lipid:spin label 100:1; Buffer and conditions as shown in Fig. 4.

other spin labels. For comparison, typical data for DPPC are included in the figure. The data suggest that motional freedom is highly restricted in the Bis-Azo PC dispersions at low temperature, but that the activation energy for molecular motion is high. A change in slope is seen at elevated temperature for data from a 16-doxyl-labelled sample after photoisomerisation. The data are similar to those for the *trans* photostationary state mixture, though at a lower temperature. Since it was shown by light scattering and calorimetry that isomerisation abolishes the phase transition, it must be assumed that these changes in slope are not directly related to occurrence of a phase transition.

A typical spectrum of 5-doxylstearate in Bis-Azo PC is shown in Fig. 5. An apparent measure of the polarity experienced by the 'doxyl' group is given [13] by the isotropic splitting constant, a'. Comparison of a' as a function of spin label positions suggests that the apparent polarity experienced by the spin probe is maximum for label in the 7-position. One normally expects a polarity gradient with the 5-doxyl label having the highest polarity [14]. As previously mentioned, the 5-doxyl label is close to the azo-group, and this might explain the observations. The a' parameter is influenced to some extent by molecular motion, in particular the rotational motion of the probe, and thus can only strictly be compared within similar environments [15].

The spin label study rather surprisingly shows that the co-operative nature of the phase transition of Bis-Azo PC is not reflected by a discontinuity in the plots of $2T_{\parallel}$ or $2T_{\perp}$ for any of the labels tested. However, changes in the height of the central peak of the spectrum from 5-doxyl stearic acid, which is sensitive to changes in linewidth, show a slight discontinuity around the temperature of the phase transition for a lipid : spin label ratio of 100 : 1 (Fig. 5b). This is also observed at a ratio of 25 : 1, but is undetectable at 400 : 1. Photoisomerisation of the lipid at a lipid : spin label ratio of 100 : 1 abolished the dis-

continuity. The presence of low concentrations of spin labels is not sufficient of itself to abolish bulk phase transitions through an 'impurity' effect. The phase transition can still be detected by light scattering when spin labels are present in samples at similar concentrations to those used in this study. It is possible that the spin label is clustering, forming microscopic domains of highly perturbed lipid. One would expect this type of behaviour to be accompanied by pronounced spectral broadening due to spin-spin interaction. Some broadening is detectable at high concentrations of spin label in the gel phase. On the other hand, the results obtained at the lowest spin label concentration suggest that the spin labels might simply be incompatible with cooperative behaviour for bulky acyl chains such as those of the azo lipid, and might locally prevent chain melting whilst having little effect on global lipid properties. A detailed study using a variety of physical techniques will be needed to discover more about the acyl chain dynamics and packing for Bis-Azo PC dispersions.

Although the spin label measurements cause local perturbation, they do consistently demonstrate increased motional freedom of probes after photoisomerisation. The fluorescence measurements appear to involve less perturbation, a surprising result considering the size of the chlorophyll *a* molecule. The study shows clearly that bilayer fluidity can be photoregulated, and the abolition of a phase transition is a particularly striking result. This opens up the possibility of many interesting experiments on reconstituted biological systems. Work is presently underway to determine the phase behaviour of mixtures of Bis-Azo PC with other lipids, with a view to optimising photo-induced changes in bilayer properties. A preliminary study has shown that gel phase vesicle fusion can be photosensitised by Bis-Azo PC, and that trapped aqueous marker dye is rapidly and totally released upon irradiation of such sensitised vesicles. A detailed study will be published separately. Apart from the scientific interest of such work, there may be aspects relevant to practical areas such as drug delivery as an adjunct to phototherapy. Additional areas of interest include 'molecular electronic' devices such as memory elements. Photochromic molecules showing hysteresis and reversible control of refractive index and absorbance are under intense study as switching elements in thin film optics. For practical applications, factors such as rate of thermal reversal of isomerisation, photostability and switching speed are of great importance. Although Bis-Azo PC is unlikely to be optimised for use in such devices, fundamental studies of molecular motion in organised assemblies underpin all practical applications.

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References

1 Quinn, P.J. and Chapman, D. (1980) CRC Crit. Rev. Biochem. 8, 1
2 Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445
3 Brown, G.H. (1971) in Photochromism, Vol. III, Techniques in Chemistry (Weissberger, A., ed), Wiley Interscience, New York
4 Sandhu, S.S., Yianni, Y.P., Morgan, C.G., Taylor, D.M. and Zaba, B. (1986) Biochim. Biophys. Acta 860, 253
5 Morgan, C.G., Thomas, E.W., Yianni, Y.P. and Sandhu, S.S. (1985) Biochim. Biophys. Acta 820, 107
6 Batzri, S. and Korn, E.D. (1973) Biochim. Biophys. Acta 298, 1015
7 Shimomura, M., Ando, R. and Kunitake, T. (1983) Ber. Bunsenges. Phys. Chem. 87, 1134
8 Kunitake, T., Nakashima, N., Shimomura, M., Okahata, Y., Kano, K. and Ogawa, T. (1980) J. Am. Chem. Soc. 102, 6642
9 Blatt, E. and Sawyer, W.H. (1985) Biochim. Biophys. Acta 822, 43
10 Lee, A.G. (1975) Biochemistry 14, 4397
11 Hubbell, W.L. and Mc Connell, H.M. (1971) J. Am. Chem. Soc. 93, 314
12 Barratt, M.D. and Laggner, P. (1974) Biochim. Biophys. Acta 363, 127
13 Seelig, J. (1970) J. Am. Chem. Soc. 92, 3881
14 Griffiths, O.H. and Jost, P.C. (1976) in Spin Labelling. Theory and Applications (Berliner, L.J., ed.), p. 453, Academic Press, New York
15 Mason, R.P., Polnaszek, C.F. and Freed, J.H. (1974) J. Phys. Chem. 78, 1329