Tetrahedron Letters,Vol.27,No.38,pp 4589-4592,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

CIRCULAR DICHROISM ACTIVE ARTIFICIAL PHOSPHOLIPIDS FOR THE STUDY OF MOLECULAR MEMBRANE DYNAMICS FOCUSED ON LIPID-LIPID INTERACTION

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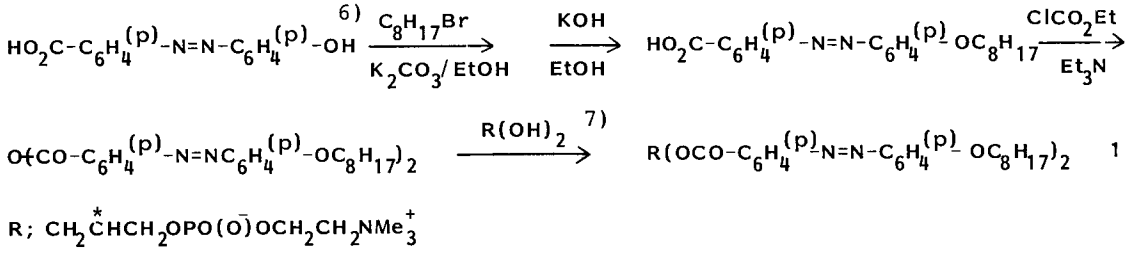
Artificial phospholipid with an azobenzene chromophore in a close proximity to the asymmetric carbon of the choline skeleton was prepared. The artificial phospholipid was miscible with the natural phospholipid and provides CD information sensitive to the membrane motion.

Membrane structure has been studied by a number of well-established physical measurements such as light scattering[1], electron microscopy[2], or X-ray diffraction[3]. However, for the study of membrane dynamics especially on the molecular level is a lack of directly, promptly and effectively responding probes[4].

We wish to report that an artificial phospholipid 1 well miscible (microscopically) with natural lipids over a wide molar fraction range affords direct and effective information concerning dynamic changes of membrane[5].

The artificial phospholipid 1 was prepared as shown in Scheme 1. The

Scheme 1



trans isomer was exclusively obtained in the dark. Observed absorption(s) at 364 nm[8] (ε 5.01 × 10^4) and CD spectrum at 382 nm[8] (θ +1.49 × 10^4) in $CHCl_3$ points to the trans azobenzene structure. 400 MHz ^1H NMR ($CDCl_3$ TMS) showed proton absorptions characteristic of the trans-azo-carboxy chromophore at δ 6.86, 6.88, 7.70, 7.76, 7.78, 7.97 and 8.07 (ppm , each in doublet with J of 8 Hz further supporting the structure. A small amount of the cis isomer easily detected by the absorption at 450 nm[9] was readily removed by a recrystallization from MeOH-Et_2O in the dark.

Artificial single wall, bimolcular liposomes were prepared from a mixture of x (25 to 100) % of 1 and (100 - x) % of purified egg lecithin in distilled

water by ultrasonic irradiation[10] followed by ultracentrifugation and Sepharose 4B gel filtration. The artificial liposomes thus prepared showed strong visible and CD absorptions, the shapes and intensities of which changed with temeprature. Both spectra showed practically the same (higher) critical temperature T_{C_2}, but no lower critical temperature, T_{C_1}, yet observed above 0° (see Fig 1). Above 40°C (T_{C_2}), the artificial liposomes containing 50 % of 1 exhibited visible absorptions at 348 nm characteristic of "freely moving" azo chromophore (F state)[11] alone while at 1°C, the visible absorptions at 304 and 352 nm with a shoulder at 400 nm characteristic of the highly ordered molecular assembly of azobenzene moiety (O state)[11] and 348 nm of the F state were observed. Population of the F state determined by the intensity was ca 40 %. From 1° to 50° the observed visible spectra changed continuously with an isosbestic point at 308 nm, suggesting a smooth conversion from the O to the F state. The reverse conversion from the O to the F state followed by electronic spectra was slightly different from the forward conversion (see Fig 2) as reported for Kunitake's achiral surfactant incorporated in liposomes[12].

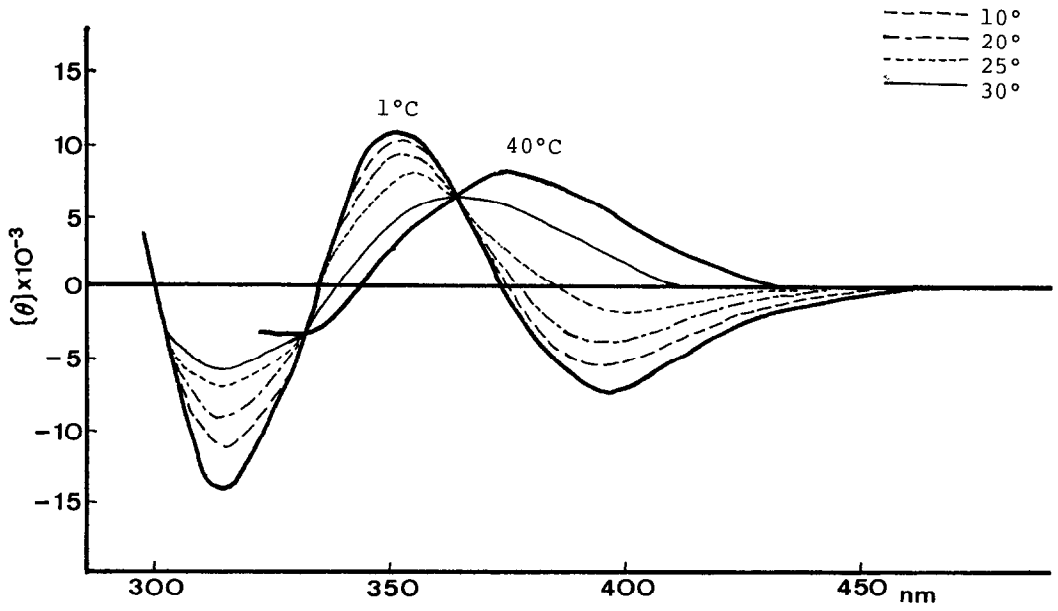


Fig 1 Circular dichroism spectra of the aqueous artificial liposomes prepared from 50% 1 and 50% egg lecithin.

1 has two important characteristics, which may be advantageous in future membrane studies. First, 1 and lecithin, a natural phospholipid, are miscible on the molecular level up to ca 30 % of 1 (mol percent, close to volume percent) — i.e. they have a strong mutual affinity — in the liposomal membrane without phase separation above 0°C, in marked contrast to ready phase separation for Kunitake's surfactant[11,12] already at the 5 % tail

content (close to volume percent). Secondly, 1 has a chiral center to provide an intense CD signal which is more informative than the electronic spectrum — with a remarkable intensity change as shown in Fig 1, and specific shape change as discussed below. The chiral center and the chromophore are located in significant sites for membrane motion or lipid-lipid interaction.

The visible spectra of the F-state were essentially the same as those of solution of 1 in $CHCl_3$ with a blue shift of 16 nm, strongly suggesting that 1 has considerable freedom of lateral motion in the F-state membrane. CD spectra of the F-state were again the same as the $CHCl_3$ solution, further supporting the proposed nature of the F-state.

The UV absorption characteristic of the O state was rather weak. Interestingly, however, CD spectra at 1° exhibited intense negative values (see Fig 1) at 313 and 395 nm, much more clearly indicating the existence of the O state.

Another important result obtained for the 1-lecithin liposomes was the remarkable stabilization of the otherwise unstable F-state (or extraordinarily slow F → O relaxation) when the reverse F → O process was followed below T_{C_2} (see Fig 2). As shown in Fig 2, the "hysteresis" was much more

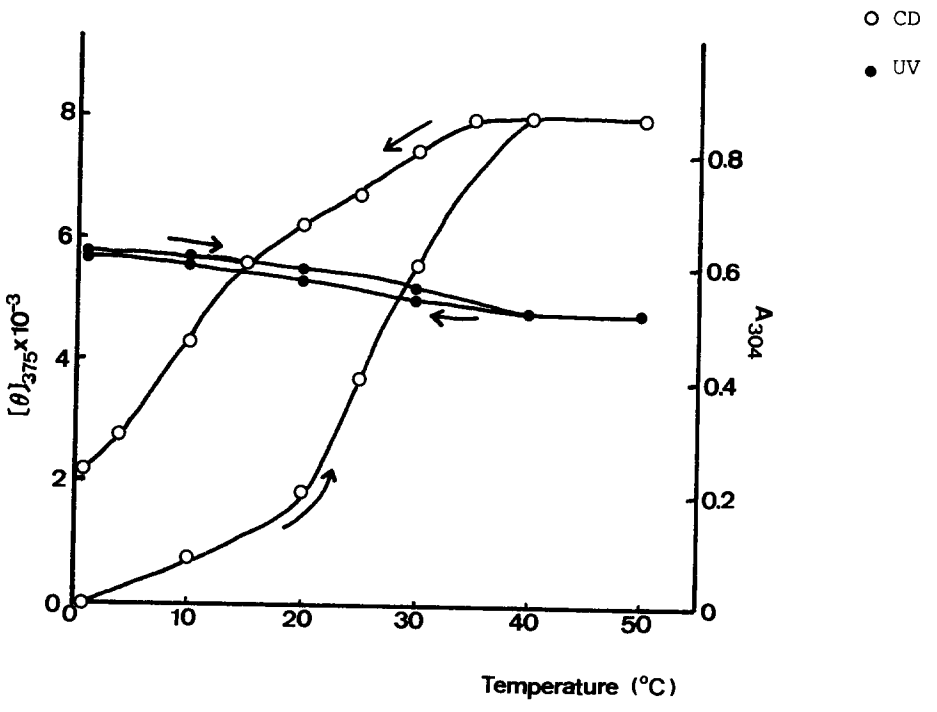


Fig 2 Hysteresis in the temperature dependent visible and circular dichroism spectra observed for the aqueous artificial liposomes prepared from 50% 1 and 50% egg lecithin.

effectively followed by CD than by electronic spectra. The observed half-life of 25 hr for the 1-lecithin liposomes (50/50) at 20° is much longer than the extrapolated[12] half-life of ca 10 min for Kunitake's. This remarkable stabilization strongly suggests an unexpectedly strong lipid-lipid interaction and may be correlated with low permeability of phospholipid membranes to water or hydrophilic ions.

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(Received in Japan 14 June 1986)