

# Effect of Sodium Cholate on the Phase Transition Temperature of Dipalmitoyl Phosphatidylcholine

Srabani Banerjee and S. N. Chatterjee

Biophysics Division, Saha Institute of Nuclear Physics, 37, Belgachia Road, Calcutta – 700 037, India

Z. Naturforsch. 38c, 302 – 306 (1983); received September 20/November 15, 1982

Liposomes, DPPC, Sodium Cholate, Phase Transition, Electron Microscopy

On treatment with sodium cholate, the phase transition temperature ( $T_c$ ) of multilamellar liposomes derived from dipalmitoyl phosphatidylcholine (DPPC) progressively decreased with increasing cholate: lipid-P molar ratio. A molar ratio of 4.5 caused a one degree depression in  $T_c$ . The unilamellar cholate vesicles of DPPC exhibited the same  $T_c$  as that of the multilamellar ones. The formation of unilamellar vesicles from the multilamellar ones by cholate treatment was investigated by electron microscopy.

## 1. Introduction

The effect of sodium cholate, a trihydroxy bile salt, on the lipid bilayer deserves attention for several reasons: (i) It has been widely used in membrane reconstitution studies which offer a most promising approach to the elucidation of the molecular organisation of the proteins and lipids in the biomembranes [1], (ii) The stability of liposomes in the presence of bile salts is important because the extent to which liposomes retain their structural integrity in the hostile environment of the gastrointestinal tract is probably crucial to their effectiveness as potential carriers of therapeutic agents [2] and (iii) Sodium cholate has been used to produce the single bilayer vesicles (cholate vesicles) from the multilamellar liposomes and the use of these vesicles as another model membrane system has been advocated [3]. On the other hand, the nature of the interaction of sodium cholate with lipid bilayers has not been investigated adequately.

The alteration of the conductance of black lipid membranes by sodium cholate was reported by Bangham and Lea [4]. Rowland and Woodley [2] demonstrated that liposomes derived from various lipids released more than 80% of their entrapped  $^{125}\text{I}$ -labelled polyvinylpyrrolidone in the presence of 10 mM bile salts. This paper reports, in this context, the effect of sodium cholate on the phase transition parameters of dipalmitoyl phosphatidylcholine (DPPC) forming multilamellar liposomes. This study has further ensured that the DPPC forming the single-bilayer cholate vesicle has the normal phase transition temperature.

Reprint requests to S. N. Chatterjee.

0341-0382/83/0300-0302 \$ 01.30/0

## 2. Materials and Methods

### 2.1 Materials

DL- $\alpha$ -dipalmitoyl phosphatidylcholine (DPPC) and sodium cholate were purchased from Sigma Chemical Company, USA.

2.2 Liposome preparation. Liposomes were prepared following in general the method described by Bangham *et al.* [5] and Mandal *et al.* [6]. A chloroform solution of dipalmitoyl phosphatidylcholine was rotary evaporated to yield a thin film. The film was resuspended in phosphate buffer of pH 7.0 and ionic strength  $i = 0.01$ , by vortexing in a nitrogen atmosphere at a temperature well above the transition temperature of the lipid so as to ensure the formation of well sealed multilamellar liposomes.

2.3 Preparation of unilamellar cholate vesicles. Unilamellar cholate vesicles or liposomes were prepared by treatment of multilamellar liposomes with sodium cholate following in general the method of Brunner *et al.* [3]. Cholate was then removed from the vesicle preparation by Sephadex G-50 chromatography followed by extensive dialysis or by a second chromatography.

2.4 Electron Microscopy. For electron microscopy by negative staining [7, 8], the lipid vesicles were dispersed in 0.16 M ammonium acetate, pH 7.0 and then mixed with an equal volume of 0.5% ammonium molybdate. One drop of this mixture was deposited on a carbon coated grid, the excess liquid being taken off by a filter paper after 5–10 min. The preparation was examined by a Siemens electron microscope Elmiskop-I at an instrumental magnification of 8000 X.

2.5 Phase transition study. The liposomes in 2 mM phosphate buffer (pH 7.0) were treated with



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

sodium cholate at different cholate/lipid-P molar ratios at room temperature and the reaction was monitored by noting the absorbance at 450 nm with time. After about 30 min, when no further decrease in absorbance was noted, the liposome cholate mixture was subjected to phase transition study in a Zeiss PMQ II Spectrophotometer provided with a thermostat controlled cell holder. The transition was noted by measuring the absorbance of the sample at 450 nm [9] with increasing temperature. The temperature corresponding to the absorbance  $A = A_{\min} + \Delta A/2$  was defined as the transition temperature corresponding to the particular lipid-cholate mixture, where  $A_{\min}$  was the minimum value of absorbance after transition and  $\Delta A$  the change in absorbance. The extent of co-operativeness in the phase transition was estimated from the measurement of  $\Delta T$ , the temperature interval through which the absorbance changed during phase transition.

### 3. Result

The kinetics of the interaction of sodium cholate with multilamellar liposomes at room temperature is shown in Fig. 1. The absorbance at 450 nm decreased with time exponentially and the reaction completed by about 30 min. Similar reaction kinetics were obtained for different values of cholate: lipid-P molar ratio, except that the overall decrease in absorbance was different for the different values of the ratio.

The absorbance *vs.* temperature plots shown in Fig. 2 are illustrative of the thermal phase transi-

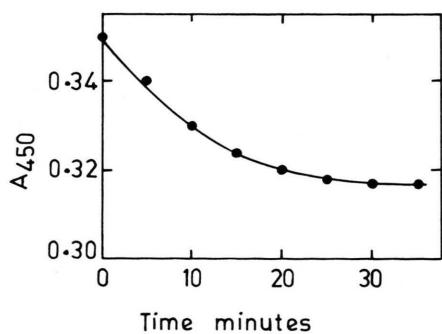


Fig. 1. Kinetics of the interaction at room temperature of sodium cholate with multilamellar DPPC liposomes (in 2 mM phosphate buffer, pH 7.0) at cholate ( $\mu\text{mol}$ ): lipid-P ( $\mu\text{mol}$ ) ratio of 21.

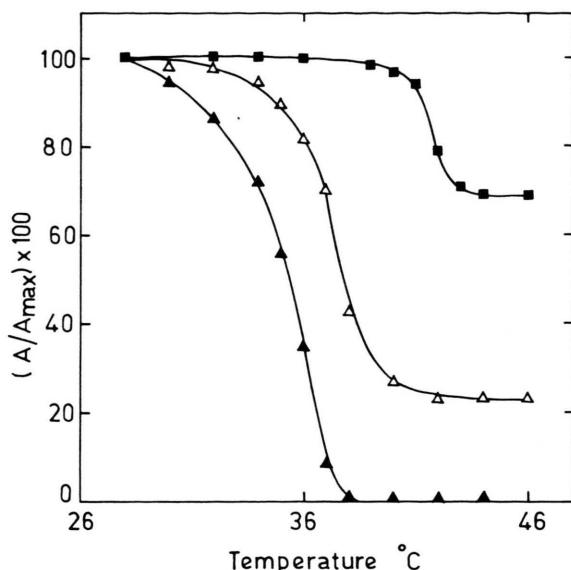


Fig. 2. Phase transition profiles of DPPC multilamellar liposomes (in 2 mM phosphate buffer, pH 7.0) at the following cholate ( $\mu\text{mol}$ ): lipid-P ( $\mu\text{mol}$ ) ratios: 0.0 (■—■), 21.78 ( $\triangle$ — $\triangle$ ) and 43–53 ( $\blacktriangle$ — $\blacktriangle$ ).

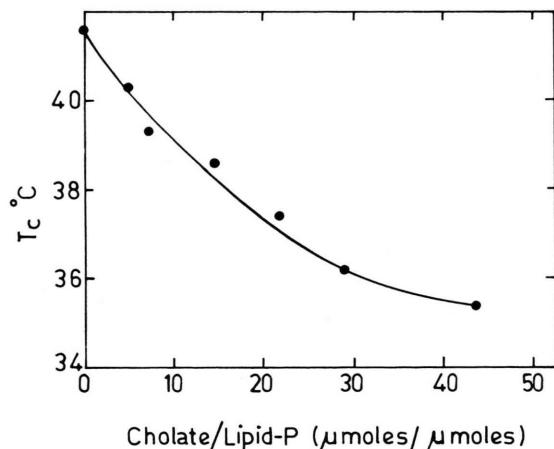


Fig. 3. The phase transition temperature of multilamellar DPPC vesicles on interaction with sodium cholate, as a function of cholate ( $\mu\text{mol}$ ): lipid-P ( $\mu\text{mol}$ ) ratio.

tions undergone by the lipid molecules in the presence of varying amounts of cholate. The transition temperature gradually decreased with increasing value of the cholate: lipid-P molar ratio (Fig. 3), but the overall decrease in absorbance,  $A_{450}$ , increased with increasing values of the ratio. It was found that a one degree depression in the transition temperature ( $\Delta T_c = 1$ ) resulted with the cholate/lipid-P molar ratio of 4.5. The measured values of the

Table I. Parameters describing the phase transition in 2 mM phosphate buffer (pH 7.0) of i) multilamellar DPPC liposomes in the presence of varying amounts of cholate and ii) unilamellar cholate vesicles (DPPC) after removal of the cholate by methods described in the text. The critical micellar concentration of sodium cholate was in the range 12–15 mM.

Concentration of sodium cholate [mM]	Cholate/Lipid-P molar ratio	Phase transition temperature $T_c$ [°C]	Depression in the phase transition temperature $\Delta T_c$ [°C]	Percentage decrease in absorbance $\Delta A$	Temperature interval over which transition occurs $\Delta T$ [°C]
<i>Multilamellar liposomes</i>					
0.0	0.0	41.6	0	31.25	6.0
1.63	5.09	40.3	1.3	60.00	15.5
2.32	7.25	39.3	2.3	61.54	15.0
4.66	14.50	38.6	3.0	67.39	14.0
6.97	21.78	37.4	4.2	76.92	13.0
9.29	29.03	36.2	5.4	83.50	12.0
13.93	43.53	35.4	6.0	99.30	10.0
<i>Unilamellar cholate vesicles</i>					
0.0	0.0	41.6	—	41.67	9.0

different parameters describing the thermal phase transition characteristics of the liposomal lipid molecules are presented in Table I. In the presence of cholate, the values of  $\Delta T$ , the temperature interval over which the phase transition occurred, were significantly higher than the control value. The DPPC in the single-bilayer vesicles derived by cholate treatment (cholate vesicles) and after extensive dialysis exhibited a phase transition temperature which was not significantly different from the control value (untreated multilamellar liposomes). Electron micrograph (Fig. 4) of cholate treated

(cholate: lipid-P = 1.16) multilamellar liposomes describes how the unilamellar vesicles may be produced from the multilamellar ones by such treatment. Arrows in Fig. 4 show the protrusion of the lipid bilayer resulting presumably from its enhanced fluidity produced by cholate treatment. Vesicles harvested similarly but treated with a higher cholate: lipid-P ratio (~4) showed (Fig. 5) that many of the multilamellar liposomes have already been transformed into fairly uniform small unilamellar vesicles of most frequent size around 300 Å. Attempts to record electron micrographs of

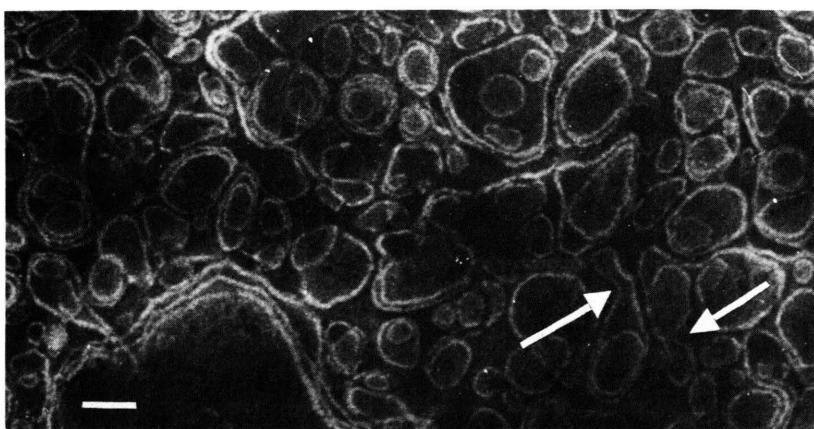


Fig. 4. Electron micrograph describing the formation of unilamellar vesicles from multilamellar ones by cholate treatment (cholate: lipid-P = 1.16). The multilamellar liposomes were treated with sodium cholate at room temperature (cholate: lipid-P = 1.16) for 30 min and then passed through a Sephadex G-50 column. The fraction obtained at the void volume was prepared for electron microscopy by negatively staining with ammonium molybdate. Bar = 730 Å.

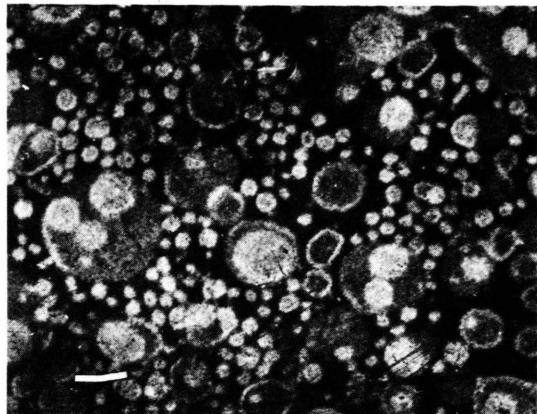


Fig. 5. Electron micrograph of sodium cholate treated (cholate: lipid-P  $\simeq$  4.0) multilamellar liposomes. The vesicles were harvested and prepared for electron microscopy as in Fig. 4. Bar = 1400 Å.

multilamellar liposomes treated with cholate at a very high cholate: lipid-P ratio ( $\sim 40$ ) did not succeed presumably because of the difficulty in removing cholate and hence its interference with the specimen preparation.

#### 4. Discussion

Phase transition has been followed in this work from a measurement of absorbance,  $A_{450}$  as described by Hill [9]. To our experience, this technique is simpler and yet yields consistent results. Using this technique, we found the transition temperature ( $T_c$ ) of DPPC multilamellar liposomes as 41.6 °C, which is in good agreement with the value obtained by other techniques [10–12]. The absorbance of the lipid dispersion at 450 nm decreased sharply at the crystalline to liquid crystalline phase transition temperature. This decrease in absorbance resulted from the increase in the volume occupied by the lipid molecules. The abrupt volume increase accompanying phase transition in the lipid bilayer was also detected by Träuble and Haynes [13] by dilatometric measurements.

Increasing amounts of cholate was found to cause a progressive reduction of the phase transition temperature of DPPC liposomes. Stillwell *et al.* [14] also showed by absorbance measurements that retinol and retinoic acid, both possessing "detergent shape", depressed the phase transition temperature of dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine liposomes. This lowering of phase transition temperature by cholate may be attributed to its labilizing effect and its ability to perturb the bilayer packing. Previous workers also demonstrated by fluorescence studies that Triton X-100 [15] and bile salt [16] incorporation fluidises the phospholipid matrix. Bangham and Lea [3] reported that cholate also increases the electrical conductivity of black lipid membranes.

The present study also revealed that sodium cholate broadened the range of transition *i.e.* enlarged the temperature range ( $\Delta T$ ) over which both fluid and gel phases could co-exist. At higher cholate concentrations the transition was sharper presumably because of the faster action of cholate, but the transition temperature interval ( $\Delta T$ ) was always greater than that observed for untreated multilamellar liposomes. Similar broadening of the phase transition temperature interval ( $\Delta T$ ) on introduction of foreign materials into DPPC liposomes was reported earlier by Eliasz *et al.* [17].

Sodium cholate has thus been found to increase the fluidity of the dipalmitoyl phosphatidylcholine and also to decrease the co-operativeness in its phase transition. This is in agreement with the electron microscopic observation (Fig. 4) indicating the mechanism of formation of the unilamellar vesicles from the multilamellar ones by cholate treatment. The most frequent size (300 Å) of vesicles obtained after treatment at the cholate: lipid-P molar ratio of 4.0 agreed reasonably with the particle size of unilamellar liposomes derived by cholate treatment and assayed by Sepharose-4B chromatography [3, 18].

- [1] S. Razin, *Biochim. Biophys. Acta* **265**, 241–296 (1972).
- [2] R. N. Rowland and J. F. Woodley, *Biochim. Biophys. Acta* **620**, 400–409 (1980).
- [3] J. Brunner, P. Skarbal, and H. Hauser, *Biochim. Biophys. Acta* **455**, 322–331 (1976).
- [4] J. A. Bangham and E. J. A. Lea, *Biochim. Biophys. Acta* **511**, 388–396 (1978).
- [5] A. D. Bangham, M. M. Standish, and J. C. Watkins, *J. Mol. Biol.* **13**, 238–252 (1965).
- [6] T. K. Mandal, S. Ghose, P. Sur, and S. N. Chatterjee, *Int. J. Radiat. Biol.* **33**, 75–79 (1978).
- [7] D. Papahadjopoulos and N. Miller, *Biochim. Biophys. Acta* **135**, 624–638 (1967).
- [8] S. M. Johnson, A. D. Bangham, M. W. Hill, and E. D. Korn, *Biochim. Biophys. Acta* **233**, 820–826 (1971).
- [9] M. W. Hill, *Biochim. Biophys. Acta* **356**, 117–124 (1974).
- [10] H. J. Hinz and J. M. Sturtevant, *J. Biol. Chem.* **247**, 6071–6075 (1972).
- [11] P. N. Strong and R. B. Kelly, *Biochim. Biophys. Acta* **469**, 231–235 (1977).
- [12] E. J. J. Van Zoelen, C. H. De Jesus, E. De Jong, M. Mulder, M. C. Blok, and J. De Gier, *Biochim. Biophys. Acta* **511**, 335–347 (1978).
- [13] H. Träuble and D. H. Haynes, *Chem. Phys. Lipids* **7**, 324–335 (1971).
- [14] W. Stillwell, M. Ricketts, H. Hudson, and S. Nahmias, *Biochim. Biophys. Acta* **688**, 653–659 (1982).
- [15] A. Alonso, R. Saez, and F. M. Goni, *FEBS Lett.* **137**, 141–145 (1982).
- [16] R. Narayan, R. Paul, and P. Balaram, *Biochim. Biophys. Acta* **597**, 70–82 (1980).
- [17] A. W. Eliasz, D. Chapman, and D. F. Ewing, *Biochim. Biophys. Acta* **448**, 220–230 (1976).
- [18] Srabani Banerjee and S. N. Chatterjee, *Ind. J. Biochem. Biophys.*, in press.