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Temperature-induced micellar–lamellar transformation in binary mixtures of saturated phosphatidylcholines with sodium cholate

Alla I. Polozova, Gennady E. Dubachev, Tatyana N. Simonova, Leonid I. Barsukov*

Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117871 Moscow V-437, Russian Federation

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Abstract The transition states of binary mixtures of dipalmitoyl- and dimyristoylphosphatidylcholines with sodium cholate at the reversible temperature-induced micellar–lamellar transformation were characterized by turbidimetry, electron microscopy, ^{31}P NMR and differential scanning calorimetry. This transformation is triggered by the phospholipid acyl chain melting, and appears to include two structural pathways: (i) from discoidal mixed micelles to network-like structures composed of long interlaced rod-like micelles, then to multilayer membrane structures, and finally to multilamellar vesicles; and (ii) from discoidal micelles to membrane fragments and finally to unilamellar vesicles.

Key words: Saturated phosphatidylcholine; Sodium cholate; Micellar-lamellar transition; Electron microscopy; NMR; Differential scanning calorimetry

1. Introduction

The mixed systems composed of phosphatidylcholines and bile salts are currently the subject of close inspection [1–4]. However, there is no complete description of their behaviour, especially in the micellar–lamellar transition region (see e.g. [5] for review).

Earlier [6], we found that binary mixtures of sodium cholate (ChNa) with saturated phospholipids, such as dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC), can undergo reversible micellar–lamellar transformation upon heating. This transformation occurs in a narrow range of lipid/detergent ratios and appears to be triggered by the phospholipid acyl chain melting. Here we describe the transition states of the DPPC–ChNa and DMPC–ChNa systems during the transformation by means of several techniques including turbidimetry, electron microscopy, ^{31}P NMR and differential scanning calorimetry (DSC).

2. Materials and methods

DPPC, DMPC and ChNa were purchased from Serva. Lipid purity was checked by thin-layer chromatography. Lipid–detergent mixtures were prepared by dispersing dry lipid film with 10 mM Tris buffer solution (pH 8.0) containing 1 mM EDTA, 75 mM NaCl, and appropriate amounts of ChNa. The lipid was deposited as a thin film on the walls of a glass tube from a chloroform stock solution by solvent evaporation at 45°C. After that, lipid films were dried under an oil pump vacuum of about 0.1 Torr at 20°C for at least 2 h to remove all residual solvent.

The mixtures were equilibrated at 45°C for 1 h and then kept at room temperature for at least 15 min before the measurements. Due to this treatment all samples were brought to a quasi-steady state with reproducible turbidity vs. temperature measurements.

The effective detergent/lipid ratio, R_e (detergent/lipid molar ratio in the mixed aggregates), was determined from the slope of the phase boundary between mixed micelles and lamellar structures as previously described [6].

The turbidimetric measurements were carried out at 450 nm on a Hitachi 220A spectrophotometer in a thermostated cell compartment fitted with a magnetic stirrer, the samples being heated at a constant rate of 0.33°C/min. The temperature of a sample was monitored by a thermocouple element inserted directly into the cuvette.

For electron microscopy studies, the preparations were equilibrated at a given temperature for 30 min, and then they were either negatively stained with 2% w/v aqueous solution of uranyl acetate, or freeze-fractured (cryofixation was performed in liquid propane followed by liquid nitrogen treatment) and replicated with Pt-C in a JEE-4C freeze-etching unit at 10^{-7} Torr and −160°C. The replicas were cleaned with 75% H_2SO_4 and then washed out with distilled water. The samples were examined in a Jeol EM 100CX-2 electron microscope.

For NMR experiments, the samples were prepared in buffer solutions containing 20% ^2H_2O. ^{31}P NMR spectra were recorded at 81 MHz using a Bruker MSL-200 spectrometer.

DSC measurements were carried out on a DASM-4A (Pushino, Russia) microcalorimeter with a scan rate of 0.25°C/min. The first heating scan was made at the rate of 4°C/min to ensure equilibration. Under these conditions all the repeated heating scans were completely reproducible.

3. Results

Binary mixtures of ChNa and saturated phospholipid (DPPC or DMPC) capable of micellar–lamellar transformation abruptly increase their turbidity upon heating [6]. Mixed systems experiencing such a transformation fall into a relatively narrow range of R_e (effective detergent/lipid molar ratio in the mixed structures): from 0.15 to 0.33 for DPPC and from 0.14 to 0.33 for DMPC. The typical temperature dependence of optical density is shown in Fig. 1 for a mixture of 20 mM DMPC and 9 mM ChNa. A drastic increase in turbidity occurs at about 35°C over a relatively narrow temperature increment (1–2°C) so that the initially low optical density levels out at the values of typical lamellar systems. This effect is reversible, and lowering the temperature leads the system from a cloudy appearance back to the optically transparent state. The similar behaviour is observed for the DPPC-ChNa mixed systems as well, although in the other temperature range. In the case of

*Corresponding author. Fax: (7) (095) 335 71 03.
E-mail: libar@ibch.siobc.msk.SU

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; ChNa, sodium cholate; DSC, differential scanning calorimetry; R_e, effective detergent/lipid molar ratio in the mixed structures.

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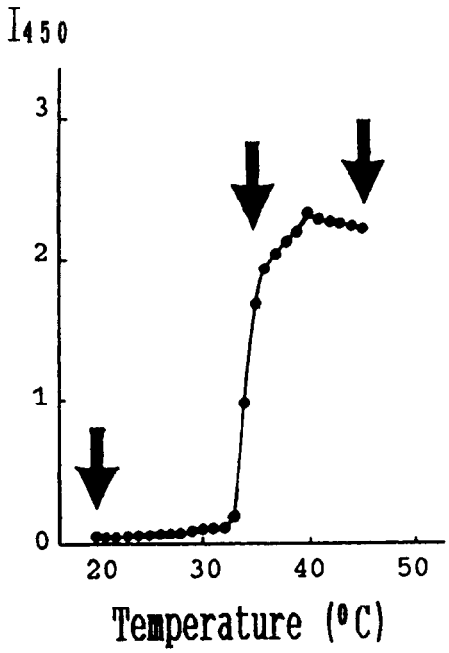

Fig. 1. Temperature dependence of optical density (I_{450}) for mixture of 20 mM DMPC and 9 mM ChNa. Heating rate was 0.33°C/min. The arrows indicate the points corresponding to the electron microscope images presented in Fig. 2.

DMPC the peculiar turbidity changes are detected at temperatures exceeding 20°C, while for mixed systems containing DPPC these changes start at about 38°C.

It should be pointed out that the turbidity alteration is preceded by a sharp viscosity increase capable of being detected visually. At the first step, when the enhancement of viscosity is observed, the optical density remains at the same low level. As the optical density increases the mixed system stays in a highly viscous state, and it becomes fluid again only when the turbidity change is complete.

Micrographs of the mixture of 20 mM DMPC and 9 mM ChNa at different temperatures are shown in Fig. 2. It is clear from Fig. 2A,B that there are no large lamellar structures in samples negatively stained or freeze-fractured from 20°C. Heating the samples up to 45°C results in the appearance of membrane liposomal structures 100–600 nm in diameter (Fig. 2E,F). The unusual network-like structures co-existing with extended membrane fragments of various sizes are observed at 35°C, which is the temperature corresponding to the intermediate state of the transition, when the mixed system is already in the turbid, but still viscous, state (Fig. 2C,D).

^{31}P NMR data also demonstrate that the heating induces large structural rearrangements in the mixed systems studied (Fig. 3). The initially narrow peak at 20°C for a mixture of 20 mM DMPC and 9 mM ChNa becomes widened at 31°C, corresponding to the temperature of the beginning of turbidity alterations. Further temperature enhancement gives rise to the superposition of two signals, one of which is compatible with typical asymmetric spectra of extended bilayers.

In order to elucidate a relationship between the main phase transition of the saturated phospholipids used and the temperature-induced micellar–lamellar transformation of the mixed systems, DSC measurements were performed. It was found that at least a partial transition of phospholipid from the gel phase to the liquid-crystalline state is necessary for the micellar–lamellar transformation to be initiated (Fig. 4). The superposition of thermograms and corresponding turbidimetric curves also reveals a correlation between the effective detergent/lipid ratio, R_e, and the temperature of the beginning of the micellar–lamellar transition: the higher the ratio, the higher the temperature and, accordingly, the larger fraction of lipid that has to melt for the transformation process to be initiated.

4. Discussion

In this study, the behaviour of mixed systems composed of sodium cholate and saturated phospholipids has been monitored as a function of temperature and detergent concentration by turbidity measurements, electron microscoopy, ^{31}P NMR and DSC. The jumps in turbidity values were taken as indirect evidence of profound structural rearrangements in these systems induced by temperature at appropriate ranges of effective detergent/lipid ratios: $0.14 < R_e < 0.33$ and $0.15 < R_e < 0.33$ for systems containing DMPC or DPPC, respectively. Electron microscopy and ^{31}P NMR studies have shown that the aggregation state of mixed systems with higher and lower detergent/lipid ratios does not depend on the temperature. So, the mixed systems with $R_e > 0.33$ remain in the micellar state over the whole temperature range studied, showing the patterns on electron micrographs similar to ones presented in Fig. 2A,B, and invariably having a narrow NMR signal. Likewise, the temperature does not affect the mixed systems containing relatively low amounts of ChNa ($R_e < 0.14$ for DMPC and $R_e < 0.15$ for DPPC). These systems, according to electron microscopy and ^{31}P NMR data, retain their lamellar state independently of temperature variations.

The structural rearrangements observed for systems with intermediate R_e values are probably related to the increase in the head group area of lipid molecules after the gel-to-liquid-crystalline phase transition. In the case of pure DMPC, a lateral expansion of the interfacial molecular area is estimated as 32%, while for pure DPPC it is about 18% [7–9]. It seems plausible that such an enhancement associated with lipid chains melting results in the additional exposure of acyl chains to water, which at a fixed sodium cholate concentration should lead to a shift in the equilibrium between mixed micelles and lamellar structures in favour of the latter (such a mechanism has been postulated earlier [10] to explain the fusion of melittin/saturated PC disc micelles on warming above the lipid phase transition temperature). If so, one can now perceive why the temperature-induced micellar–lamellar transformation takes place in the narrow range of effective detergent/lipid ratios. It is clear that in only the limited range of detergent/lipid effective ratios can a small shift of equilibrium give rise to the actual phase boundary intersection.

The complicated features of ^{31}P NMR spectra and DSC thermograms of mixed systems undergoing the temperature-induced structural transformation (Figs. 3 and 4) imply that this transformation is a rather sophisticated process, involving two or more intermediate steps. Probably, the specific interactions of sodium cholate with saturated phospholipids resulting

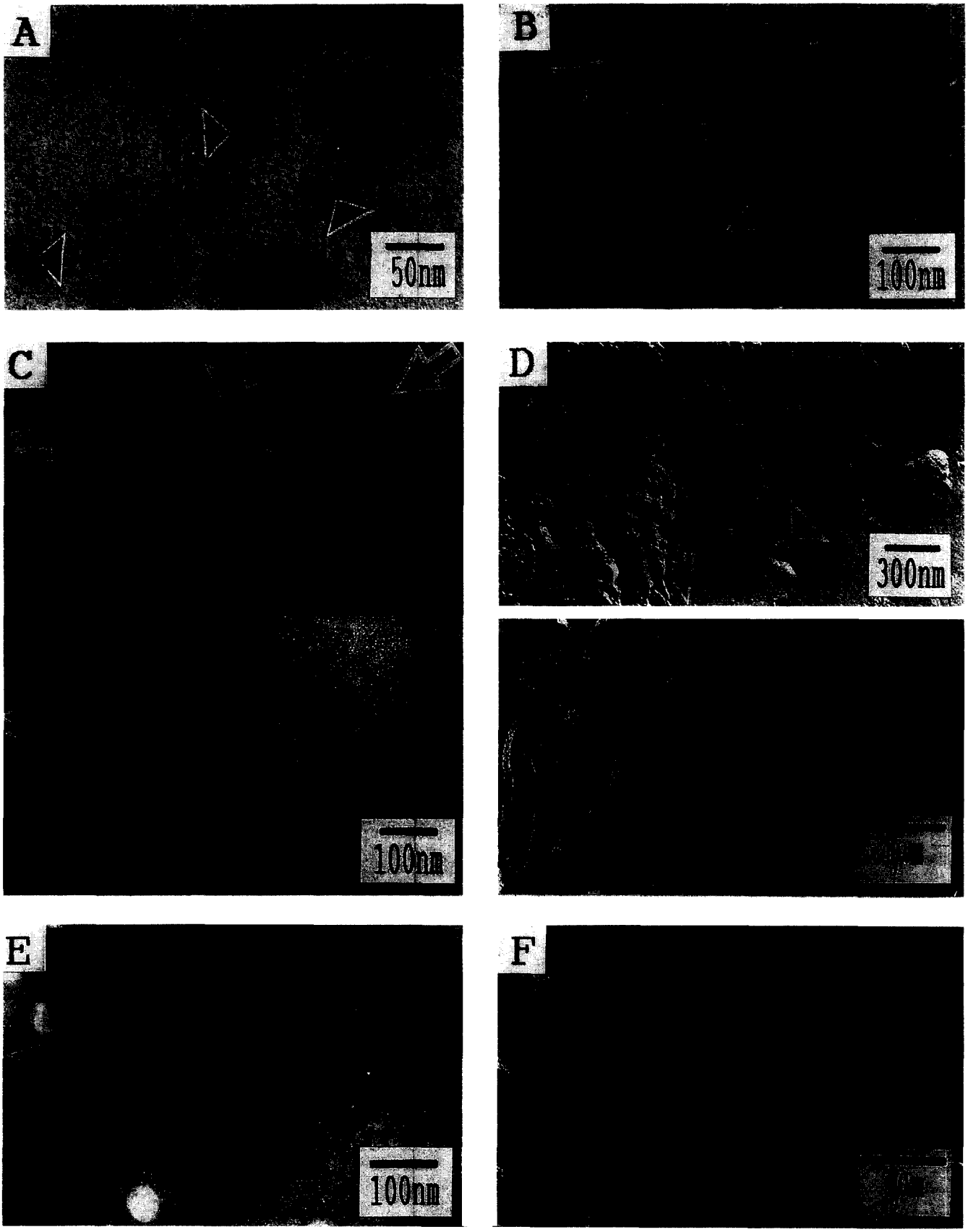

Fig. 2. Electron micrographs of mixture of 20 mM DMPC and 9 mM ChNa at 20°C (A,B), 35°C (C,D) and 45°C (E,F). Negative staining with uranyl acetate (A,C,E) and freeze-fracture (B,D,F). Structures observed are marked as follows: arrowheads, discoidal mixed micelles and membrane fragments; double arrowheads, multilayers; thick arrows, network-like structures; thin arrows, vesicles.

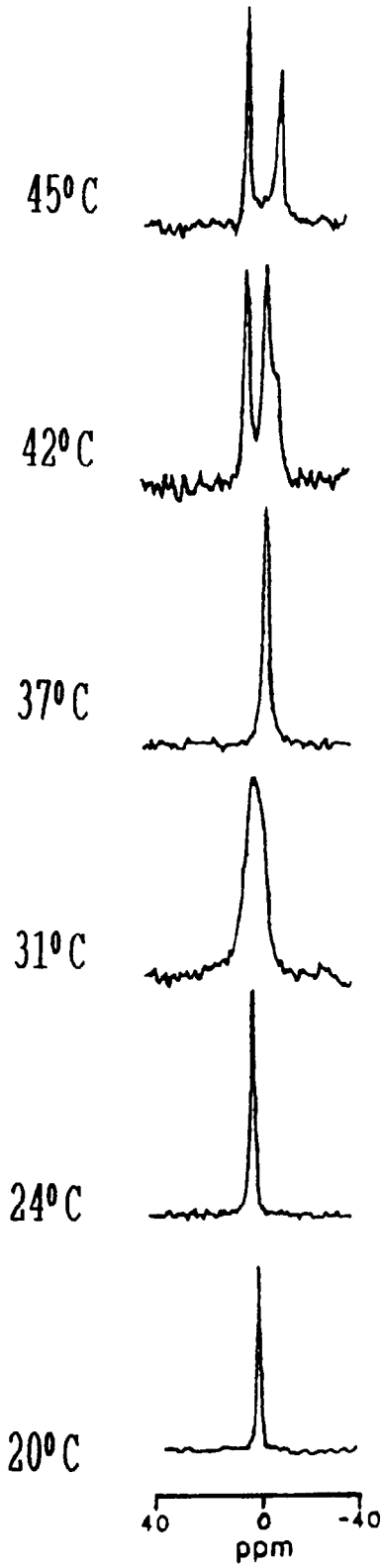

Fig. 3. Temperature dependence of ^{31}P NMR spectra for a mixture of 20 mM DMPC and 9 mM ChNa.

in multicomponent DSC thermograms are dramatically affected by temperature as the lipid molecules undergo the gel-to-liquid-crystalline phase transition. The consequences of such alterations in the specific detergent–lipid interactions could be a reduction in detergent solubilization ability, as well as detergent–lipid microdomain reorganization. The change of microdomain organization, in its turn, could result in whole structural rearrangements such as mixed micelle shape transformation.

Current views on the structural organization of mixed micelles formed by interactions of bile salts with phospholipids are based on two principally different models: the mixed disc model [11] and the capped-rod model [12]. We think it is possible to suggest that, below the gel-to-liquid-crystalline phase transition temperature, the discoid shape of mixed micelles is preferable for mixtures of saturated phospholipids with sodium cholate, while acyl chain melting initiates the rod-like micelle formation. Thus the network-like structures (see Fig. 2C,D) detected by electron microscopy at intermediate temperatures may be considered as formed by the interlaced long rod-like micelles, the existence of which can explain the temporary increase in viscosity of mixed systems. Therefore the first change in the ^{31}P NMR signal (see Fig. 3) can be ascribed to the initial step of the structural transformation of mixed micelles.

According to the data of electron microscopy (Fig. 2E,F) and ^{31}P NMR (Fig. 3), further heating brings about the formation of large lamellar structures. It is conceivable that the association of rod-like micelles, resulting in the formation of network-like structures, is followed by their transformation to the extended multilayers. This assumption is supported by the fact that the morphogenetic relationship between network-like and multilamellar structures has been observed on some electron micrographs [13].

On the other hand, membrane fragments co-existing with network-like structures have been found on electron micrographs of samples negatively stained or freeze-fractured from the intermediate temperature (35°C) (Fig. 2C,D). Probably, another pathway for the structural transformation in mixed systems studied may be via the fusion of discoid micelles with lipid acyl chains melted to the larger bilayer fragments. Therefore the small unilamellar vesicles also found on the electron micrographs may result from bending and closure of these fragments.

Seemingly, the reversible temperature-induced micellar–lamellar transformation is a common property of mixed systems composed of saturated phospholipids and some appropriate surfactants. A similar temperature-triggered transformation has been described earlier for mixtures of the amphipathic peptide melittin with DPPC [14] or DMPC [10,15], and for lysolecithin and DPPC mixtures [16]; but the mixed systems containing sodium cholate and related detergents are of particular interest because these amphipaths are widely used in membrane research for solubilization and reconstitution procedures. The systems reported in this paper give an opportunity to model different stages of the membrane reconstitution process solely by temperature variations, and to study intermediate structures and transient states during membrane self-assembly without changing the actual detergent/lipid ratio in the incubation medium. They are also promising for the development of an alternative technique for incorporation of membrane proteins into the lipid bilayer. Our preliminary experiments have shown that functionally active bacteriorhodopsin proteo-

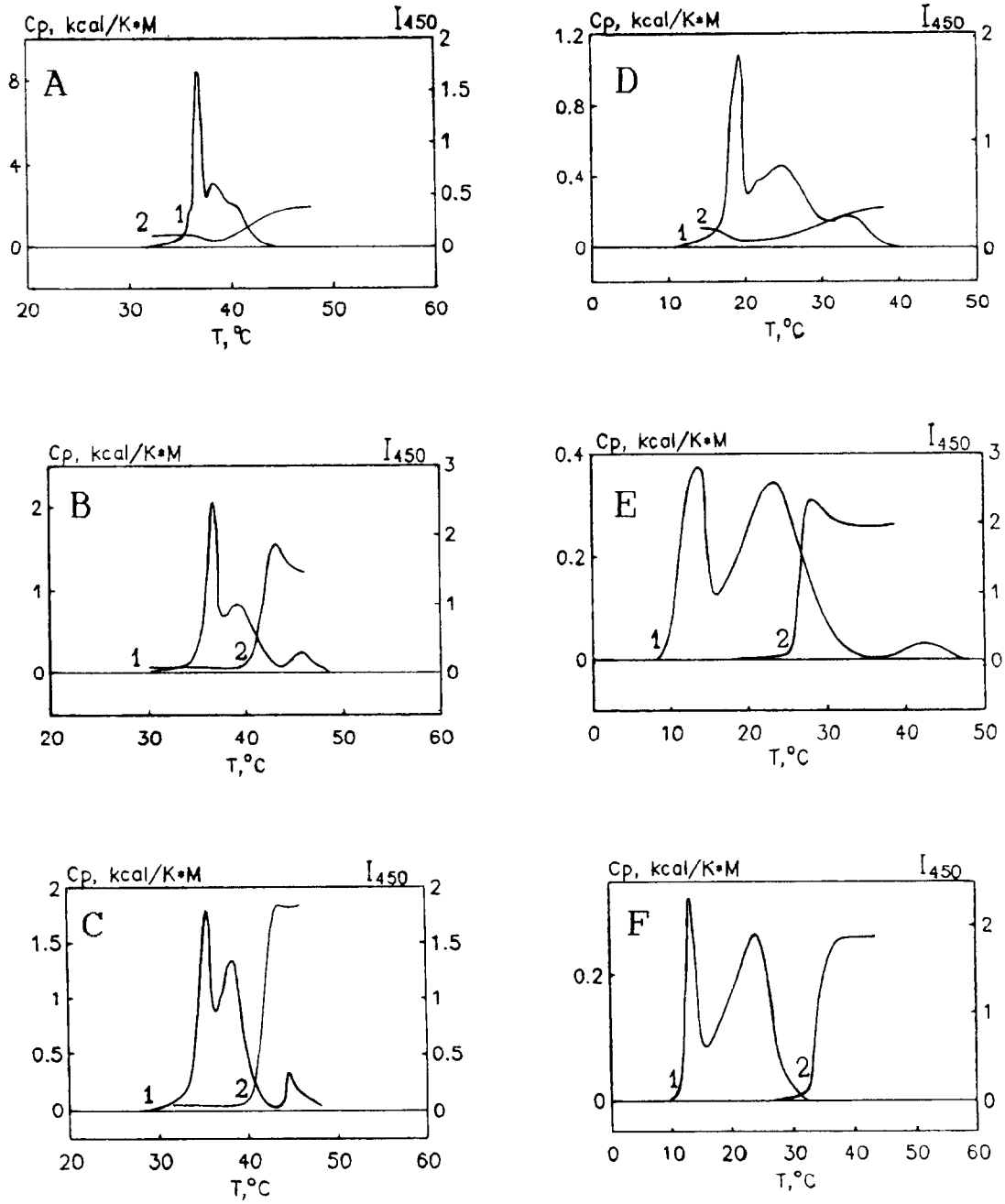

Fig. 4. DSC transition curves (1) and turbidimetric curves (2) for a mixture of 10 mM DPPC (A,B,C) or 20 mM DMPC (D,E,F) with different amounts of ChNa: 6 mM (A), 7 mM (B), 8 mM (C,E), 5 mM (D), 9 mM (F).

liposomes can be prepared by temperature-controlled membrane reconstitution.

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