BBA Report

EFFECT OF Ca^{2+} ON THERMOTROPIC PROPERTIES OF SATURATED PHOSPHATIDYLCHOLINE LIPOSOMES

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The effect of various concentrations of calcium ion (Ca^{2+}) on dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC) and mixed DPPC/DSPC (1:1) liposomes was studied by differential scanning calorimetry. Ca^{2+} concentrations of 10 mM and above split the main transition peak of DPPC into two distinguishable components, and, at 30 mM and above, also resulted in the disappearance of a pre-transition peak. The effect of Ca^{2+} on DSPC liposomes was even more dramatic in that it induced a more definitive split in the main transition peak and caused the loss of the pretransition peak at only 10 mM concentration. The thermograms of the DPPC/DSPC mixed liposomes were unaltered in the presence of Ca^{2+}, even at a concentration of 50 mM. Whether or not Ca^{2+} is able to alter thermograms of phosphatidylcholine liposomes appears to be dependent on the degree of molecular order of the bilayer prior to interaction with Ca^{2+}.

The interaction of divalent cations, particularly Ca^{2+}, with phospholipids in natural and model membrane systems has received wide attention. Such interactions are believed to play an important role in membrane structure and function [1]. It is generally accepted that Ca^{2+} binds strongly to negatively charged phospholipids but there is some controversy as to whether Ca^{2+} binds to neutral phospholipids such as phosphatidylcholines.

Liposomes have been widely used as a membrane model system to study the effects of Ca^{2+} on membrane properties such as permeability [2,3] and fusion characteristics [4]. Differential scanning calorimetry (DSC) has increased our understanding of the possible physical changes of bilayers such as gel → liquid crystalline phase transitions,

phase separation, and membrane fusion which result from these interactions [4–7].

It is difficult to study experimentally the interaction of Ca^{2+} with naturally occurring phospholipids by DSC since these lipids have low phase transition temperatures, usually below 13°C. The majority of investigations of the interactions of Ca^{2+} with phospholipid liposomes employ mixed lipid systems consisting of naturally occurring anionic phospholipids and a synthetic, saturated phosphatidylcholine which has a well defined, experimentally accessible transition temperature. These systems readily facilitate experimental observations of fluidity changes and phase separations following exposure of these systems to Ca^{2+} [8,9]. The resultant changes in the thermograms on exposure of these mixed liposomes to Ca^{2+} has been attributed solely to the interaction of Ca^{2+} with the anionic component of the mixed systems. Whereas this assumption is probably correct for Ca^{2+} concentrations below 10 mM, we

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will show evidence that higher concentrations influence the thermograms of pure, saturated phosphatidylcholines.

There are reports in the literature which suggest that Ca^{2+} does not interact with phosphatidylcholines [10,11], but recent studies employing NMR [12,13], radiotracers [14], spectrophotometry [15], and X-ray diffraction [16] leave little doubt that Ca^{2+} interacts with the phosphate groups of phosphatidylcholine monolayers and bilayers, imparting a charge to them. Simon et al. [17] report that Cd^{2+} salts specifically interact with DPPC liposomes. On the other hand, Ca^{2+} salts, at concentrations of 0.1–100 mM, showed no significant concentration effects on the transition temperature, enthalpy, or peak width of the thermograms.

Saturated lecithins, particularly DPPC and DSPC, are frequently incorporated into liposomes to be used as model membranes and as potential drug delivery systems. As part of our studies of their membrane properties, the effect of various concentrations of Ca^{2+} (1–50 mM) on DPPC, DSPC and mixed DPPC/DSPC liposomes by DSC has been examined. For these investigations DPPC and DSPC were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. No detectable impurities in either of these phospholipids were evident by TLC. Their purity was further confirmed by pre-transition and main transition peaks of their DSC thermograms. The lipids were dissolved in chloroform and stored under N_2 at $-20°C$ until used. Other chemicals used in these studies were of reagent grade and water was triply distilled using an all glass still.

Lipid containing chloroform solutions were transferred to a 10-ml round-bottom flask and the solvent was evaporated under N_2. During this procedure, the flask was rotated by hand to produce a thin layer of lipid at the bottom of the flask. The flask, containing the thin lipid film was stored overnight under vacuum to ensure removal of residual solvent. The lipid film was then suspended at 42°C (DPPC) or 58°C (DSPC) in a sufficient amount of 10 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA to yield a final concentration of about 30 μmol lipid per ml by vortexing for 10 min. Liposomes thus formed were found to be multilamellar as determined by negative stain electron microscopy.

An aliquot of these liposomol dispersions was incubated with various concentrations of $CaCl_2$ (0–50 mM final concentration) for 1 h at the phase transition temperatures of the lipids.

Thermograms were obtained using a Perkin-Elmer DSC-2C scanning calorimeter. Samples were placed in sample pans for volatile matter and were properly sealed. Each sample was usually of 16 μl and contained approx. 0.3–0.6 μmol lipid. An equal amount of buffer was placed in the reference pan. All the scans were obtained at a heating rate of 5°C/min. and a range setting of 1 mcal/s. Indium standard and water were used to calibrate the calorimeter. After each run, the amount of lipid in the sample pan was estimated following the methods of Fiske and SubbaRow [18]. All experiments were performed in triplicate.

The thermograms for DPPC, DSPC and DPPC/DSPC (1:1) in the absence and presence of Ca^{2+} are shown in Fig. 1. In the absence of Ca^{2+}, DPPC shows a pre-transition peak at 36°C and a sharp main transition peak at 41°C (Fig. 1A), whereas DSPC shows a pre-transition peak at 55°C and a sharp main transition peak at 57°C (Fig. 1B). These values are in good agreement with the literature [8,9,19]. DPPC/DSPC shows a single, comparatively broader transition peak midway between the main transition peaks of the pure phospholipid components (50.5°C) (Fig. 1C). There is no indication of pre-transition peaks or of main peaks corresponding to that of the individual components of the mixture, indicating good molecular mixing [6].

Incubation of DPPC with 1 mM and 5 mM Ca^{2+} at 42°C did not alter the thermogram. When the Ca^{2+} concentration was increased to 10 mM, the main transition peak was split into two distinguishable components centered at 41°C and 42.5°C. Further increases in Ca^{2+} concentration (30 mM and 50 mM) resulted in sharper resolution of the split peaks, slight upward peak temperature shifts (42°C and 43°C), and disappearance of the pre-transition peak (Fig. 1A).

DSPC liposomes were even more sensitive to Ca^{2+}. Only 10 mM Ca^{2+} was necessary to eliminate the pre-transition peak. Furthermore, Ca^{2+} (10 mM–50 mM) induced a more definitive split in DSPC systems as compared to DPPC (Fig. 1B).
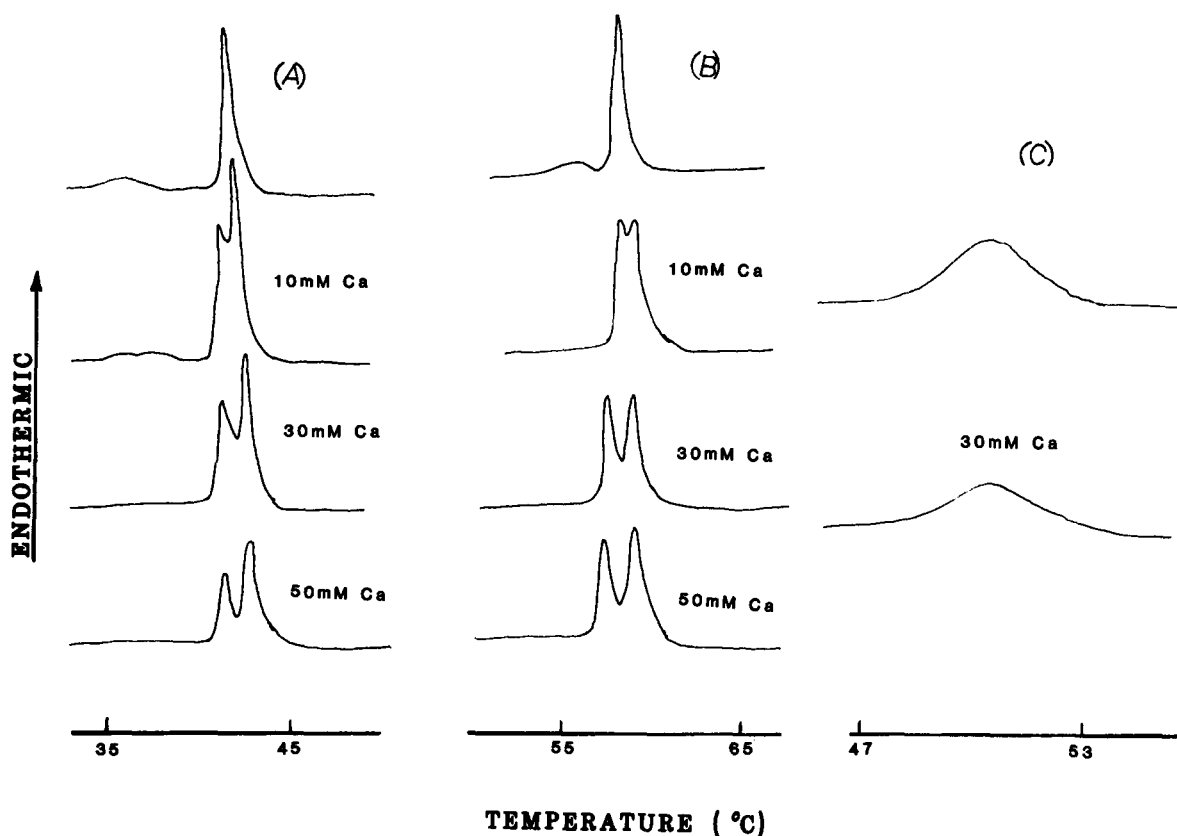
Fig. 1. Thermograms of (A) DPPC, (B) DSPC and (C) DPPC/DSPC (1:1) liposomes dispersed in 10 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA, in the presence and absence of Ca^{2+}. Heating rate was 5°C/min.

Incubation of the mixed DPPC/DSPC liposomes with Ca^{2+} at 50°C (the transition temperature of the mix) or at 58°C (the transition temperature of DSPC) was without effect on the thermogram (Fig. 1C).

Our data clearly show that Ca^{2+} affects the physical properties of DPPC and DSPC bilayers. It has been reported that at 30 mM Ca^{2+}, about 1 in 20 DPPC molecules bear a Ca^{2+} [20]. These rather weak ionic interactions alter molecular packing within the bilayer resulting in observed changes in thermal properties. Since the polar head group is the same for each of the systems tested, the absence of an observed effect by Ca^{2+} in the DPPC/DSPC system may be the result of less molecular cooperativity of the lipids in the bilayer prior to Ca^{2+} addition, as is indicated by its broad endothermic peak. Slight changes in

packing arrangements induced by Ca^{2+} will therefore not be as evident as in the case of the pure lipids which show very sharp transition peaks.

Most of the recent studies [13,15,16,20], including the study reported here, clearly demonstrate that Ca^{2+} does interact with neutral phospholipids. A highly ordered gel state for phosphatidylcholine appears to be needed to clearly observe the perturbation effect of Ca^{2+}. The use of more disordered systems such as monolayer studies using naturally occurring phospholipids [11,14], or our mixed synthetic phosphatidylcholine systems do not possess the molecular packing features required to observe physical changes due to phosphatidylcholine-Ca^{2+} interactions.

Natural membranes contain a mixture of lipids of varying degree of saturation, chain length, and polar groups, leading to a disordered packing

arrangement as compared to a pure saturated phospholipid. Thus, caution should be exercised in interpreting results of Ca^{2+}-membrane interactions since any Ca^{2+}-phosphatidylcholine interactions that may be present in these systems will not be easily observed.

References

1 Urry, D.W. (1978) Ann. N.Y. Acad. Sci. 307, 3–27
2 Van Dijck P.W.M., Ververgaert, P.H.J.T., Verkleij, A.J., Van Deenen, L.L.M. and De Gier, J. (1975) Biochim. Biophys. Acta 406, 465–478
3 Mandersloot, J.G., Gerritsen, W.J., Leunissen-Bijvelt, J., Van Echteld, C.J.A., Noordam, P.C. and De Gier, J. (1981) Biochim. Biophys. Acta 640, 106–113
4 Papahadjopoulos, D., Vail, W.J., Newton, C., Nir, S., Jacobson, K., Poste, G. and Lazo, R. (1977) Biochim. Biophys. Acta 465, 579–598
5 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504–519
6 Papahadjopoulos, D. (1977) J. Colloid Interface Sci. 58, 459–470
7 Portis, A., Newton, C., Pangborn, W. and Papahadjopoulos, D. (1979) Biochemistry 18, 780–790
8 Papahadjopoulos, D., Poste, G., Schaeffer, B.E. and Vail, W.J. (1974) Biochim. Biophys. Acta 352, 10–28
9 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152–161
10 Rojas, E. and Tobias, J.M. (1965) Biochim. Biophys. Acta 94, 394–404
11 Hauser, H. and Dawson, R.M.C. (1967) Eur. J. Biochem. 1, 61–69
12 Hauser, H., Phillips, M.C., Levine, B.A. and Williams, R.J.P. (1975) Eur. J. Biochem. 58, 133–144
13 Hauser, H., Hinckley, C.C., Krebs, J., Levine, B.A., Phillips, M.C. and Williams, R.J.P. (1977) Biochim. Biophys. Acta 468, 364–377
14 Seimiya, T. and Okhi, S. (1973) Biochim. Biophys. Acta 298, 546–561
15 Colbow, K., Avramovic-zikic, O. and Wessel, S. (1981) J. Colloid Interface Sci. 82, 233–239
16 Lis, L.J., Lis, W.T., Parsegian, V.A. and Rand, R.P. (1981) Biochemistry 20, 1771–1777
17 Simon, S.A., Lis, L.J., Kauffman, J.W. and MacDonald, R.C. (1975) Biochim. Biophys. Acta 275, 317–326
18 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
19 Phillips, M.C., Ladbrooke, B.D. and Chapman, D. (1970) Biochim. Biophys. Acta 196, 35–44
20 Lis, L.J., Parsegian, V.A. and Rand, R.P. (1981) Biochemistry 20, 1761–1770