Biochimica et Biophysica Acta 860 (1986) 713–716
Elsevier

BBA 70336

BBA Report

Anisodamine induces the hexagonal phase in phospholipid liposomes

Fen Hwang *, Yu-wei Wu and De-cheng Wen

Institute of Biophysics, Academia Sinica, Beijing (China)

(Received March 24th, 1986)

Key words: Cardiolipin; Dioleoylphosphatidylcholine; Liposome; Lipidic particle; Hexagonal H_{II} phase; Freeze-fracture; Electron microscopy

The effects of anisodamine on the polymorphic phase behaviour of cardiolipin and dioleoylphosphatidylcholine liposomes have been investigated by freeze-fracture electron microscopy. Anisodamine induces the formation of lipidic particles in cardiolipin liposomes at pH 7.0 and hexagonal H_{II} tubes at pH 8.8. When the molar ratio of anisodamine and dioleoylphosphatidylcholine is 4 to 1, lipidic particles can be observed in the fracture faces.

The lipids in biological membranes are arranged in a bilayer structure. In recent years, ^{31}P-NMR and freeze-fracture electron microscopy studies have reported that at physiological temperature, some lipids, such as unsaturated phosphatidylethanolamine (PE) [1,2], monoglucosyldiacylglycerol [3], cardiolipin (in the presence of Ca^{2+}), prefer hexagonal H_{II} structure, which can play important roles in physiological functions of cells, for example, cell fusion, transbilayer movement of lipids, etc. The transition from bilayer to non-bilayer phase can be modulated by factors as temperature, pH, drugs and divalent ions. Anisodamine is newly isolated from Chinese medicinal herbs and synthesized first by Chinese scientists. This drug, showing an inhibiting effect on the cholinergic nerve function as well as improvement on microcirculation, is extensively used in clinic, especially in case of toxic shock and organophosphorus intoxication. But the mechanism of this drug is still unclear. In previous paper, we reported that anisodamine increased the fluidity of neutral phospholipid dipalmitoylphosphatidylcholine (DPPC) liposomes [5]. Recently, we found that anisodamine can promote the transition of bilayer to H_{II} in phosphatidylethanolamine (PE) liposomes by using ^{31}P-NMR and freeze-fracture electron microscopy [6,7]. In this study, it has been shown that anisodamine can also induce the hexagonal H_{II} phase in cardiolipin and dioleoylphosphatidylcholine (DOPC) liposomes. Lipidic particles appeared on the fracture faces at pH 7.0 and at alkaline pH anisodamine can also induce hexagonal H_{II} tubes in cardiolipin liposomes. Anisodamine induces formation of lipidic particles in DOPC liposomes when anisodamine/DOPC ratio is 4 to 1 (mol/mol) or higher.

Cardiolipin and DOPC were obtained from Sigma Co. and showed a single spot by thin-layer chromatography. Anisodamine was obtained from Chengdu First Pharmaceutical Factory.

Preparation of liposomes. The appropriate amount of cardiolipin or DOPC (dissolved in chloroform) were dried under vacuum and left overnight. The lipid films were resuspended in 10 mM Tris buffer (pH 7.0 or 8.8) containing 100 mM NaCl. The final concentration of phospholi-

* To whom correspondence should be addressed.

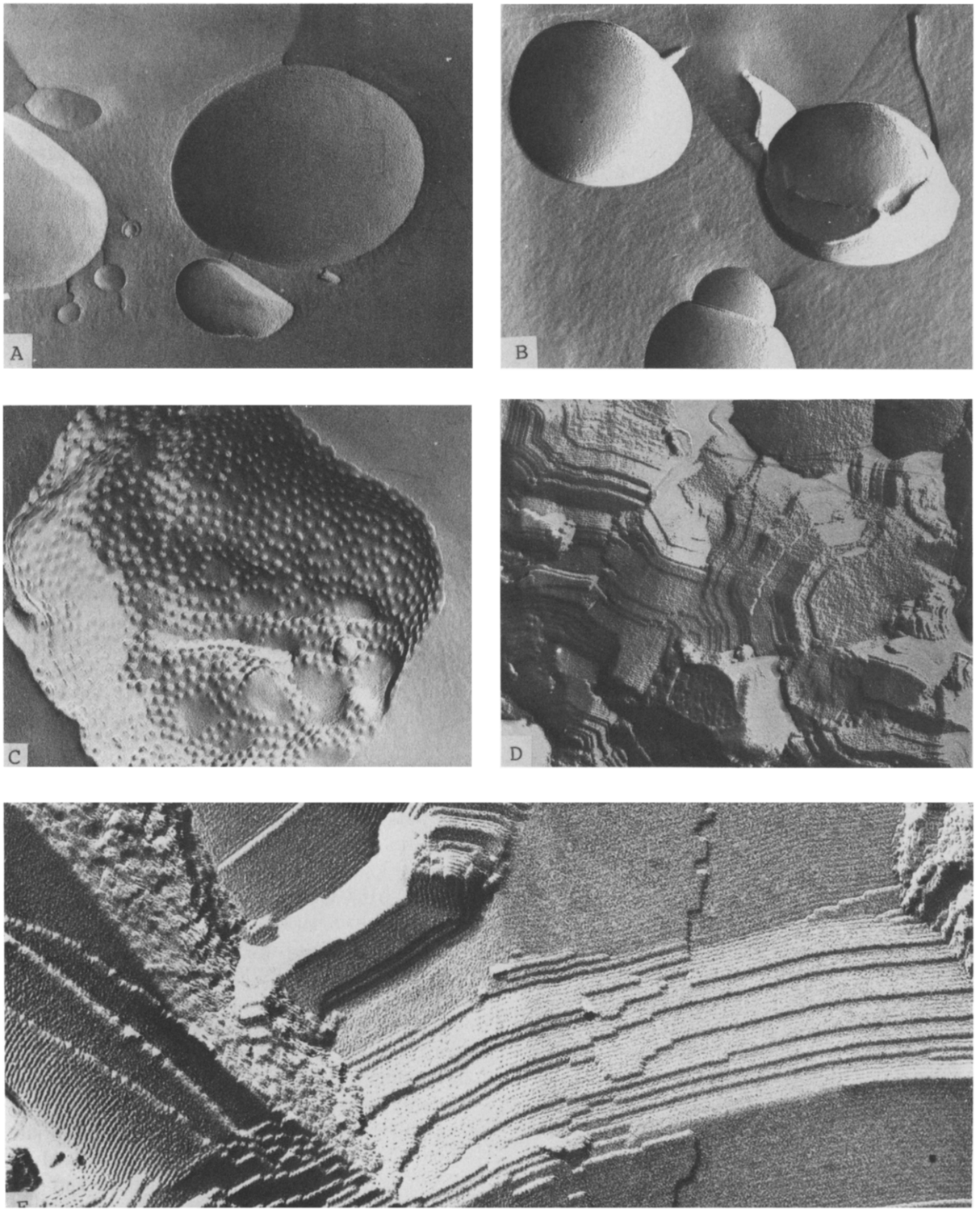

Fig. 1. Freeze-fracture micrographs of cardiolipin dispersed in buffer containing 10 mM Tris, 100 mM NaCl. (A) in the absence of anisodamine at pH 7.0 (47000×); (B) in the absence of anisodamine at pH 8.8 (47000×); (C) containing 30 mol% anisodamine at pH 7.0 (60000×); (D) containing 30 mol% anisodamine at pH 8.8 (60000×); (E) in the presence of 10 mM Tris, 1 M $CaCl_2$ ratio Ca^{2+}/cardiolipin 2:1 at pH 7.0 (120000×).

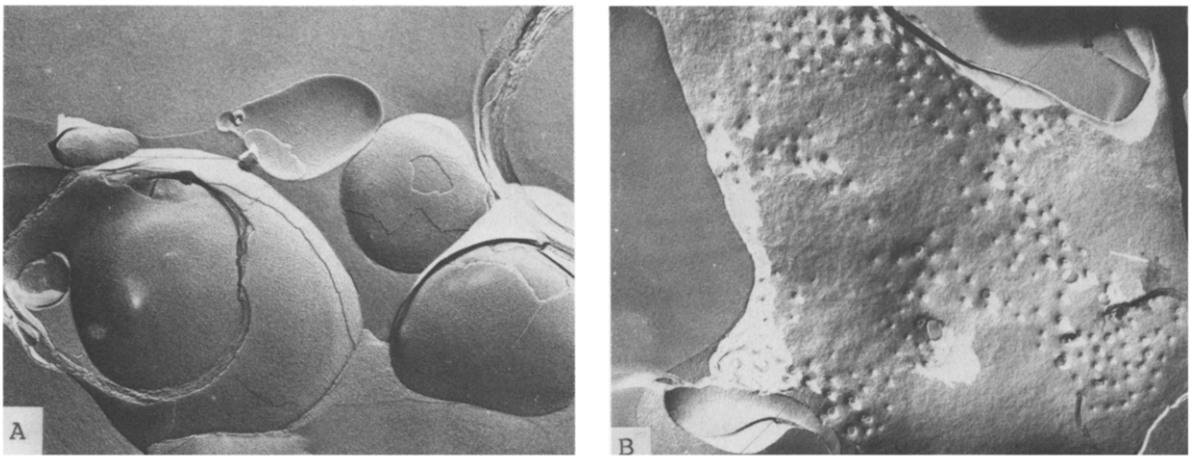

Fig. 2. Freeze-fracture micrographs of dioleoylphosphatidylcholine dispersed in buffer containing 10 mM Tris, 100 mM NaCl. (A) in the absence of anisodamine at pH 7.0 (29000×); (B) anisodamine/dioleoylphosphatidylcholine (molar ratio 4:1) at pH 7.0 (29000×).

pid was 40 mg/ml. Liposomes were prepared by dispersing with a YKH-1 Vortex mixer for 12 min at a temperature higher than the phospholipid transition temperature. Then 30 mol% of anisodamine was added to phospholipid liposomes, for cardiolipin liposomes 1 M of $CaCl_2$ (Ca^{2+}/lipid = 2:1 in molar ratio) was added. Different concentrations of anisodamine (drug/lipid = 1:1, 2:1, 3:1, 4:1, 4.5:1 in molar ratio) were added into DOPC liposomes. Pure cardiolipin or DOPC dispersion was used as control.

Freeze-fracture electron microscopy was performed according to established procedures [5]. Glycerol was added into the liposomes with a final concentration of 20%. Aliquots of the samples were equilibrated at the required quenched temperature for 15 min prior to freezing in the liquid nitrogen, fractured by a BAF400D freeze-fracture device and shadowed by platinum-carbon at a vacuum of $5 \cdot 10^{-6}$ mbar. The replicas were washed with chloroform/methanol and examined under JEM-100CX electron microscopy.

In general, cardiolipin liposomes are organized in extended bilayers. When cardiolipin liposomes contain 30 mol% of anisodamine, at pH 7.0, lipidic particles with 10 nm in diameter are found on the freeze-fracture faces. At pH 8.8, the hexagonal H_{II} tubes is 7.3 nm (Fig. 1, A, B, C, D and E). In our results, the repeat distance between tubes of the hexagonal H_{II} phase in cardiolipin containing Ca^{2+} is 5.2 nm; it is in good agreement with previous study [8]. In biomembranes, PC and sphingomyelin are typical bilayer-forming lipids which play important role in maintaining the basic structure. When different molar ratio of anisodamine were added into DOPC, lipidic particles with a size about 13 nm can be observed when the molar ratio of anisodamine and DOPC is 4 to 1 (Fig. 2, A and B). This result showed that transition from bilayer to hexagonal H_{II} phase in unsaturated PC occurred only at a high concentration of anisodamine.

Many experiments showed that PE liposomes contain unsaturated fatty acyl chain, adopting hexagonal H_{II} phase at physiological temperature. The more unsaturated the PE species, the more readily the hexagonal H_{II} phase is adopted. Ca^{2+}, dibucaine and chlorpromazine induce the formation of hexagonal H_{II} phase in cardiolipin liposomes [4]. Gramicidin, trichloroethylene induce PC and DOPC liposomes to hexagonal H_{II} phase, respectively, but high concentration of drug were required [9,10]. All these phenomena are closely related to the physiological function of biomembrane and molecular mechanism of drug.

The preference of a lipid to adopt a particular phase can be explained in terms of different molecular shapes. Lipids with a small polar head group and relatively large hydrocarbon area are in 'cone shape' and thus prefer the hexagonal H_{II}

phase [11]. It has been shown that [12] the transition from the lamellar liquid-crystalline to the nonlamellar hexagonal H_{II} phase in lipids makes the increasing of concentration of *gauche* bonds in the acyl chains. Such a major structural rearrangement triggers the formation of the non-bilayer phase. According to this suggestion, the possible mechanism of anisodamine with cardiolipin and DOPC liposomes may be considered as follows. Firstly, anisodamine increases the content of *gauche* conformers and the fluidity of phospholipid membranes. Secondly, anisodamine is a drug of polyheterocyclic structure. The interaction of anisodamine with membrane is mainly with the polar head group of lipid bilayer by electrostatic force [5], thus the conformation of the head group of phospholipids are changed, resulting in the formation of non-bilayer structure. Further investigations are in progress.

We would like to thank Professor Su-min Wang for her help and fruitful discussion.

References

1 Cullis, P.R. and De Kruijff, B. (1978) Biochim. Biophys. Acta 513, 31–42
2 Cullis, P.R. and Hope, M.J. (1978) Nature 271, 672–674
3 Wieslander, A., Ulmius, J., Lindblum, G. and Fontell, K. (1978) Biochim. Biophys. Acta 512, 241–253
4 Cullis, P.R., Verkleij, A.J. and Ververgaert, P.H.J.T. (1978) Biochim. Biophys. Acta 513, 11–20
5 Hwang, F., Wang, S.M. and Hu, C.C. (1983) Biochim. Biophys. Acta 736, 220–225
6 Hwang, F., Wu, Y.W. and Wen, D.C. (1985) Kexue Tongbao (Chinese) 30, 614–616
7 Hwang, F. and Wang, S.M. (1985) Kexue Tongbao (Chinese) 30, 1107–1109
8 Van Venetië, R. and Verkleij, A.J. (1981) Biochim. Biophys. Acta 645, 262–269
9 Killian, J.A., Verkleij, A.J., Leunissen-Bijvelt, J. and De Kruijff, B. (1985) Biochim. Biophys. Acta 812, 21–26
10 Forrest, B.J. and Rodham, D.K. (1985) Biochim. Biophys. Acta 814, 281–288
11 Cullis, P.R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420
12 Mantsch, H.H., Martin, A. and Cameron, D.G. (1981) Biochemistry 20, 3138–3145