Cardiolipin vesicles can accommodate cholesterol up to 0.80 mole fraction, i.e. one molecule per cardiolipin fatty acid chain

Jacques Gallay, Béatrice de Foresta and Michel Vincent

Equipe de Recherche no. 64 du Centre National de la Recherche Scientifique, Unité d'Enseignement et de Recherche Biomédicale des Saints-Pères, 45, rue des Saints-Pères, 75270 Paris cedex 06, France

Received 16 August 1985

Cardiolipin-cholesterol interactions were studied by time-resolved fluorescence spectroscopy with 1,6-diphenyl-1,3,5-hexatriene as probe. The residual anisotropy parameter, r_∞ (reflecting the fatty acid chain packing), was measured in the liquid crystalline phase as a function of cholesterol addition. Two main results are reported: (i) a slight increase of the order parameter, S, computed from the r_∞ value as $S=(r_\infty/r_0)^{\frac{1}{2}}$, in the physiological concentration range of cholesterol; (ii) a sharp enhancement of S from a cholesterol mole fraction (X_{chl}) of 0.20 and up to X_{chl} of 0.80. This is in contrast to unsaturated lecithin systems for which a continuous increase of the order parameter was monitored, culminating at $X_{chl}=0.50$, the well-known maximum level of incorporation of cholesterol into lecithin model membranes.

Cardiolipin *Dioleoylphosphatidylcholine* *Cholesterol* *Time-resolved fluorescence anisotropy* *Order parameter* *1,6-Diphenyl-1,3,5-hexatriene*

1. INTRODUCTION

Precise knowledge of cholesterol function implies detailed understanding of its interactions with the main components of biological membranes: the phospholipids. Phosphatidylcholine and phosphatidylethanolamine-cholesterol interactions have been widely studied obviously because these lipids are major species in membranes [1,2]. Less attention has been paid to other species, like cardiolipin [3], probably because this phospholipid is almost exclusively present in sterol-poor membranes like inner membranes of mitochondria and bacterial membranes [4,5]. However, one might wonder if there is a relationship between the presence of this phospholipid at a high level and the relative low amounts of cholesterol in these membranes and if sterol-cardiolipin interactions occur with the same magnitude as in lecithins for instance.

In this respect, we have undertaken a physicochemical study of the effect of cholesterol on the dynamics of a cardiolipin bilayer, using the time-resolved fluorescence anisotropy technique with DPH as probe. This allowed us to evaluate the stoichiometry of the interaction and the effect of cholesterol on the degree of packing of the fatty acid chains of cardiolipin by measuring the residual anisotropy (r_∞) of the anisotropy decay. For comparison, the same measurements were performed on unsaturated phosphatidylcholines in the liquid crystalline phase.

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine

2. MATERIALS AND METHODS

2.1. *Chemicals*

Bovine heart cardiolipins were purchased from Sigma (St Louis, MO) and kept in ethanol

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

(10 mg·ml^{-1}) under an argon atmosphere at −20°C. DOPC was from Serdary (Canada) and cholesterol from Serva (FRG). DPH was from Koch Light (Colnbrook, Bucks). All other reagents were of the highest grade commercially available.

2.2. *Vesicle preparation and labelling*

Ethanol injected vesicles were prepared according to Kremer et al. [6] by injecting 0.1 ml ethanol containing cardiolipin or DOPC and the appropriate amount of cholesterol into 3 ml Hepes buffer, pH 7.4, 0.2 mM EDTA, 0.144 M NaCl at room temperature. Prior to injection, the buffer was extensively bubbled with argon. Labelling was achieved by dissolving the fluorescent probe (DPH) in the injected volume (probe/lipid molar ratio = 1:450).

2.3. *Fluorescence measurements*

Nanosecond decay measurements were performed using a time correlated single photon counting instrument as described [7]. Analysis of the data was performed according to described methods [8]. Data were interpreted according to the distribution function theory [9,10].

3. RESULTS AND DISCUSSION

Nanosecond time-resolved fluorescence anisotropy decay of DPH in cardiolipin vesicles exhibits, as in many membrane model systems [11–14], a non-vanishing value of the anisotropy a long time after the excitation pulse. This residual anisotropy (r_∞) displays similar values in cardiolipin and in unsaturated lecithins in the liquid crystalline phase, as exemplified in table 1 where cardiolipin and DOPC are compared. This indicates that in both systems the acyl chain packing is comparable.

Upon incorporation of cholesterol in cardiolipin vesicles, the value of r_∞ does not change up to a cholesterol mole fraction $\bar{X}_{chol} = 0.20$ in strong contrast to DOPC where this mole fraction corresponds to one third of the maximum effect (table 1). Above this mole fraction, r_∞ increases sharply in cardiolipin and the effect is continuous up to $\bar{X}_{chol} = 0.80$, corresponding to 1 cholesterol molecule/acyl chain. At this high mole fraction, the value of the r_∞ parameter is significantly higher than in DOPC for $\bar{X}_{chol} = 0.50$. In contrast to the large effect observed on the r_∞ parameter, in both lipid systems the apparent correlation time (Φ) remains at the same value within experimental error (table 1). As found also in most of the membrane systems studied, $r_{0effective}$, the extrapolated zero time value of the anisotropy exhibits values lower than the fundamental anisotropy value determined in isotropic media in absence of rotation [15]. This probably reflects the occurrence of very fast rotation not resolved on the instrument [16].

The existence of non-zero r_∞ values has been interpreted as reflecting the occurrence of rotational barriers within the lipid matrix, the principal axis of symmetry of the fluorophore being non-randomly distributed inside the membrane [9,10,17,18]. From the r_∞ value, an average orien-

Table 1

Fluorescence anisotropy decay parameters of DPH in cardiolipin and in DOPC vesicles at 25°C

Cholesterol mole fraction (X_{chol})	0	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80
Cardiolipin									
$r_{0effective}$	0.285	0.281	0.271	0.272	0.319	0.294	0.308	0.316	0.266
Φ (ns)	1.9	2.1	2.0	2.3	1.9	2.3	2.2	1.7	1.6
r_∞	0.015	0.015	0.023	0.042	0.055	0.077	0.115	0.151	0.205
DOPC									
$r_{0effective}$	0.294	0.321	0.349	0.258	0.315	0.277			
Φ (ns)	2.3	1.9	1.9	3.2	2.4	2.9			
r_∞	0.011	0.027	0.044	0.079	0.114	0.162			

A nonexponential model of the form $r_{(t)} = (r_{0effective} - r_\infty)\exp(-t/\Phi) + r_\infty$ was used

tational order parameter (S) can be computed as $S = (r_\infty/r_0)^{1/2}$ [9,10], r_0 being the fundamental anisotropy in the absence of rotational motion (r_0 = 0.384) [15]. As DPH exhibits a similar cross-section to a lipid acyl chain, the average orientational order parameter, S, of the probe reflects to a large extent the average packing of the acyl chain.

As a function of cholesterol addition in the cardiolipin vesicles, the variation of S exhibits a biphasic curve (fig.1). A slight increase in S value is observed in the physiological low range of cholesterol mole fractions (below X_{chol} = 0.20). This is in strong contrast to the situation in DOPC where a mole fraction of 0.30 corresponds to the cholesterol half-maximum effect. Above this mole fraction, the order parameter value in cardiolipin starts to increase linearly until X_{chol} = 0.80, the highest value tested. In DOPC, the variation of S as a function of the sterol mole fraction is linear over the whole range of concentration (fig.1). Such a linear variation of S as a function of cholesterol addition up to X_{chol} = 0.50 has also been observed with ESR in unsaturated lecithins [19,20]. This observation indicates that the interactions of cholesterol with the acyl chains are not cooperative: whatever the range of sterol mole fraction, equivalent increments of cholesterol produce a similar ordering effect in unsaturated PC.

The 'latence range' of cholesterol mole fractions in cardiolipin vesicles as well as the effect at high mole fractions were also observed on the total intensity decay parameters of DPH. As in PC systems [12–14], the total emission decays are multiexponential in either the absence or presence of cholesterol. As the ordering of the acyl chains remained unchanged in cardiolipin vesicles up to a cholesterol mole fraction of 0.20, a very slight change of the mean excited state lifetime value is observed (fig.2). Above this mole fraction, a larger increase of this parameter occurs. In DOPC, in contrast, the increase in the mean excited state lifetime value is linear over the whole range of sterol mole fractions.

In conclusion, these results evidence that cardiolipin can accommodate very large amounts of cholesterol, much larger than reported for PC or PE systems [1]. However, the cholesterol effect on

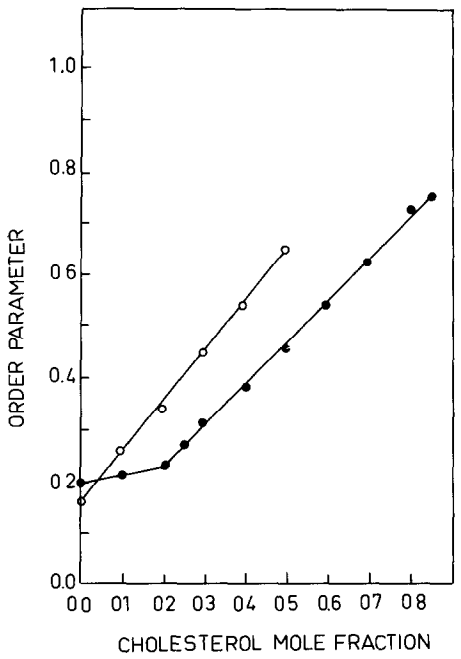

Fig.1. Variation of the average orientational order parameter of DPH in cardiolipin vesicles (●) and in DOPC (○) at 25°C as a function of the cholesterol mole fraction.

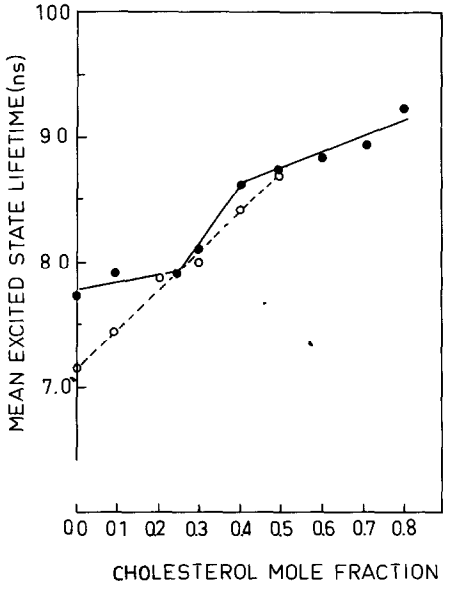

Fig.2. Variation of the mean excited state lifetime $\langle\tau\rangle = \Sigma a_i\tau_i^2/\Sigma a_i\tau_i$ [14] of DPH in cardiolipin vesicles (●) and in DOPC (○) at 25°C as a function of the cholesterol mole fraction.

the lipid ordering occurs only at relatively high mole fractions. This may indicate that cholesterol-cardiolipin interactions are weaker than lecithin-cholesterol interactions. This would in turn explain the relative low level of cholesterol in cardiolipin rich membranes as the sterol molecule could be exchanged more readily from these membranes to sterol transport proteins.

ACKNOWLEDGEMENT

This work was partially supported by Institut National de la Santé et de la Recherche Médicale.

REFERENCES

[1] Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132.
[2] Bloch, K.E. (1983) CRC Critical Rev. Biochem. 14, 47–92.
[3] Boggs, J.M. and Hsia, J.C. (1973) Can. J. Biochem. 51, 1451–1459.
[4] Ioannou, P.V. and Golding, B.T. (1979) Prog. Lip. Res. 17, 279–318.
[5] Brückdorfer, K.R. and Graham, J.G. (1976) in: Biological Membranes (Chapman, D. and Wallach, D.F.H. eds) vol.3, pp.103–152, Academic Press, New York.
[6] Kremer, J.M.H., Esker, M.W., Pathmamanoharan, C. and Wiersema, P.H. (1977) Biochemistry 16, 3932–3935.
[7] Vincent, M., Gallay, J., De Bony, J. and Tocanne, J.-F. (1985) Eur. J. Biochem. 150, 341–347.
[8] Vincent, M. and Gallay, J. (1984) Biochemistry 23, 6514–6522.
[9] Heyn, M.P. (1979) FEBS Lett. 108, 359–364.
[10] Jähnig, F. (1979) Proc. Natl. Acad. Sci. USA 76, 6361–6365.
[11] Kawato, S., Kinosita, K. jr and Ikegami, A. (1978) Biochemistry 17, 5026–5031.
[12] Stubbs, C.D., Kouyama, T., Kinosita, K. jr and Ikegami, A. (1981) Biochemistry 20, 4257–4262.
[13] Dale, R.E., Chen, L.A. and Brandt, L. (1977) J. Biol. Chem. 252, 7500–7510.
[14] Chen, L.A., Dale, R.E., Roth, S. and Brandt, L. (1977) J. Biol. Chem. 252, 2163–2169.
[15] Gallay, J., Vincent, M. and Alfsen, A. (1982) J. Biol. Chem. 257, 4038–4041.
[16] Veatch, W.R. and Stryer, L. (1977) J. Mol. Biol. 117, 1109–1113.
[17] Lipari, G. and Szabo, A. (1980) Biophys. J. 30, 489–506.
[18] Kinosita, K. jr, Kawato, S. and Ikegami, A. (1977) Biophys. J. 20, 289–305.
[19] Semer, R. and Gelerinter, E. (1979) Chem. Phys. Lipids 23, 201–211.
[20] Dahl, C. (1981) Biochemistry 20, 7158–7161.