



Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics

Publication details, including instructions for authors and
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<http://www.tandfonline.com/loi/gmcl17>

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Version of record first published: 22 Sep 2006.

To cite this article: A. E. Sokolova, A. E. Grinfeldt, A. A. Lev & Yu. M. L'vov (1990): Lipid Mesophase Organization and Single Ionic Channel Phenomenon, *Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics*, 193:1, 133-137

To link to this article: <http://dx.doi.org/10.1080/00268949008031815>

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LIPID MESOPHASE ORGANIZATION AND SINGLE IONIC CHANNEL PHENOMENON

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Abstract A correlation between organization of lipid mesophases and single channel phenomenon was investigated by a comparison of small angle X-ray diffraction data and recording of current fluctuations in the bilayers formed from correspondent lipid systems. Transition from lamellar (L_α) to hexagonal (H_{II}) phase in the presence of classical channel forming compound - gramicidin A had been proved. It was found that egg phosphatidylcholine (egg PC) with brominated fatty acid chains (BR-egg PC) exhibited both H_{II} phase in X-ray scattering and single channels conductance fluctuations in the lipid bilayers without addition of any channel forming substance.

INTRODUCTION

A relationship between gramicidin (Gr A) induced hexagonal (H_{II}) phase formation in the human erythrocyte membranes and the membranes ionic leakage had been recently shown.¹ It was of interest to compare the ability of Gr A and its tryptophan-N-formylated analog (TNF-Gr A) to induce lamellar (L_α) to hexagonal (H_{II}) phase transition in multilayer liposomes and appearance of single channel fluctuations in bilayers prepared from the same lipids and treated by these two peptides.

SMALL ANGLE X-RAY DIFFRACTION STUDIES

Studies of water dispersions of egg PC and dioleoyl

phosphatidylcholine (DOPC) by small angle X-ray scattering showed that egg PC and DOPC liposomes gave rise to reflections with repeat distances which relate as $1:1/2:1/3\dots$, consistent with multilamellar (L_α) organization.² Addition of Gr A at a 1:10 and a 1:5 molar ratio to these PCs resulted in appearance hexagonal (H_{II}) phase specific system of reflections $1:1/\sqrt{3}:1/2:1/\sqrt{7}$ (Figure 1, Table I). No hexagonal (H_{II}) phase formation was registered in DOPC multilayer liposomes in the presence of TNF-Gr A. At a 1:10 molar ratio of that substance to DOPC the reflections appeared to be characteristic for L_α lamellar structure (Figure 1A).

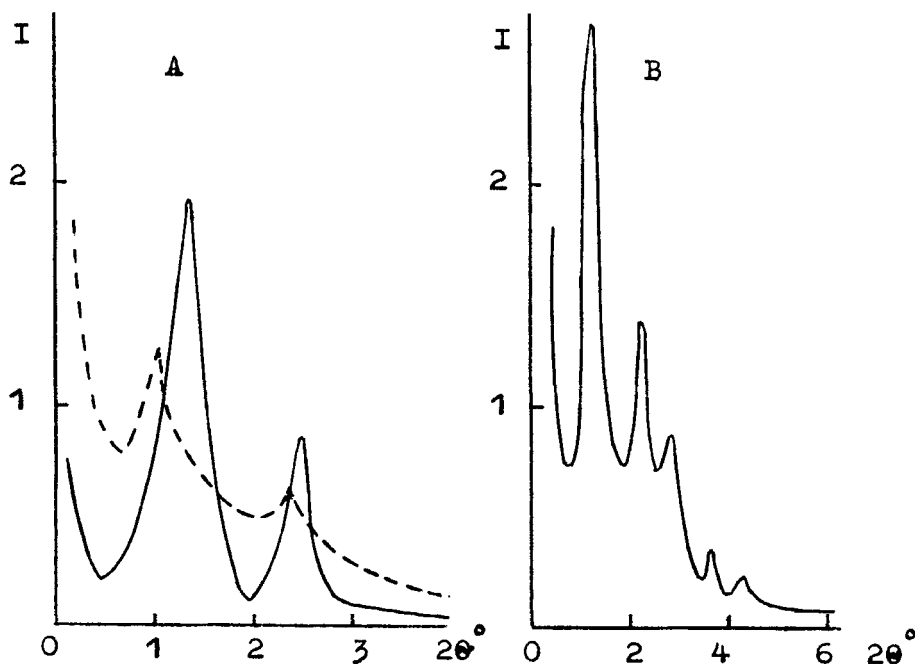


FIGURE 1 Small angle X-ray diffraction patterns of aqueous lipid dispersions. A - egg PC without peptide (solid line) and DOPC with TNF-Gr A (dotted line), lamellar (L_α) phase. B - egg PC with Gr A, hexagonal (H_{II}) phase. 1:10 peptide to lipid molar ratio.

X-ray studies on BR-egg PC showed H_{II} phase without addition of any modifier when in the case of brominated DOPC (BR-DOPC) $L\alpha$ phase was found (Table I).

TABLE I Small angle X-ray diffraction characteristics of phosphatidylcholine (PC) mesophases.

PC sample and molar PC/peptide ratio	Reflection ratio and structure of mesophase	Repeat distance (nm)
egg PC	1:1/2 ($L\alpha$)	6.3
+ Gr A (10/1)	1:1//3:1/2:1//7 (H_{II})	6.3
+ Gr A (5/1)	1:1//3 (H_{II})	7.2
DOPC	1:1/2:1/3:1/4 ($L\alpha$)	5.3
+ Gr A (10/1)	1:1//3 (H_{II})	7.0
+ Gr A (5/1)	1:1//3 (H_{II})	7.1
+ TNF-Gr A (10/1)	1:1/2 ($L\alpha$)	6.1
BR-egg PC	1:1//3 (H_{II})	6.3
BR-DOPC	1:1/2 ($L\alpha$)	6.1

SINGLE IONIC CHANNEL STUDIES

Montal-Mueller bilayer lipid membranes were studied by voltage-clamp method. Bilayers formed of DOPC and egg PC in the presence of a minute amount of Gr A exhibited single channels with the conductance of 13.8 and 6.2 pS consequently. When Gr A was substituted by its Trp-N-formylated analog single channel conductance drastically decreased (1.9 pS) (Table II, Figure 2). Studies of bilayer membranes from BR-egg PC showed well pronounced cation specific single channels with the conductance of 9.4 pS. When bilayers were formed of BR-DOPC

single channels appeared to be not well pronounced and their conductance was much smaller (3.1 pS) (Table II, Figure 2).

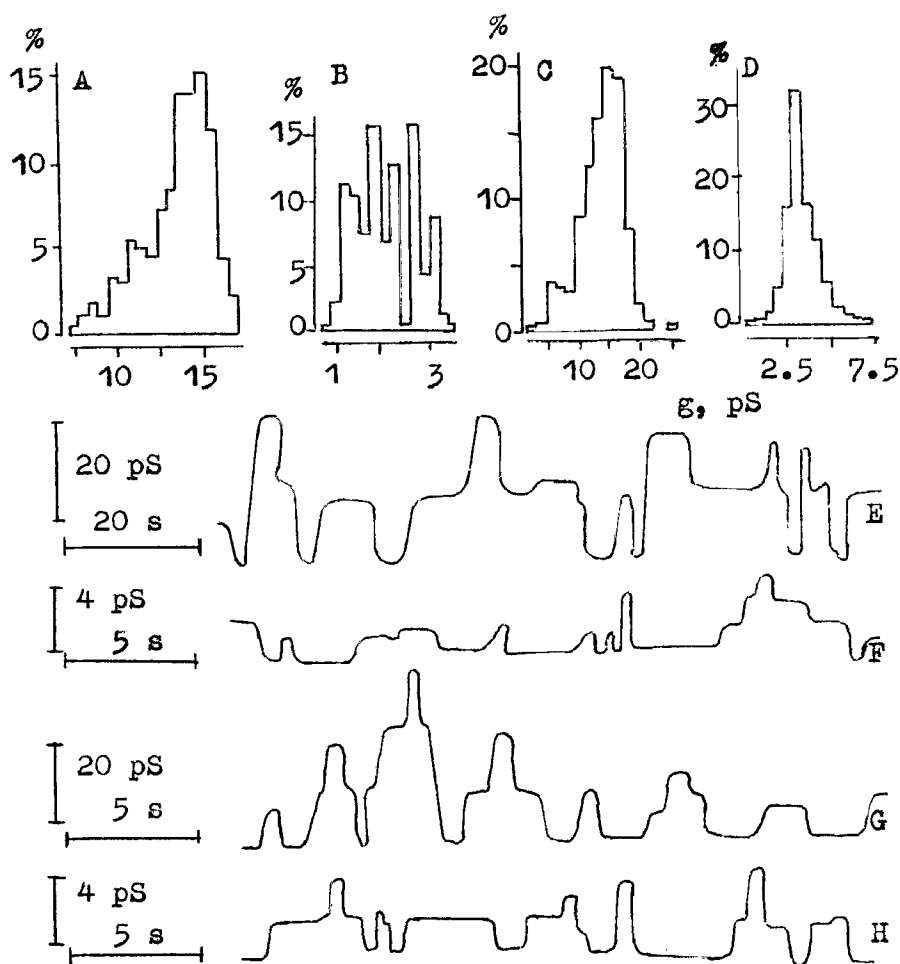


FIGURE 2 Distribution of single ionic channels conductances (g) for PC bilayers and records of single channels. A,E - DOPC+Gr A; B,F - DOPC+TNF-Gr A; C,G - BR-DOPC; D,H - BR-egg PC; 0.1 M RbCl, t° 22°C.

TABLE II Single ionic channels conductance for diacylphosphatidylcholine bilayers.

Lipid	Peptide and its concentration in membrane bathing solution (M)	Channel conductance, pS, 0.1 M RbCl
egg PC	no peptide	no channels
	Gr A, $1 \cdot 10^{-12}$	6.2 ± 0.2
DOPC	no peptide	no channels
	Gr A, $1 \cdot 10^{-12}$	13.8 ± 0.2
	TNF-Gr A, $2 \cdot 10^{-11}$	1.9 ± 0.1
BR-egg PC	no peptide	9.4 ± 0.2
BR-DOPC	no peptide	3.1 ± 0.04

CONCLUSION

In three cases presented (egg PC+Gr A, DOPC+Gr A and BR-egg PC) where a formation of hexagonal (H_{II}) phase was found by X-ray scattering the studies on bilayers including the same components revealed well pronounced single channel phenomena. Contrary to that systems possessing no H_{II} phase formation showed either no or very low conductivity single channels. Thus some correlation between the ability of lamellar ($L\alpha$) to hexagonal (H_{II}) phase transition and single channel phenomena was found irrespective of the cause of changes in the lipids.

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