Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics

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

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Version of record first published: 13 Dec 2006.

To cite this article: Maria Szógyi , Tibor Cserháti & Barna Bordas (1987): Phospholipid - Amino Acid Interactions, Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics, 152:1, 267-278

To link to this article: http://dx.doi.org/10.1080/00268948708070957

Mol. Cryst. Liq. Cryst., 1987, Vol. 152 pp. 267-278
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Printed in the United States of America

PHOSPHOLIPID - AMINO ACID INTERACTIONS

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Abstract Interaction of 17 amino acids with dipalmitoyl phosphatidylethanolamine and with dipalmitoyl phosphatidylcholine was studied by differential scanning calorimetry. Amino acids influenced the main transition temperature, the enthalpy of main transition and the half width of main transition suggesting the existence of amino acid-phospholipid interactions. Stepwise regression analysis proved that the effect of amino acids depended equally on their lipophilicity and on the pK values of α amino and α carboxyle groups that is the effect observed is the results of the interplay of hydrophobic and hydrophilic interactions. Principal component analysis proved that tryptophan exerted the highest impact on the thermal behaviour of phospholipids.

INTRODUCTIONS

Phospholipid-protein interaction play a considerable role in cell function (1,2). A major class of membrane proteins reacts both with the polar and apolar part of the lipid. Poly-L-lysine, which is commonly used as a model for extrinsic membrane proteins, has a pronounced bilayer stabilizing effect in model membranes of cardiolipin due to its electrostatic interactions with the lipid headgroups (3, 4). It was recently reported that phosphatitylserine accelerated the phosphorylation of rat brain Ca^{2+}-activated and phospholipid-dependent protein kinase (5) and the magnitude of the helical hydrophobic moment around proline residues is reduced in lipid-binding proteins (6). It was further

established that polyamines, spermine, spermidin and putrescine stabilized membrane (7).

Not only proteins but also the polypeptide antibiotics as gramicidins influence the structure of membrane phospholipids (8). The tryptophan residues in the gramicidin molecule play an important role in the lipid structure modulating effect of the peptide (9,10,11). However, according to our knowledge the type of interactions (electrostatic or hydrophobic) and the relative impact of individual amino acid-residues on the strength of interaction have not been studied in detail.

In computerized quantitative structure-activity relationship approach of molecular interactions it has become apparent that the interaction can rarely be coupled to a single parameter, but should rather be envisaged as a results of interplay of various parameters. In these types of experiments the results are determined by a great number of variables which can be measured, but there may be hidden factors which can be extracted by principal component analysis (12). This multivariate method is specially useful when the variables show significant intercorrelations. Up till now this method has received relatively little attention in the study of molecular interactions.

The objectives of our work were to determine the amino acid-phospholipid interactions by differential scanning calorimetry (DSC) and to correlate the change of phase transition parameters of phospholipids with some molecular parameters of amino acids.

MATERIAL AND METHODS

Dipalmitoyl phosphatidylethanolamine (DPPE) and dipalmitoyl phosphatidylcholine (DPPC) were purchased from Sigma Chem.

Co. (USA) and were used without further purification. Amino acids of analytical purity were purchased from Reanal (Hungary).

For DSC measurements samples were made by mixing the dry lipid and amino acids with twice distilled water with a vigorous vortex mixer for 30 minutes. The lipid:water weight ratio was 1:4, whereas the amino acid:phospholipid molar ratio was 1:10. The measurements were carried out in a DuPont 910 DSC-cell at a heating rate of $5^{o}C$/min. and in the sensitivity range of 0,1-0,2 mW/cm. The equipment was calibrated using indium. The main transition temperature (T_m) and the half width of the transition ($\Delta T_{1/2}$) were determined. The enthalpies (ΔH_m) were calculated from the area below the endotherms.

The R_M values of amino acids characterizing their lipophilicity were determined by reversed-phase thin-layer chromatography (13,14,15). Silufol UV_{254} plates (Kavalier, Czechoslovakia) were impregnated with 5 % paraffin oil in n-hexane as described in ref.16. Amino acids were dissolved in water:2-propanol (1:4 vol.) at a concentration of 1 mg/cm^3, 3 mm^3 of each solution were spotted on the plates. Twice distilled water was applied as eluent. After development the plates were dried at $105^{o}C$ and the spots were detected by the ninhydrin reagent (7).

Each experiment (DSC, and lipophilicity determinations) was run in quadruplicate.

To find the molecular parameters of amino acids influencing the phase transition parameters (T_m,$\Delta T_{1/2}$,ΔH_m) of phospholipids correlations were calculated between the T_m, $\Delta T_{1/2}$ and ΔH_m values of DPPC and DPPE as dependent and the R_M, $pK_{\alpha NH_2}$, $pK_{\alpha COOH}$, and pI values of amino acids as

independent variables. The dissociation constants and isoelectric points of amino acids were taken from refs 18 and 19.

As the character of correlation (linear or quadratic) between the variables mentioned above is not well known stepwise regression analysis (20) was applied to select the independent variables ($pK_{\alpha NH_2}$, $pK_{\alpha COOH}$, pI, R_M) that significantly influence the phase transition parameters of DPPC and DPPE. Stepwise regression analysis was carried out six times taking the T_m, $\Delta T_{1/2}$ and ΔH_m values of DPPE and DPPC separately as dependent variables. The independent variables were in each case the linear and quadratic forms of $pK_{\alpha NH_2}$, $pK_{\alpha COOH}$, pI and R_M values. The number of accepted independent variables was not limited, their partial F value was set above 1. To take into consideration all DSC results simultaneously the data were evaluated by principal component analysis (PCA). To not lose information by the normalisation PCA was carried out on the covariance matrix instead of the correlation matrix. Nonlinear mapping of PC loadings and variables was also carried out (21). The phase transition parameters of DPPE and DPPC form clusters on the nonlinear map of PC loadings according to their similar response to the effect of amino acids or they are widely separated when their responses are widely different. On the nonlinear map of PC variables the amino acids influencing similarly the phase transition parameters of DPPE and DPPC form groups whereas the amino acids with different effects are separated.

RESULTS AND DISCUSSION

The phase transition parameters of DPPC and DPPE as well as the R_M values of amino acids are compiled in Table I. The

coefficient of variation of R_M values never exceed 6 %. Each amino acid modifies one or more of the phase transition parameters of DPPC and/or DPPE, however, compared to the effect of other membrane damaging agents (22), this influence is fairly low. Except glutamic acid each amino acid decreased the main transition temperature and the enthalpy of main transition and increased the half width of transition. These findings prove that amino acids interact with DPPC and DPPE. The amino acids act as structural defects loosening the lipid packing density and increasing the desorganization of lipid bilayers. The stability enhancing effect of glutamic acid can be explained by the assumption that the two carboxyle groups probably interact simultaneously with the quaternary amino groups of two DPPC molecules (hydrophilic interaction) resulting in the stabilization of the phospholipid structure. Our supposition is supported by the fact that no stabilizing effect was observed with DPPE.

The results of stepwise regression analysis are compiled in Table II. In case of the half width of DPPC and that of the enthalpy of main transition of DPPE the independent variables applied in the calculations did not influenced significantly the phase transition parameters mentioned above. In these cases either other molecular parameters of amino acids are responsible for the effect (ΔH_m of DPPE) or the effect is negligible ($\Delta T_{1/2}$ of DPPC). The lipophilicity was selected as unique independent variable to influence significantly (over 95 % significance level) the enthalpy of main transition of DPPC and the main transition temperature of DPPE. The lipophilicity exerts significant impact also on the other two thermotropic variables (main transition temperature of DPPC and half width of transition of DPPE). These results suggest that the amino acid - phospho-

lipid interaction bases on hydrophob-hidrophob interactions. However, the presence of other independent variables than lipophilicity in equations A and D (Table II) suggests that the actual situation may be more complicated than outlined above. Besides of lipophilicity the main transition temperature of DPPC is influenced by the alkalinity of the α amino groups, by the acidity of the α carboxyle groups and by the isoelectric point of amino acids. This finding indicates that the alkaline α amino group of amino acids may interact with the acidic group of DPPC and the acidic α carboxyle group forms probably hydrogen bond with the quaternary amino group of DPPC. In the case of half width of transition of DPPE only the acidity of the carboxyle group exerts a significant impact probably interacting with the amino group of DPPE.

Summarizing the results of stepwise regression analysis we established that the amino acid - phospholipid interaction is governed equally by hydrophobic and hydrophilic interactions, the hydrophobic one seems to be the stronger. The apolar side chain of amino acids penetrate among the hydrocarbon chains of lipid molecules, their polar groups form hydrogen bond with the polar head groups of phospholipids.

The results of principal component analysis are shown in Table III. The first principal component (PC) explains more than half of the total variance. It means that may exist a single background variable with the help of which about half of the change of phase transition parameters can be explained. We have to stress that the PCA does not prove the existence of real background parameters, it only indicates that within the set of measured data their existence is mathematically possible. The PC loadings show that the T_m and ΔH_m values of DPPC and DPPE are similarly influenced

by the amino acids as well as the behaviour of $\Delta T_{1/2}$ values deviates considerably from that of T_m and ΔH_m values and also from each other.

The two dimensional nonlinear map of PC loadings (Fig.1.) shows that each phase transition parameter is influenced differently by the amino acids. However, the phase transition parameters of DPPE and DPPC form two loose clusters indicating that the type of polar head groups partially determines the character of interaction. As the $\Delta T_{1/2}$ values are the most widely separated this is the parameter differing more strongly in DPPE-amino acid and DPPC-amino acid interactions.

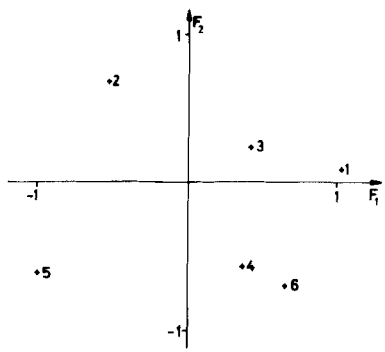

FIGURE 1. Two dimensional nonlinear map of PC loadings.
No of iteration: 5, Max. error of mapping: 0.02.
1. T_m of DPPC 2. $\Delta T_{1/2}$ of DPPC 3. ΔH_m of DPPC
4. T_m of DPPE 5. $\Delta T_{1/2}$ of DPPE 6. ΔH_m of DPPE

The two dimensional nonlinear map of PC variables (Fig.2.) shows that - taking into consideration all the six DSC parameters - tryptophan and lysine exert the highest impact (their points are the most widely separated from the point

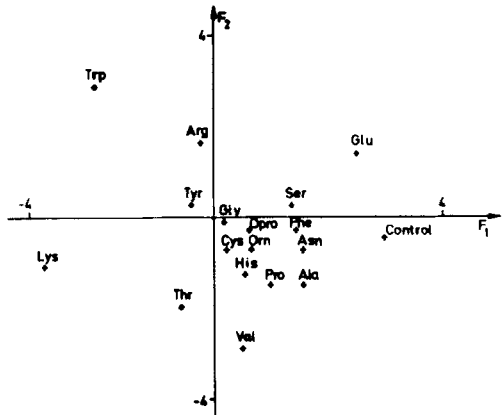

FIGURE 2. Two dimensional nonlinear map of PC variables. No. of iteration: 11, Max. error of mapping:$2{,}65.10^{-2}$.

of control). The map clearly prove that the amino acids do not form clusters according to their structural characteristics. Neither the aliphatic nor the aromatic amino acids separate adequately that is their effect on the phase transition parameters of DPPE and DPPC cannot be predicted on the basis of their chemical structure.

TABLE I. Effect of amino acids on the phase transition parameters of DPPC and DPPE and their lipophilicity (R_M) values

	DPPC						DPPE						
	T_m °C		$\Delta T_{1/2}$ °C		ΔH_m mJ/mg		T_m °C		$\Delta T_{1/2}$ °C		ΔH_m mJ/mg		R_M
	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	
Control	41.7	0.3	2,6	0.2	54.9	1.2	66,0	0.5	2,2	0.2	52.1	0.4	
Trp	40.7	0.1	2.9	0.2	45.4	0.5	63.8	0.3	2.7	0.1	39.4	1.7	0.37
Lys	40.7	0.3	2.3	0.2	38.5	1.6	64.3	0.6	2.6	0.2	47.2	1.2	1.43
His	41.4	0.4	2.4	0.2	54.3	1.5	65.2	0.4	2.5	0.1	52.3	1.2	0.73
Gly	41.2	0.6	2.5	0.1	54.7	1.5	64.7	0.8	2.5	0.1	49.3	0.9	-0.93
Asn	41.7	0.3	2.5	0.1	54.8	1.6	65.4	0.4	2.4	0.2	51.3	1.9	-0.72
Phe	41.8	0.3	2.5	0.1	53.5	2.1	64.9	0.1	2.4	0.1	49.0	2.2	0.12
Val	41.1	0.1	2.5	0.1	54.5	2.1	66.1	0.1	2.6	0.3	53.3	1.4	-0.32
Ser	41.5	0.1	2.6	0.1	54.9	1.3	65.4	0.2	2.4	0.2	52.2	1.0	-0.93
Glu	42.5	0.2	2.7	0.1	57.0	1.4	65.2	0.3	2.4	0.2	52.1	1.0	-1.14
Thr	41.2	0.4	2.4	0.2	54.0	2.8	65.0	0.7	2.7	0.1	49.4	1.2	-0.72
Tyr	40.9	0.3	2.6	0.1	52.0	2.8	64.9	0.1	2.5	0.3	51.3	2.5	-0.29
Ala	41.6	0.1	2.4	0.1	54.8	0.6	65.6	0.4	2.4	0.1	48.1	1.7	-0.63
Pro	41.7	0.2	2.4	0.2	57.2	3.6	64.8	0.4	2.5	0.3	51.4	2.7	-0.13
Opro	41.4	0.2	2.5	0.1	54.5	1.6	65.0	0.2	2.5	0.1	47.0	4.2	-0.72
Cys	41.3	0.2	2.4	0.2	54.9	1.3	64.6	0.2	2.4	0.2	49.4	1.2	-0.64
Orn	41.4	0.2	2.4	0.1	53.7	3.5	64.8	0.4	2.4	0.2	50.9	1.6	1.27
Arg	40.8	0.2	2,7	0.1	54.4	2.2	64.2	0.3	2.4	0.2	46.0	2.0	1.44

TABLE II. Results of stepwise regression analysis

A. DPPC $T_m = a + b_1.pK_{\alpha NH_2} + b_2.pI + b_3.(pK_{\alpha COOH})^2 + b_4.R_M^2$

B. DPPC $\Delta H_m = a + b_1.R_M$

C. DPPE $T_m = a + b_1.R_M$

D. DPPE $\Delta T_{1/2} = a + b_1.R_M + b_2.(pK_{\alpha COOH})^2 + b_3.R_M^2$

	Parameters of equation			
	A	B	C	D
a	41.08	52.8	64.9	2.2
b_1	0.25	-2.83	-0.33	$6,2.10^{-2}$
b_2	-0.24			$7,0.10^{-2}$
b_3	-0.16			$-7,5.10^{-2}$
b_4	0.32			
b_1' %	15.76			29.50
b_2' %	44.77			42.62
b_3' %	17.70			27.88
b_4' %	21.77			
s	0.29	3.95	0.49	$7,3.10^{-2}$
s_{b_1}	0.12	1.15	0.13	$2,6.10^{-2}$
s_{b_2}	0.05			$1,7.10^{-2}$
s_{b_3}	0.07			$3,2.10^{-2}$
s_{b_4}	0.14			
r	0.8350	0.5374	0.5116	0.7786
F	6.91			6.67
t_1	2.09			2.40
t_2	4.54			4.08
t_3	2.36			2.32
t_4	2.22			
$r_{95\%}$		0.4821	0.4821	
$F_{99\%}$	5.41			5.74
$t_{90\%}$	1.78			
$t_{95\%}$	2.18			2.16
$t_{99\%}$				3.01
$t_{99,9\%}$	4.32			

TABLE III. Results of principal component analysis (PCA)

No of PC	Eigenvalue	Sum of total variance explained %
1	3.21	53.53
2	1.21	73.63
3	0.58	83.08

Principal component loadings

Variable	No of principal component		
	1	2	3
T_m DPPC	0.80	0.26	-0.09
$\triangle T_{1/2}$ DPPC	-0.27	0.89	0.28
$\triangle H_m$ DPPC	0.79	0.29	0.22
T_m DPPE	0.82	-0.17	0.32
$\triangle T_{1/2}$ DPPE	-0.70	-0.34	0.56
$\triangle H_m$ DPPE	0.85	-0.36	0.12

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