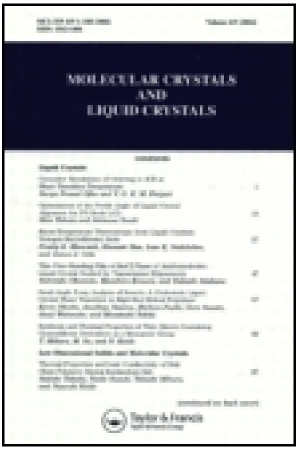

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PHOSPHOLIPID-PAPAIN INTERACTIONS

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Abstract Interaction of six synthetic phospholipids with papain was studied by differential scanning calorimetry. Papain and phospholipid influenced mutually each other's main transition temperature, the enthalpy of main transition and the half with of the transition suggesting the existence of papain—phospholipid interactions. Multivariate methods proved that the length of apolar fatty acid chains of phospholipids is responsible for the effect, indicating hydrophobic interaction between papain and phospholipids. The efficacy of phospholipids increases with growing length of fatty acid chain, the grade of methylation of polar head group of phospholipids influences to a lesser extent the interaction. The presence of phospholipids has a negligible effect on the proteolytic activity of papain.

INTRODUCTION

Phospholipid—protein interactions play a considerable role in cell function (1,2,3). Liposomes fuse with virus particles (4), this fusion activity is strongly dependent on the lipid composition (5). α-lactalbumin, lysosyme and ribonuclease induced the fusion of phosphatitylserine/ phosphatidylethanolamine vesicles (69). Phospholipids influence considerably glucon synthase activity too (7, 8). Polycationic compounds as polylysine are able to increase the permeability of the outer membrane of Gram--negative bacteria (9). It was recently reported that

phosphatidylserine accelerated the phosphorylation of rat brain Ca^{2+}-activated and phospholipid-dependent protein kinase (10) and the magnitude of the helical hydrophobic moment around proline residues is reduced in lipid-binding proteins and peptides (11).
Although the phospholipid—protein interaction has been studied on various objects, the type of interaction (hydrophilic or hydrophobic) and the relative impact of polar head group and alkyl chain length of phospholipids on the interaction have not been elucidated in detail.

The objectives of our work were to investigate the phospholipid-papain interaction by differential scanning calorimetry (DSC) and to correlate the change of phase transition parameters with the structural characteristics of phospholipids. To extract maximal information of our results the data matrix (the change of phase transition parameter caused by phospholipid-protein interaction) was evaluated by multivariate methods as principal component analysis (12) spectral mapping technique (13) and cluster analysis (12).

MATERIAL AND METHODS

Dilauroylphosphatidylcholine (DLPC), dimyristoylphoshatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylethanolamine (DPPE), N-monomethyl-dipalmitoylphosphatidylethanolamine (MMDPPE) and papain were purchased from Sigma Chem. Co (USA) and used without further purification. Other reagents were of analytical purity.
For DSC measurements samples were made by mixing the dry lipids and papain with twice distilled water for 30 min.

with a vortex mixer. The lipid:water weight ratio was 1:4, whereas the papain:phospholipid molar ratio was 1:10. The measurements were carried out in a DuPont 910 DSC-cell at a heating rate of $5^0C/min$ and in the sensivity range of 0.1—0.2 mW/cm. The main transition temperature (T_m), the half width of the transition ($\Delta T_{1/2}$) and the enthalpy of main transition (ΔH_m) of phospholipids, and the two transition temperatures (T_{p_1}, T_{p_2}) of papain were determined. The effect of phospholipids on the proteolytic acitvity of papain was determined on caseine substrate. Phospholipid were sonicated in 0.16 M phosphate buffer (pH = 7.0) for 10 min. than papain was added to the dispersion, after 2 hours incubation the activity was determined by the biuret reaction (14). Phsopholipid:papain molar ratio was 100-10:1. Each experiment (DSC and determination of proteolytic activity) was run in quadruplicate.

In order to find the molecular substructures of phospholipids influencing the five phase transition parameters mentioned above correlations were calculated between the change of phase transition parameters as dependent and the length of apolar fatty acid chain (number of carbon atoms) and the grade of methylation of polar head group of phospholipids as independent variables. As the character of correlation (linear, quadratic or logarithmic) between the variables was not previously established, stepwise regression analysis (15) was applied to select the independent variables (length of fatty acid chain, grade of methylation of head group of phospholipids) that significantly influence the phase transition parameters. Stepwise regression analysis was carried out five times taking the T_m, $\Delta T_{1/2}$, ΔH_m, T_{p_1} and T_{p_2} values separately as dependent variables. The

independent variables were in each case the linear, quadratic and logarithmic forms of alkyl chain length and the linear and quadratic forms of the grade of methylation of head groups. The number of accepted independent variables was not limited, their partial F value was set to F=2.
To take into consideration all phase transition results simultaneously the data matrix was evaluated by principal component analysis, by spectral mapping technique and by cluster analysis.

RESULTS AND DISCUSSION

The DSC curves of DPPC, papain and DPPC-papain mixture are shown in Fig.1. Papain and DPPC mutually modify each other's phase-transition parameters. The pretransition of DPPC disappears, the main transition temperatures increase and the peaks are wider. All these changes indicate papain-DPPC interaction. The phase transition parameters are compiled in Table I. Each phase transition parameter of each phospholipid-papain mixture were modified compared to the pure compounds proving the existence of papain-phospholipid

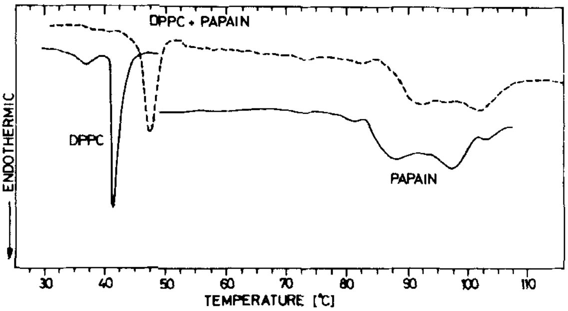

FIGURE 1. DSC curves of DPPC, papain (——), and DPPC:papain 10:1 molar ratio (– – –)

interaction. The increasing half with of the transition and of decreasing enthalpy of phospholipids indicates that papain decreases the intermolecular order of phospholipids by interacting with them.

The results of stepwise regression analysis are compiled in Table II. The change of T_{p_1} and T_{p_2} did not depend significantly on the fatty acid chain length and on the grade of methylation of head group of phospholipids. The longer is the fatty acid chain the lower is the effect of papain on the phase transition temperature and on the half width of the transition of phospholipids (Eqs.1 and 2 in Table II). These findings suggest that the papain-phospholipid interactions are mainly governed by hydrophobic interactions, the apolar fatty acid chains adsorb on the hydrophobic substructures in papain. The path coefficients (b_i'%) of Eq.3. indicate that the length of fatty acid chain has a higher impact and the grade of methylation is of secondary importance.

This result supports our previous supposition concerning the hydrophobic character of interaction. In the principal component analysis the first principal component explains 79.1% of total variance (eigenvalue: 4.75). It means that may exist a single background variable with the help of which about 80 % of the change of phase transition parameters can be explained. We stress that this result does not prove the existence of real background parameters, it only indicates that within the set of measured data its existence mathematically possible. The second principal component explains 18.7 % of total variance (eigenvalue 1.13) which indicates the overhelming role of only one background variable.

The two dimensional map PC variables is shown in Fig.2.

The phase transition parameters which are similarly influenced by all the six phospholipids are near to each other

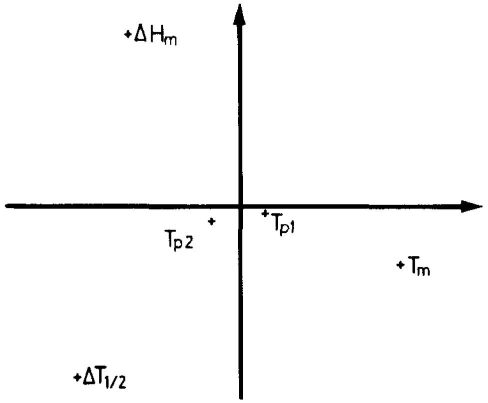

FIGURE 2. Two dimensional map of PC variables T_m: main transition temperature of phospholipids, $\Delta T_{1/2}$: half width of the main transition of phospholipids, ΔH_m: enthalphy of main transition of phospholipids, T_{p_1} and T_{p_2}: transition temperatures of papain

on the map as well as the parameters influenced differently are widely separated from each other. The phase transition parameters of phospholipids (T_m, $\Delta T_{1/2}$ and ΔH_m) are well separated, it means that papain influence differently the parameters mentioned above. Oppositely, the T_{p_1} and T_{p_2} values are near to each other indicating that phospholipids exert similar effect on both transition temperatures of papain. The result of cluster analysis (clustering of phospholipids taking into consideration simultaneously all the five phase transition parameters) is shown in Fig.3. Our data clearly show that the grade of methylation has a negligible effect on the efficacy of phospholipids (the points of DPPE, MMDPPE and DPPC are near to each other) and the length of apolar fatty acid chains differentiate markedly the phospholipids proving again the importance of hydrophobic forces in the papain-phospholipid interactions.

The potency values calculated by the spectral mapping

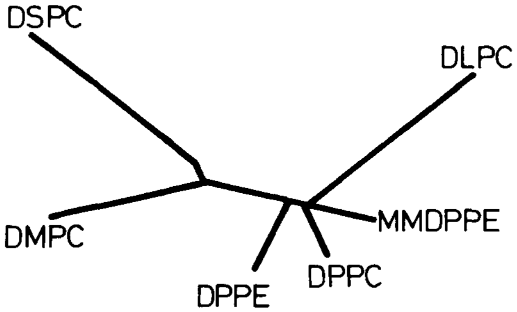

FIGURE 3. Result of cluster analysis

technique support our previous findings. The overall efficiency of phosphatidylcholines increases with growing length of apolar fatty acid chains (potency values: DLPC=6.65; DMPC=12.55 and DSPC=13.56) however the grade of methylation of polar head group influence to a lesser extent the efficacy (potency values: DPPE=8.55; MMDPPE=9.25; DPPC=10.54). DLPC, DMPC did not influenced significantly the proteolytic activity of papain even at the highest molar ratio. DPPE, MMDPPE, DPPC and DSPC decreased the proteolytic activity of papain with 13.7; 15.2; 12.8 and 14.3% respectively at 100:1 molar ratio. However these effects were significant, they are negligible from practical point of view. The results of activity measurements suggest that the adsorption of phospholipid on the surface of papain molecules takes place far from the activity centrum of papain. This interaction makes more stable the papain structure but does not influence markedly its proteolytic activity.

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TABLE I. Phase transition parameters of phospholipid-papain mixtures

Composition	T_m °C	$\Delta T_{1/2}$ °C	ΔH_m mJ/mg	T_{p_1} °C	T_{p_2} °C
Papain	-	-	-	88.0±1.1	96.8±2.5
DLPC	0.5±0.7	1.6±0.2	10.3±3.4	-	-
Papain-DLPC	5.5±0.7	3.1±0.1	8.2±2.8	91.5±2.1	99.8±2.5
DMPC	24.2±0.9	1.4±0.2	45.0±4.2	-	-
Papain DMPC	28.7±0.3	2.9±0.1	34.5±2.1	93.8±0.8	102.5±0.5
DPPC	41.7±0.3	1.4±0.2	55.7±2.5	-	-
Papain DPPC	46.0±0.5	2.3±0.4	45.0±6.6	91.3±2.5	101.2±1.8
DSPC	58.4±0.4	2.5± 0.1	70.7±3.1	-	-
Papain-DSPC	60.7±0.8	3.2±0.2	54.7±4.2	94.3±1.5	101.8±1.6
DPPE	66.8±1.3	1.7±0.2	55.3±1.5	-	-
Papain-DPPE	70.5±0.5	2.4±0.1	48.7±3.5	92.5±0.5	100.3±0.6
MMDPPE	59.7±1.6	1.9±0.1	56.0±2.0	-	-
Papain-MMDPPE	64.2±0.3	2.6±0.2	48.0±2.1	91.2±1.8	101.0±0.7

TABLE II. Effect of fatty acid chain length (L) and grade of methylation of head group of phospholipids (M) on the change of phase transition parameters of papain-phospholipid mixtures $n = 6$, $r_{95\%} = 0.8114$

1. $T_m = 7.28 - (1,35\pm0.4).10^{-2}L^2$ $r_{calc.} = 0.8447$
2. $\Delta T_{1/2} = 3.61 -(0.17\pm0.05)L$ $r_{calc.} = 0.8777$
3. $\Delta H_m = -81.65 + b_1M + b_2\log L$

$F_{calc} = 14.3$ $F_{95\%} = 9.55$

	M	logL
b_i	1.92	72.74
s_{b_i}	0.64	13.96
$b_i'\%$	36.47	63.53
t	2.99	5.21
$t_{90\%}$	2.35	
$t_{95\%}$	3.18	

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