EFFECT OF PROTEIN BINDING ON PHOSPHOLIPASE C HYDROLYSIS OF AQUEOUS PHOSPHOLIPID DISPERSIONS

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Summary

Formation of ionic complexes with basic proteins (lysozyme, cytochrome c) does not allow phospholipid hydrolysis by phospholipase C. On the other hand phospholipids in hydrophobic complexes with lipid-depleted mitochondria (LDM) and intact submitochondrial membranes (ETP) are rapidly hydrolysed by the phospholipase. Phospholipids in ternary complexes of basic proteins with LDM-phospholipid complexes or with ETP are however also hydrolysed. These results suggest that the lipid bilayer has a greater dynamic character in membranes than in phospholipid vesicles.

The effect of phospholipases on phospholipid hydrolysis in natural membranes is often taken as an indication that the phospholipid polar heads are available to the attack of the enzyme on the surface of the membrane (1-5). The experimental results have been discussed in terms of models for membrane structure, and the suggestion has been advanced that a continuous layer of protein cannot cover the phospholipid bimolecular leaflet (2,6). Others have however pointed out that the dynamic character of the membranes might account for phospholipase action even if phospholipids are disposed in a Danielli type of membrane (7,8). There is however very little experimental knowledge of the effect of lipid-protein interactions on the action of phospholipases on aqueous phospholipid dispersions. This note is a preliminary account of our investigations to clarify this important point.

Sonicated phospholipid vesicles (9) have been allowed to in-

teract with soluble basic proteins (lysozyme, cytochrome c, and protamine) and the ionic insoluble complexes (10) have been treated with phospholipase C from Clostridium welchii. Table I shows that all three basic proteins inhibit phospholipase action, whereas serum albumin (which does not form ionic complexes at neutral pH) has little effect. On the other hand, when hydrophobic complexes have been made between phospholipids and lipid-depleted submitochondrial particles ETP (LD-ETP), there is a high hydrolytic action of phospholipase C; similar results are clearly shown when intact ETP are treated with the phospholipase (Table II).

The LDM-phospholipid complexes or intact ETP have then been treated with basic proteins with resulting formation of ternary complexes where the negative charges of the phospholipids neutralize the cationic groups in the basic proteins (11). Surprisingly, these complexes are hydrolyzed by phospholipase C at very high rates in contrast with the ionic binary complexes of basic proteins with phospholipids (Table III).

Two considerations can be derived from the results of this investigation. First, phospholipase C hydrolysis per se cannot be a sufficient tool for determination of the phospholipid location in natural membranes. Secondly, the availability of phospholipids to the action of phospholipase in the presence of a basic protein screen is much higher in membranes than in phospholipid vesicles. This effect could give some indication on the mechanism of phospholipase C action. If we accept the premise that basic proteins interact with phospholipids in reconstituted or natural membranes in the same way as they do in protein-free phospholipid vesicles (11), then a protein layer covering the membrane cannot be a sufficient hinder to phospholipase action. We must then suggest that either phospholipid exchange or Brownian motion of membrane subunits (8) allow exposure of phospholipids in the water medium and are involved in phospholipase action. Such dynamic character of the bimolecular leaflet is significantly higher in membranes in comparison with phospholipid vesicles and

Table I - Effect of soluble proteins on phospholipase C hydrolysis of Asolectin vesicles.

Addition	Rate of hydrolysis (°)
-	100
Lysozyme	10
Cytochrome c	10
Asolectin	80
then lysozyme (°°)	0
Protamine	4
Albumin	75

(°) Hydrolysis has been followed at 37° in a pH-Stat by NaOH titration of hydrogen ions liberated by hydrolysis at constant pH of 7.2. The incubation medium contained 130 µg of Asolectin phosphorus, 5 mM NaCl and 1 mM $CaCl_2$ in a total volume of 6 ml. Lysozyme and cytochrome c were 250 nmoles, albumin was 100 nmoles, and protamine 2.5 mg. After equilibration, 400 µg of phospholipase C were added and hydrolysis recorded for 20 min. The rate of digestion was calculated from the initial curve when the rate of hydrolysis was linear and expressed as nEq of NaOH/min.

(°°) Lysozyme was added after the initial rate of hydrolysis had been recorded.

Table II - Phospholipase C hydrolysis of reconstituted and intact membranes.

Particle (°)	Rate of hydrolysis
Asolectin	150
RM	262
R-ETP	125
ETP	125

(°) The experimental conditions were the same as in the legend of Table I. The phosphorus content of the different particles hydrolysed was the following: Asolectin: 130 µg; RM, i.e. mitochondrial membranes reconstituted from lipid depleted mitochondria and phospholipids (12): 90 µg; R-ETP, i.e. membranes reconstituted from lipid depleted ETP and phospholipids: 130 µg.

Table III - Effect of basic proteins on phospholipase C hydrolysis of ETP and of reconstituted membranes.

Particle	Addition	Rate of hydrolysis
RM	-	75
RM	Lysozyme	87
RM	Cytochrome c	75
R-ETP	-	125
R-ETP	Lysozyme	187
R-ETP	Cytochrome c	156
ETP	-	137
ETP	Lysozyme	162
ETP	Cytochrome c	137

Experimental conditions were the same described in Tables I and II.

must be conferred to the phospholipid by hydrophobic interaction with membrane proteins.

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