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SPECIFICITY REVERSAL IN PHOSPHOLIPASE A_2 HYDROLYSIS OF LIPID MIXTURES

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SUMMARY

The activity and specificity of phospholipase A_2 from cobra venom (*Naja naja naja*) toward binary mixtures of phosphatidylcholine and phosphatidylethanolamine in mixed micelles with the nonionic surfactant Triton X-100 were examined. In mixtures containing 5-50 mol % phosphatidylcholine, the rate for phosphatidylethanolamine hydrolysis was enhanced greatly over that for phosphatidylcholine. This is in marked contrast to previous studies with individual phospholipid species in mixed micelles where phosphatidylcholine was found to be the preferred substrate and phosphatidylethanolamine was found to be a very poor substrate. Possible explanations for this specificity reversal are considered.

INTRODUCTION

Phospholipase A_2 (EC 3.1.1.4) has been used widely for studies on the distribution of phospholipids in surfaces of biological membranes (1) and for investigations on the mechanism of enzymatic catalysis of substrate localized at the lipid-water interface of model membranes (2,3). In order to properly evaluate these studies, it is important that pure enzyme be used and that the substrate specificity of the enzyme be understood. In our laboratory, we have prepared a pure, homogeneous form of phospholipase A_2 from cobra venom (*Naja naja naja*) having a low molecular weight (4,5) and have examined the substrate specificity of this enzyme toward individual lipid species in mixed micelles with the nonionic surfactant Triton X-100 (6). It was found that the apparent substrate specificity of this enzyme was phosphatidylcholine>> phosphatidylethanolamine.

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We now report new findings on the activity and substrate specificity of the cobra venom phospholipase A_2 toward binary mixtures of phosphatidylcholine and phosphatidylethanolamine in mixed micelles with Triton. A surprising observation is that in these mixtures, the specificity of the enzyme is reversed, becoming phosphatidylethanolamine>phosphatidylcholine, and that the rate of hydrolysis of phosphatidylethanolamine is enhanced dramatically in the presence of small amounts of phosphatidylcholine. Two possible explanations for the observed reversal in the specificity of this enzyme are considered.

MATERIALS AND METHODS

Mixed micelles were prepared from an aqueous solution of Triton X-100 (Rohm and Haas) and dry samples of egg phosphatidylcholine prepared by the method of Singleton et al. (7), and/or phosphatidylethanolamine prepared by transesterification of egg phosphatidylcholine (Avanti Biochemicals), by brief mixing in a Potter-Elvehjem homogenizer. Final concentrations in the assay mixture were: 4 mM total phospholipid, 16 mM Triton X-100, 10 mM $CaCl_2$, and 25 mM Tris-HCl, pH 8.0. Samples (1.00 ml) were preincubated at 40° for 1 min in a shaking water bath and the reaction was initiated by the addition of 0.31 μg phospholipase A_2 prepared from lyophylized cobra venom, *Naja naja naja* (Pakistan) obtained from the Miami Serpentarium and purified as described elsewhere (4,5). The reaction was quenched after 2 min by the addition of 0.5 ml chloroform-methanol-acetic acid (1:2:0.5, v:v:v) and the phases were separated by the addition of 0.3 ml chloroform. Aliquots of the organic layer were spotted and the substrates and their hydrolysis products were separated by one dimensional thin-layer chromatography on Beckmann silica gel G thin-layer plates (20 x 20 x 0.25 cm) with chloroform-methanol-water (65:25:4, v:v:v) as the developing solvent. The lipids were visualized by iodine vapors and the substrate phospholipid(s) and lysophospholipid product(s) were scraped from the plates. Lipid phosphorus was determined by a modification (8) of the method described by Turner and Rouser (9). For phosphatidylethanolamine alone, the pH-stat procedure (10) was employed using the same assay conditions, except that the buffer was omitted and 3.1 μg of enzyme was utilized in a 2.00 ml assay. Phosphatidylcholine alone was run under the same conditions, and the results were normalized to those obtained by thin-layer chromatography. In all cases, the average of duplicate determinations and the average deviation are reported.

RESULTS

The specific activity of cobra venom phospholipase A_2 toward binary mixtures of phosphatidylethanolamine and phosphatidylcholine in mixed micelles, where the amount of each lipid present in the mixture was varied from 0 to 100 mol %, is presented in Table 1. A dramatic enhancement of the rate of phosphatidylethanolamine hydrolysis in the presence of as little as 5 mol % phosphatidylcholine occurs. At a 50-50 mixture of phosphatidylcholine and

TABLE 1: THE ACTIVITY OF PHOSPHOLIPASE A_2 TOWARD BINARY MIXTURES OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN MIXED MICELLES

Phosphatidylcholine Present (mol %)	Specific Activity (μmol min^{-1} mg^{-1})	
	Phosphatidylcholine	Phosphatidylethanolamine
0	--	46 ± 2[a,b]
5	a	355 ± 28
10	a	550 ± 37
50	320 ± 38	680 ± 20
90	680 ± 34	160 ± 20
100	810 ± 16	--

[a]Amount of lysophospholipid produced was too low to determine accurately by the experimental procedure utilized (less than about 75 μmol min^{-1} mg^{-1}).

[b]For this single phospholipid, pH-stat procedures were employed.

phosphatidylethanolamine, phosphatidylethanolamine was the preferred substrate, but at 90% phosphatidylcholine in the mixed micelle, phosphatidylcholine is hydrolyzed at rates comparable to this lipid alone in mixed micelles (100 mol % phosphatidylcholine) while the rate of hydrolysis for phosphatidylethanolamine has dropped significantly. In similar experiments with phospholipid mixtures in mixed micelles at other mol ratios of Triton/phospholipid and other total concentrations of phospholipid (M.F. Roberts, M. Adamich, and E.A. Dennis, unpublished experiments), phosphatidylethanolamine was consistantly the preferred substrate suggesting that the specificity reversal is not a peculiarity of the specific experimental conditions reported in Table 1.

DISCUSSION

The results presented here show that the specificity of phospholipase A_2 toward lipids in surfaces of mixed micelles, when more than one kind of lipid is present, is not simple and that the presence of the second lipid can alter the activity and substrate specificity of the enzyme toward both lipids. The implications of these findings for studies on the distribution of phospholipids in surfaces of natural membranes, where a variety of lipids are present, are far-reaching, and early studies of membrane lipid asymmetry utilizing these enzymes may be in need of further evaluation.

The mechanism by which the presence of phosphatidylcholine, in mixed micelles with phosphatidylethanolamine and Triton X-100, leads to a reversal in the apparent substrate specificity of the enzyme, and to an enhanced rate of phosphatidylethanolamine hydrolysis, can be explained in two ways:

1. Phosphatidylcholine may interact directly with phosphatidylethanolamine to cause changes in the conformation or steric arrangement at the lipid-water interface of the latter lipid rendering it a more suitable substrate for the enzyme. Such an interaction could involve a specific complex of phosphatidylcholine and phosphatidylethanolamine, or a clustering or patching of the phospholipids. Interactions between phosphatidylcholine and phosphatidylethanolamine (11) and cluster formation of phospholipids have been suggested to occur in bilayers containing mixtures of phospholipids (12).

2. Alternatively, the two different lipids may not interact directly and are randomly distributed in the mixed micelle surface. In this case, the phosphatidylcholine could exert its effect on phosphatidylethanolamine hydrolysis by a subtle alteration of the surface properties of the micelles or of the association of the enzyme with the interfacial phospholipid. An attractive possibility would be that the two lipids affect differently two separate steps in the enzymatic reaction. According to the "dual phospholipid" model proposed by Roberts _et al_. (3) for the mechanism of action of phospholipase A_2, monomeric enzyme first binds to a phospholipid molecule in the

lipid-water interface causing aggregation of the enzyme. In a second, distinct step, the Michaelis complex is formed with another phospholipid molecule and catalysis occurs. If phosphatidylcholine is preferred in the first step, but phosphatidylethanolamine works equally well if not better in the second step, the kinetic preferences shown in Table 1 would be explained.

Clearly, before a strong case can be made for the function of phosphatidylcholine as either a structural and/or a catalytic "primer" for phosphatidylethanolamine hydrolysis by phospholipase A_2, it will be necessary to investigate the effects of other lipids and more complex lipid mixtures on the action and substrate specificity of this enzyme.

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