

## SHORT COMMUNICATIONS

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## Phospholipase A activity of rat-liver mitochondria

Microsomal<sup>1,2</sup> and mitochondrial<sup>3,4</sup> fractions of several tissues have been demonstrated to acylate lysolecithin so as to form lecithin. A monoacyl-diacyl phosphoglyceride cycle may play a part in the fatty acid renewal of the phospholipids from these subcellular structures and in addition this process may be involved in the  $\text{Ca}^{2+}$ -induced swelling of mitochondria<sup>5</sup>. Apart from the fatty acid transacylases<sup>1,2</sup> such a cycle requires the participation of one or more phospholipases catalysing the hydrolysis of the fatty acid ester linkages. Although tissue homogenates are known to exhibit phospholipase A (EC 3.1.1.4) activity<sup>6</sup>, so far only indirect information suggested the presence of such an enzyme in mitochondria<sup>7</sup>.

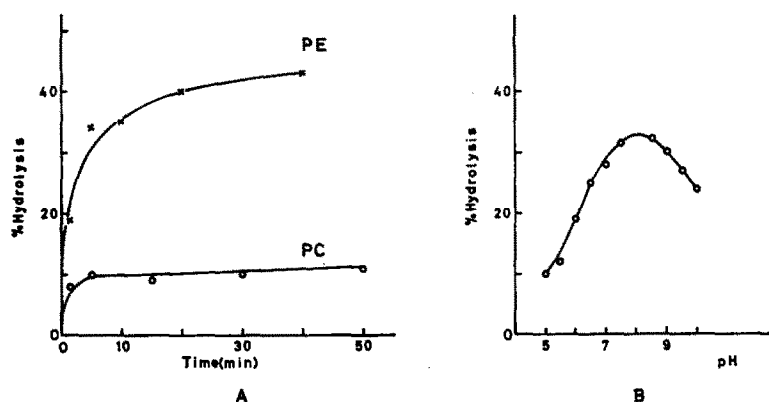


Fig. 1. (A) Enzymic hydrolysis of phosphatidylethanolamine (PE) and lecithin (PC) by mitochondrial suspensions. The  $^{32}\text{P}$ -labelled substrates were solubilized by ultrasonic irradiation, and incubated separately with a mitochondrial suspension of  $D_{550}$  0.60 in 5 ml of 0.125 M KCl-0.02 M Tris buffer (pH 7.4) ( $\text{Ca}^{2+}$ ,  $10^{-3}$  M) at  $25^\circ$ . Mitochondrial fractions were obtained from a 10% homogenate of rat liver in 0.25 M sucrose-0.02 M Tris buffer (pH 7.4), according to a slight modification of the method of SCHNEIDER<sup>8</sup>. The lipids were extracted from the incubation mixture as described by BLIGH AND DYER<sup>14</sup>. Hydrolysis was determined by measuring the radioactivity of the spots of intact phospholipid and lysoderivative on silica-impregnated paper chromatograms<sup>9</sup>. (B) The effect of pH on the hydrolysis of phosphatidylethanolamine by a solubilized phospholipase A preparation from rat-liver mitochondria. Methods and conditions were the same as in (A) except that, for incubations at low pH, use was made of a 0.02 M phosphate buffer. Incubation time, 30 min.

In the present study mitochondrial preparations from rat liver<sup>8</sup> were incubated with samples of pure  $^{32}\text{P}$ -labelled phosphatidylethanolamine and phosphatidylcholine prepared biosynthetically from rat liver<sup>6</sup>. Chromatography on silica-impregnated paper<sup>9</sup> and comparison with reliable reference substances showed the formation of lysoderivatives. Under the conditions used (Fig. 1) a further breakdown of the reaction products by lysophospholipase (EC 3.1.1.5) activity appeared to be limited. In order to ascertain that the conversion observed was brought about by a phospholipase A

and did not involve a transacylase, experiments were performed with substrates containing a  $^{14}\text{C}$ -labelled fatty acid constituent. Apart from lysoderivatives free fatty acids could be detected as hydrolysis products only.

Quantitative assays demonstrated that the phospholipase A present in the mitochondrial fraction more readily attacks phosphatidyl ethanolamine than the choline analogue (Fig. 1). Similar observations have been made on phospholipase A from pancreas<sup>10, 11</sup> but, in contrast to this enzyme, the phospholipase from the mitochondrial fraction was not significantly activated by the addition of deoxycholate. Recently it was reported that a phospholipase A from post-heparin plasma which is very active towards phosphatidylethanolamine does not act on lecithin<sup>12</sup>. It will be of interest to study, in more detail, the substrate specificity of the enzyme from the mitochondrial fraction and to verify whether the differences observed are not to be attributed to a different extent of re-acylation of the lysocompounds formed.

After ultrasonic vibration of mitochondrial suspensions and centrifugation for 30 min at  $130000\times g$ , the phospholipase activity was almost completely recovered in the supernatant fraction. As regards the properties of this enzymic preparation it can be noted that, after passage through a Sephadex G-25 column or dialysis against distilled water, the addition of  $\text{Ca}^{2+}$  gave only a slight activation. When compared with snake-venom phospholipase A, the enzyme studied is rather heat-labile. Treatment at  $80^\circ$  for 15 min reduced the activity to about 15%, but after heating at  $55^\circ$  for 15 min about 70% of the activity remained. The enzyme is active within a wide pH-range, having an optimum activity between pH 7 and 8 (Fig. 1). Below pH 6 the activity rapidly decreases, but at pH 9 a significant enzymic hydrolysis of phosphatidylethanolamine still could be observed. When compared with snake-venom and pancreatic tissue the phospholipase A capacity of the mitochondrial fraction from rat liver appears to be rather limited. The enzyme solubilized from the mitochondria of 300 mg of rat liver was found to hydrolyse about 0.1 mg of phosphatidylethanolamine.

It is worth noting that after incubation of the mitochondria for 1 h, which hydrolysed completely the labelled substrate added (in trace amount), a breakdown of the endogenous phospholipids was not detected. In support of this, no substantial formation of lysoderivatives was observed after incubation of rat-liver mitochondria for 1 h which had their phospholipids labelled following injection of radioactive phosphate into the living animal. Also, the solubilized phospholipase A preparation was found on chromatography with Sephadex to contain phospholipids *e.g.* lecithin and phosphatidylethanolamine, but whereas added substrates were hydrolysed no breakdown of the native constituents was detected by the methods used. Although the interference of a re-acylation reaction can not be ruled out completely, it seems most plausible that the endogenous phospholipids, both in the intact mitochondria and in the solubilized fraction, are present in the form of membraneous lipoproteins, and are not readily accessible to the phospholipase. Snake-venom phospholipase A is well known not to attack the phospholipids present in intact erythrocytes, and even after lysis erythrocytes of some (but not all) animal species appear to be not very susceptible to this enzyme<sup>13</sup>.

Quite recently Rossi *et al.*<sup>7</sup> reported that, during aging of rat-liver mitochondria, the phosphoglycerides were converted into lysoderivatives; while, after a period of 1 h, this degradation was not significant, a most pronounced breakdown was observed after aging for 24 h, at  $22^\circ$ . Although we were able to obtain similar results, these

experiments do not render conclusive evidence about the presence of phospholipase A activity in our opinion. We observed degradation of phosphoglycerides into lysoderivatives and water-soluble phosphodiesteres when mitochondria were stored for 22 h in 0.25 M sucrose at 25°, this treatment giving rise to a lowering of the pH from 7.0 to 4.7. However a breakdown was not apparent, or was greatly limited, when the incubation mixture was maintained at pH 7.4. Furthermore mitochondria previously heated for 15 min at 100° revealed a similar breakdown of their phospholipids only when the pH of the medium was permitted to reach a value of about 5 (*e.g.* because of bacterial contamination). Under similar conditions, incubation of isolated phospholipids gave rise to a non-enzymic breakdown as well. These results cast some doubt on the interpretation given by Rossi *et al.*<sup>7</sup>, although our experiments involving incubation for a short time with labelled substrates, indeed demonstrated the presence of phospholipase A activity in a mitochondrial fraction. However, further investigations are required to establish whether the phospholipase A studied originates from mitochondria or lysosomes, this forming a pre-requisite for the evaluation of the physiological significance of this enzyme.

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