SC 2337

The enzymic hydrolysis of ultrasonically irradiated lecithin by phospholipase A

It has long been known that aqueous emulsions of natural lecithin are attacked only very slowly by the phospholipase A (EC 3.1.1.4) of snake venoms. However, if the aqueous phase is saturated with diethyl ether the substrate is readily hydrolysed by the enzyme[1]. It has recently been shown that the probable explanation of this effect is that the ether penetrates into the lecithin particle dispersing it into smaller units as well as preventing orientation of the liberated fatty acids at the lipid–water interface[2,3]. The net result of these two effects is that a greater surface area of lecithin molecules is available for enzymic attack throughout the reaction.

The recent demonstration that permanent dispersions of lecithin sols could be obtained by ultrasonic irradiation[4] suggested that these might be suitable as substrates for phospholipase A in aqueous media without the necessity of adding ether.

A preparation of ovolecithin[3] (20 mg) was emulsified with 2 ml water and irradiated with ultrasound (Mullard 60 W) for 20 min in an ice bath. The almost clear solution was diluted with 2 ml 0.05 M collidine buffer (pH 6.5), and stored at 4° until required. The dispersion was stable, no turbidity appearing in the course of several days. Analysis by chromatography on silicic acid-impregnated paper[5] showed that no significant hydrolysis to lysolecithin and fatty acid had occurred even after 60 min irradiation. Cobra (*Naja naja*) venom was used as a source of enzyme, activity being measured by the decrease in fatty acyl ester bonds[3].

When lecithin emulsion which had not been ultrasonically disintegrated was used

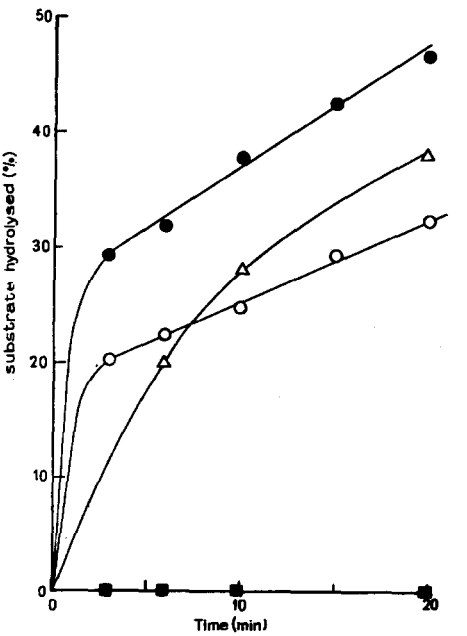

Fig. 1. Hydrolysis of ovolecithin by cobra venom phospholipase A. The incubation medium contains 2.5 μg venom, 40 μg of lecithin phosphorus, 0.5 mM $CaCl_2$ in 1 ml of 0.02 M collidine buffer (pH 6.5). ■—■, large lecithin particles; △—△, large lecithin particles plus 0.1 ml ether; ○—○, ultrasonically irradiated lecithin; ●—●, ultrasonically irradiated lecithin plus 0.1 ml ether.

as substrate little enzymic activity could be detected (Fig. 1). Ultrasonically irradiated lecithin on the other hand was hydrolysed by the enzyme, there being a rapid initial hydrolysis in the first few minutes followed by a slower but constant liberation of fatty acid (Fig. 1).

The addition of 10% (v/v) of ether stimulated the hydrolysis of the normal lecithin emulsions. It also activated the breakdown of the ultrasonically treated lecithin increasing both the extent of the initial rapid hydrolysis and also the slower constant rate of hydrolysis which follows (Fig. 1). In other experiments it was shown that ether had a similar effect on the hydrolysis of ultrasonically irradiated lecithin even when added after the initial rapid hydrolysis had taken place (Fig. 2).

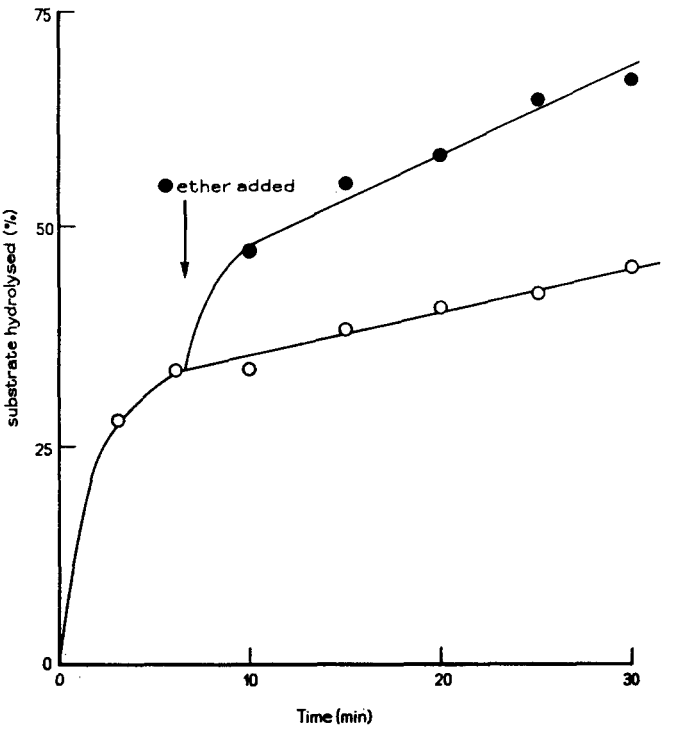

Fig. 2. Hydrolysis of ultrasonically irradiated lecithin by cobra venom. ●—●, 0.1 ml ether added after 6 min incubation. Other details as in Fig. 1.

The degree of dispersion of lecithin particles obtained by ultrasonic disintegration can also be obtained by the addition of certain anionic detergents. Thus on adding 0.25% sodium dodecylsulphate to the incubation medium the optical transmission of a lecithin emulsion is increased to about the same value as that obtained by ultrasonic irradiation. However, the hydrolysis by phospholipase A is not stimulated. The reason for this is illustrated in Fig. 3 which compares the degree of dispersion produced by the detergent and its inhibitory action on the hydrolysis of ultrasonically treated lecithin. It can be seen that inhibition of the enzyme occurs with low concentrations of sodium dodecylsulphate and is complete with 0.075% in the incubation medium.

The present observations and those of a previous investigation[3] emphasize that the essential physico-chemical requisite for substantial hydrolysis of a lecithin by

venom phospholipase A is not the surface ζ-potential of the substrate but an efficient contact between enzyme and the susceptible acyl ester bond which must be maintained as the reaction proceeds. The large particles of a normal lecithin emulsion will present only a small percentage of lecithin molecules at the lipid–water interface and the subsequent reaction will be blocked by the accumulation of liberated fatty acid at the interface. Electron-microscope photographs of ultrasonically treated lecithin (kindly taken by Mr. R. W. HORNE) reveal that the particles have been broken down into micromicelles and individual bimolecular leaflets of lecithin with a consequent enormous increase in the surface area available for enzymic attack. A similar effect is probably obtained by decreasing the chain length of synthetic lecithin. These so-called "soluble" lecithins are susceptible to phospholipase-A attack[6].

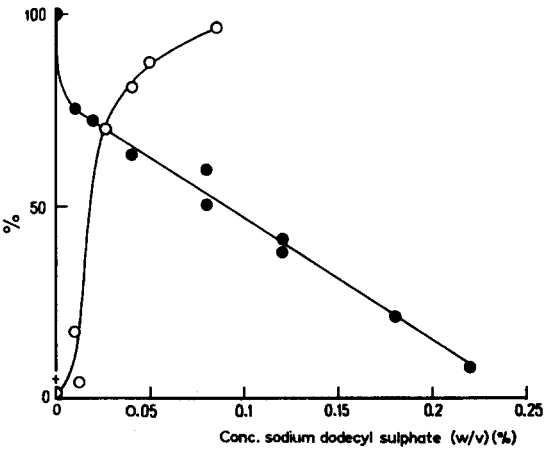

Fig. 3. The inhibitory effect of dodecylsulphate on the hydrolysis of ultrasonically irradiated lecithin and its clearing action on large lecithin particles. Incubation conditions as in Fig. 1, except $CaCl_2$ = 1 mM. O—O, % inhibition of hydrolysis of ultrasonically treated lecithin; ●—●, light absorption expressed as % of that of original lecithin emulsion; +, light absorption of ultrasonically treated lecithin expressed as % of that of original lecithin emulsion.

Ether penetrates into the lecithin particles causing a dispersion of the lecithin particles into smaller units. At the same time it promotes the replacement of the liberated fatty acids at the lipid–water interface with fresh substrate molecules[3]. Both these actions result in greatly increased contact between enzyme and substrate molecules so again a rapid hydrolysis occurs.

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Received July 1st, 1963