SC 2283

Conversion of lipoprotein-bound lecithin to lysolecithin induced by snake-venom phospholipase A

An increase in the rate of phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) induced splitting of lecithin bound to lipoprotein over that for purified lecithin has been reported recently by CONDREA *et al.*[1] from our laboratory. In the present study we have traced both the lysolecithin and the fatty acid, formed in human serum by the action of *Vipera palestinae* venom phospholipase A, in the serum protein fractions. Serum was incubated with purified phospholipase A (ref. 2) at 37° for 30 min. A sample of the same serum was incubated with saline under the same conditions at the same time. The β-lipoprotein fraction was separated by paperelectrophoresis (barbital buffer (pH 8.6), ionic strength 0.1, 3 V/cm) and extracted with methanol–chloroform. The extract was evaporated to dryness under N_2 and the residue was dissolved in distilled water. Following desalting (Reco electric desalter Model R 1500) and drying, the residue was dissolved in absolute ethanol, then in 96% ethanol, and then chromatographed on silicic acid-treated paper. Fig. 1 shows the disappearance of lecithin and the appearance of lysolecithin in the β-lipoprotein fraction following treatment of the serum with phospholipase A. The lysolecithin spot was identified by the addition of a minute amount of ^{32}P-labelled lysolecithin[3], prepared from the liver of white rats which had been injected subcutaneously with labelled orthophosphate[4]; the spots were counted on aluminium planchettes in a Geiger–Müller counter.

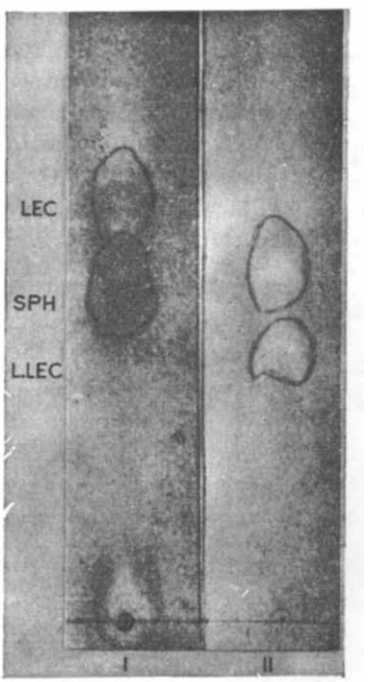

Fig. 1. Presence of lysolecithin in β-lipoprotein fraction. Silicic acid paper chromatography of chloroform–methanol extract of the electrophoretic β-lipoprotein fraction. I, untreated serum; II, phospholipase A-treated serum (L.LEC, lysolecithin; SPH, sphingomyelin; LEC, lecithin).

Lecithin and lysolecithin were estimated quantitatively on whole serum, in the β-lipoprotein fraction obtained by precipitation with dextran sulphate and in the α-lipoprotein-containing supernatant. Silicic acid paper chromatography of a chloroform–methanol extract was performed and the amount of phosphorus in the spots was determined. The amount of free fatty acid was estimated by the method of DOLE[6].

TABLE I

LECITHIN AND LYSOLECITHIN IN β-LIPOPROTEIN PRECIPITATE AND SUPERNATANT FROM SERUM TREATED WITH PHOSPHOLIPASE A

	Control serum		*Phospholipase-treated serum*	
	Lecithin (mg of P)*	*Lysolecithin (mg of P*)*	*Lecithin (mg of P*)*	*Lysolecithin (mg of P*)*
Whole serum	2.8	0.10	0	3.2
β-Lipoprotein precipitate	1.8	0.12	0	2.2
Supernatant	0.86	0.20	0	1.2

* The quantities of phosphorus relate to 100 ml of serum.

In each of six experiments performed, it was found that the amount of lysolecithin in the β-lipoprotein, precipitated from the phospholipase-treated serum, was equivalent to the amount of β-lipoprotein lecithin of the untreated serum. Similarly, the amount of lysolecithin in the supernatant from the phospholipase-treated serum was equivalent to the amount of lecithin in the supernatant of the untreated serum. An illustrative experiment is given in Table I and Fig. 2. Furthermore, the amounts of phospholipid phosphorus in the precipitate of β-lipoprotein and in the supernatant did not change by phospholipase treatment of the serum.

In the various experiments about 90 % of the unesterified fatty acids, liberated in the serum by phospholipase treatment, were found in the supernatant after precipitation with dextran sulphate. About 75–80 % of the total amount of unesterified fatty acid present in the phospholipase-treated serum was recovered in the albumin fraction, separated by precipitation with $(NH_4)_2SO_4$.

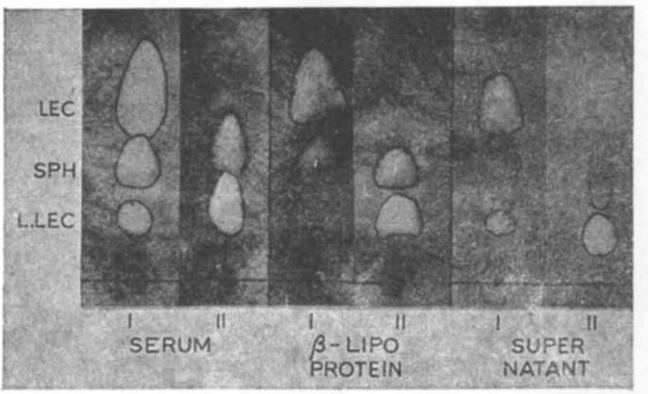

Fig. 2. Conversion of lecithin to lysolecithin. Silicic acid paper chromatography of chloroform–methanol extract of whole serum, β-lipoprotein fraction precipitated by dextran sulphate, and supernatant. I, untreated serum; II, phospholipase A-treated serum (L.LEC, lysolecithin; SPH, sphingomyelin; LEC, lecithin).

These findings show that the lysolecithin formed in phospholipase-treated serum from the lecithin of the β-lipoprotein remained a part of the lipoprotein molecule, whereas the bulk of the liberated fatty acids left the lipoprotein to become bound to the serum albumin. The same, presumably, holds true for the products of the α-lipoprotein-bound lecithin, although the presence of lysolecithin in the α-lipoprotein was not directly demonstrated.

In a previous report[1] the shift towards the anode of both the α- and the β-lipoprotein fractions, consequent upon treatment of the serum with phospholipase A, was thought to be due to their association with excess fatty acids. However, the finding of only a small part of the liberated fatty acid in the β-lipoprotein fraction prompts the consideration that the shift of this fraction towards the anode may be due to the lysolecithin still present in the molecule. Similarly, it is possible that the shift towards the anode of the α-lipoprotein band in the paperelectropherogram of phospholipase-treated serum is due to the formed lysolecithin remaining bound to the α-lipoprotein molecule. Indeed, addition of lysolecithin to serum (1 mg to 1 ml) caused a shift of both the α- and the β-lipoprotein fractions towards the anode.

This study was supported by a research grant (E-3171) from the National Institute of Health of the Public Health Service, Md. (U.S.A.).

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and The Labor Sickfund, Beilinson Hospital,
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Received March 4th, 1963

Biochim. Biophys. Acta, 70 (1963) 591–593

SC 2323

Effect of ethionine treatment on esterification *in vitro* of free [4-^{14}C]cholesterol by rat plasma

The administration of ethionine rapidly produces a fatty liver in the fasted, female rat[1,2]. The pronounced increase in liver lipids is confined to the triglycerides[3–5] and is associated with a fall in triglycerides, phospholipids and cholesterol of plasma[4–6]. Ethionine treatment has been shown to depress the incorporation of radioactive amino acids into liver proteins[7–9] and plasma lipoproteins[9]. It has been suggested that an interference with formation of plasma lipoproteins in liver is the cause of both the fatty liver and the fall in plasma lipids[6,9]. Such a mechanism can account for the accumulation of glycerides in the liver (formed from free fatty acids mobilized from adipose tissue) and for the decrease of all lipids associated with plasma lipoproteins. But it does not account for the observation that the concentration of cholesterol esters in plasma is lowered to a much greater extent by ethionine treatment than are the concentrations of free cholesterol, triglycerides and phospholipids[5].