ISOLATION OF RAT BRAIN LECITHINASE-A, SPECIFIC FOR THE α'-POSITION OF LECITHIN

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It is well established that snake venom or bacterial phospholipase A removes the fatty acids from the β-position of lecithin, but does not attack the α'-position. The fatty acids in the β-position of most lecithins are predominantly unsaturated, while those in the α'-position are saturated (Reviewed by Van Deenen and DeHaas, 1964 and Lands, 1965). Van Den Bosch et al. (1965) and Van Den Bosch and Van Deenen (1965) reported the presence, in rat liver and pancreas, of lecithinases which hydrolyze both the α' and β-position of lecithin. However, these enzymes were not separated from each other. Very recently, Vogel and Bierman (1966) have reported that post heparin human serum has a lecithinase specific for the α'-position. This hydrolytic activity had a pH optimum of 9.5 and required taurocholate. The enzyme was not purified.

The present communication reports the extraction and partial purification of a lecithinase A from rat brain. The enzyme hydrolyzed only the α'-position of lecithin, releasing saturated fatty acids and had but negligible lysolecithinase activity.

The purification of the enzyme followed the procedure employed for the isolation of β-galactosidase from rat brain (Gatt and Rapport, 1966). Rat brain was homogenized

with 9 volumes of 0.25M sucrose-10^{-3}M EDTA, and particles, which sedimented between 800 and 20,000 xg, were collected. These were suspended in sucrose-EDTA, subjected to sonic disintegration for 3-8 min at 10 kc and recentrifuged at 20,000 xg. The supernatant fluid was discarded and the precipitate was extracted with 0.5% sodium cholate in sucrose-EDTA. Cholate was removed by dialysis against 0.02M Tris buffer, pH 7.4 and the dialyzate was kept at -20°. This preparation was then dialyzed for 2 hours against 0.05M acetate buffer pH 5 and the precipitate which formed was discarded. The supernatant thus obtained had a specific activity 35 times that of the homogenate, using rat liver lecithin as substrate. Hydrolysis of lecithin by this enzyme was proportional to enzyme concentration, had a pH optimum of 4.0, a Km of 8×10^{-4}M and Vmax of 0.7 μmoles/mg protein/hour. The reaction required Triton X-100 and was inhibited by the addition of sodium cholate. Lysolecithin (prepared by hydrolysis of rat liver lecithin with Crotalus toxin) was split at less that 1% of the rate of hydrolysis of lecithin.

Evidence for the positional specificity of the enzyme

1. Egg lecithin was dispersed in Triton X-100 (1.5 mg/ml of reaction mixture), acetate buffer pH 4.2 and enzyme were added and the mixture was incubated at 37°. Fatty acids were isolated by the method of Dole (1965) and were methylated with distilled diazo methane in ether. Gas liquid chromatography on polyethylene glycol succinate showed that 95% of the fatty acids released were saturated (Table I). For comparison, data are also presented in Table I on the fatty acids released by snake venom - these had 97% of unsaturated fatty acids. With less purified preparations of rat brain lecithinase a somewhat higher proportion of unsaturated fatty acid was observed. This indicates that brain tissue also has a lecithinase which hydrolyzes the β-position of lecithin; this, apparently, is removed during the purification procedure. Evidence for the presence of the two lecithinases in brain has been presented by Robertson and Lands (1962).

Table I

Composition of Fatty Acid Hydrolyzed off Lecithin

Fatty Acid	Enzyme	
	Rat Brain Lecithinase	Crotalus Toxin
	%	%
Palmitic	72	2.5
Palmitoleic	--	1.2
Stearic	23	---
Oleic	4	71.2
Linoleic	1	25

2. Lecithin labeled with both ^{32}P and tritiated oleic acid in the β-position was synthesized from ^{32}P-lysolecithin and 9,10-di^{3}H-oleic acid, using rat liver microsomes and supernatant (modification of Robertson and Lands, 1962). It was subjected to hydrolysis by the rat brain lecithinase and by Crotalus toxin (Long and Penny, 1957). The reaction mixtures were extracted according to Folch et al. (1957) and the lower, chloroform-rich, phases were chromatographed on silicic acid columns. Fatty acids were eluted with chloroform-methanol 95:5 and the unhydrolyzed lecithin with chloroform-methanol 55:45. Lysolecithin was eluted with methanol and re-chromatographed on thin layer silica gel plates in chloroform-methanol-water 70:25:4. The silicic acid was scraped off and extracted with mixtures of chloroform-methanol and with methanol and counted in a Packard Tri-Carb liquid scintillation counter. The ratios of cpm of tritium to ^{32}P in substrate lecithin and in the lysolecithins obtained are summarized in Table II.

Table II

Ratios of $^{3}H/^{32}P$ in Lecithin and Lysolecithins

Compound	$^{3}H/^{32}P$
Lecithin (used as substrate)	2.10
Lysolecithin (obtained by hydrolysis with rat brain lecithinase)	1.95
Lysolecithin (obtained by hydrolysis with Crotalus toxin)	0.01

These experiment establish that the specificity of the rat brain lecithinase differs from that of Crotalus toxin. As the latter attacks the β-position (Van Deenen and DeHaas, 1964), the brain enzyme hydrolyzes the α'-position of lecithin.

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