ON THE SPECIFICITY OF PHOSPHOLIPASE A

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Phospholipase A (lecithinase A) from snake venom is well known for its ability to split off one of the two fatty acid radicals from lecithins with formation of lysolecithins. As to the mode of action of lecithinase A Hanahan (1954) found that this enzyme specifically attacks the γ-ester position in lecithin, which fact was independently confirmed by Long and Penny (1954). Recently, however, this specificity of lecithinase has been doubted. Marinetti (1960) assumes specificity to be connected with chain length as well such that also certain β-linked fatty acids can be liberated by snake venom phospholipase A. Tattrie (1959) even expresses the opinion that the site of hydrolysis is exclusively at the β-position of the lecithins. In the above studies mostly natural lecithins have been used and the structure of the resulting lysolecithins investigated. In order to avoid the difficulties involved in the elucidation of the structure of the enzyme-formed lysolecithins we tried a different approach.

The synthesis was undertaken of several optically pure L-α-lecithins with two dissimilar fatty acids, each having a known position. Analysis of the fatty acids, liberated from these lecithins by lecithinase A, has furnished new information about the site of attack of the enzyme on the lecithins investigated.

EXPERIMENTAL

The total synthesis of "mixed-acid" α-lecithins has already been described (de Haas and van Deenen, 1960). Meanwhile this synthesis has been modified such that optically pure α-lecithins including those with one unsaturated fatty acid have become accessible (de Haas and van Deenen, 1960[a]).

The synthetic lecithins were enzymatically degradated with lecithinase A from Crotalus adamanteus (Hans Psenner, Schlangenfarm, Innsbruck). A weighed amount (about 100 mg) of lecithin, dissolved in 1 ml of chloroform, was diluted with 100 ml of ether. To this solution was added an aqueous emulsion of 1 mg of venom in 1 ml 5×10^{-3} M $CaCl_2$ solution. The incubation was carried out at 30° for 18 hrs. The precipitated lysolecithins were centrifuged and the liberated fatty acids present in the supernatant were analysed by gas-liquid chromatography (Kögl et al. 1960). Quantitative measurement of the separated fatty acid methyl esters was made possible by introducing into each sample (before incubating with the lecithinase) a weighed amount of pure pentadecanoic or eicosanoic acid as an internal standard.

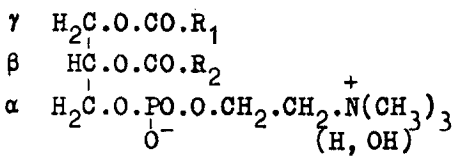
RESULTS

Table I presents the results of the enzymatic degradation of the synthetic lecithins with two dissimilar fatty acids. From the two structural isomers γ-stearoyl-β-lauroyl-α-lecithin (I) and γ-lauroyl-β-stearoyl-α-lecithin, both obtained by total synthesis, only one fatty acid was released, viz. exclusively the β-attached one. Also the fully synthetic γ-stearoyl-β-oleoyl-α-lecithin (III) yielded exclusively the β-attached oleic acid.

On account of these results we were led to the assumption that lecithinase A only attacks the acid present at the β-

Table I

Degradation of "mixed-acid" α-lecithins with phospholipase A from rattle snake venom



Compound	Configuration	Fatty acid position R_1 (γ)	R_2 (β)	Fatty acids liberated by lecithinase A R_1 (γ)	R_2 (β)
I	L	Stearic	Lauric	<1% Stearic	112% Lauric
II	DL	Lauric	Stearic	<1% Lauric	53% Stearic
III	L	Stearic	Oleic	0% Stearic	95% Oleic
IV	L	Oleic	Stearic	0% Oleic	90% Stearic
V	L	Stearic	Oleic	3% Stearic	68% Oleic*)
VI	D	Stearic	Oleic	0% Stearic	0% Oleic

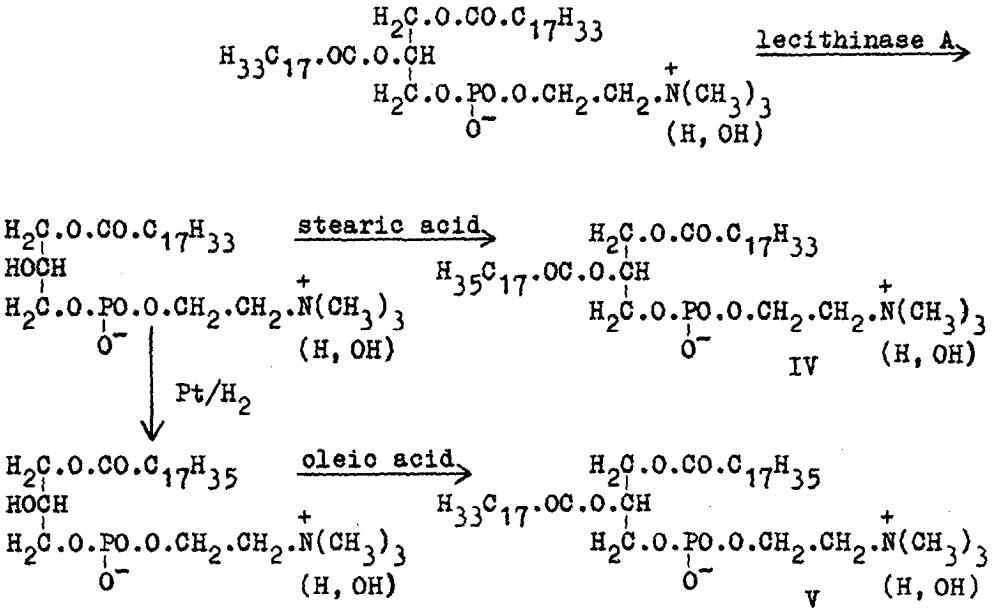
*) Since the used oleic acid in this case was contaminated with palmitic acid, also 13% of this fatty acid was recovered.

position. Although no chain length preference of phospholipase A seems to exist for saturated fatty acids, the present experiments did not preclude the possibility of a preferential hydrolysis of unsaturated fatty acids. To gain further evidence an indirect synthesis was carried out of two isomeric "mixed-acid" lecithins containing a saturated and an unsaturated fatty acid. Scheme I indicates the pathway of this partial synthesis, details of which will be published elsewhere (de Haas and van Deenen 1960[b]).

The results of lecithinase A hydrolysis of these compounds are given in table I. The isomeric lecithins IV and V yielded different fatty acids. From this it is clear that a preferential hydrolysis, either of the saturated or of the unsaturated fatty acid, does not occur, at least not in the case that both acids have the same chain length. In our experiments with "mixed-acid" α-lecithins we have observed that the L-

Scheme I

Indirect synthesis of optically pure "mixed-acid" L-α-lecithins



isomers (table I, no.I, III, IV, V) are completely hydrolysed to lysolecithins. Contrary to this the synthetic D-enantiomers resisted lecithinase A degradation completely (table I, no.VI). Consequently it was found that with the DL-compounds (table I, no.II) hydrolysis did not surpass 50 %. In addition it was found that β-lecithins (with the phosphoryl-nitrogenous moiety attached to the β-carbon atom of glycerol) could not be split with snake venom. On account of these facts and of the exclusive action of lecithinase A on the β-attached fatty acid, it is clear that the enzyme is highly stereo- and positional-specific. Experiments are in progress for a further elucidation of the mechanism of this enzyme-substrate interaction.

Although the contradictory views regarding the site of action of lecithinase A may be explained by possible isomerizations in the enzyme-formed lysolecithins, the hetero-

geneity of the various snake venom preparations cannot be precluded. As a consequence of a β-specificity of lecithinase A it seems unavoidable to revise some existing views on the structure of the plasmalogens.

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ADDENDUM

Shortly after the submittance of our paper we became acquainted with a recent publication of Hanahan et al. [J.Biol. Chem. 235, 1917 (1960)]. On account of the results obtained with lipase as a specific reagent for release of α-bound fatty acids from diglycerides derived from natural lecithins (compare Tattrie, J. Lip.Research 1, 60, 1959), the authors conclude contrary to the former statements of Hanahan that the site of attack of lecithinase A is exclusively at the β-ester position of lecithins. Evidently this agrees with our conclusive experiments on well-defined "mixed-acid" lecithins obtained by total synthesis.