ACTION OF SNAKE VENOM PHOSPHOLIPASE A_2 ON ANALOGS OF PHOSPHATIDYLCHOLINE

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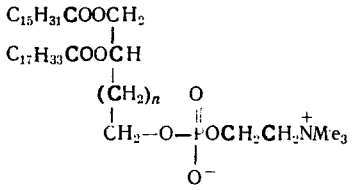
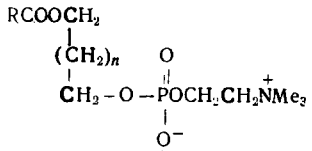
UDC 577.153

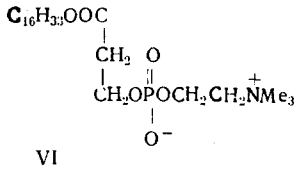
Phospholipase A_2 is an enzyme widely distributed in nature which selectively hydrolyzes phosphatidylcholine (PC) (I) with the liberation of the acid occupying position 2. It has been shown previously that of the diol analogs of PC (III-V) only the ethylene glycol analog (III) is hydrolyzed by phospholipase A_2 at an appreciable rate, while other analogs undergo enzymolysis only to an insignificant degree [1-3].



I, $n = 0$
II, $n = 2$



III, $n = 0$, $R = C_{17}H_{35}$
IV, $n = 2$, $R = C_{17}H_{35}$
V, $n = 3$, $R = C_{17}H_{33}$



VI

The low rate of enzymatic hydrolysis of diol phospholipids can be explained by several factors. In the first place, in the diol analogs of PC the fatty acid is bound to a primary hydroxyl, while in PC it is split off from a secondary hydroxyl. In the second place, PC contains, in addition to the ester group attacked, a second ester group unaffected by the enzyme but, possibly, activating the first one. In the third place, it is known that the presence of only one fatty-acid residue in the molecule of a diol phospholipid changes its physical properties, including the capacity for being included in micelles and the specific area on the surface of a micelle, which is important for the course of enzymatic hydrolysis [3, 4].

In order to investigate how the capacity of a phospholipid for being hydrolyzed by phospholipase A_2 depends on the distance between the phosphate and ester groups when two ester groups are present in its molecule, we have subjected to hydrolysis by snake venom phospholipase A_2 a pentanetriol analog of PC – rac-4-oleoyloxy-5-palmitoyloxypentylphosphorylcholine (II) – in which the phosphate group is separated from the closest ester group by two additional methylene links as compared with PC. In addition, synthetic PC – 2-oleoyl-1-palmitoyl-3-sn-glycerophosphorylcholine (I) – and also the butanediol (IV) and pentanediol (V) analogs of PC and another analog (VI) in which the diglyceride part has been replaced by the residue of β-hydroxypropionic acid esterfied with cetyl alcohol has been subjected to enzymatic hydrolysis.

As can be seen from the results given (Table 1), the hydroxypropionyl analog of PC (VI) proved to be completely stable to the action of phospholipase A_2, and the diol analogs (IV) and (V) were cleaved only slightly. At the same time, the pentanetriol analog of PC (II) was cleaved by the enzyme at an appreciable

M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 413-415, May-June, 1975. Original article submitted April 11, 1974.

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TABLE 1. Cleavage of Phospholipids by Phospholipase A_2

Phospholipid	Reaction Time	% of fatty acid split off (means of three experiments)
Phosphatidylcholine (I)	7 min	100 (oleic)
Diol analogs of PC		
butanediol analog (IV)	20 h	3
pentanediol analog (V)		1
Pentanetriol analog of PC (II)		11 (palmitic)
Hydroxypropionyl analog of PC (VI)		2 (oleic)
		0

rate, although considerably more slowly than PC itself. In this process, the acid bound to the primary hydroxyl (palmitic) was split off to a greater degree than the acid esterifying the secondary hydroxy group (oleic), while in the case of PC it is known that only the acid substituting the secondary hydroxyl is split off. These differences cannot be ascribed to the dissimilar natures of the fatty-acid residues, since it is known that in the case of PC a change within small limits of the length and degree of unsaturation of the fatty acids scarcely affects the rate of their splitting off by phospholipase A_2 [1].

Apparently, an important condition for the effective action of phospholipase A_2 is the vicinal position of the phosphate and ester groups: Under conditions close to those described in the present paper the ethylene glycol analog of PC (III) is more than 40 % hydrolyzed [2]. When, however, the ester group is remote from the phosphate group, the splitting off of the fatty acid residue from a primary hydroxyl takes place more readily than from a secondary, probably because of the smaller steric hindrance. The higher rate of enzymatic hydrolysis of the pentanetriol analog of PC (II) as compared with the diol analogs (V) and (IV) can be explained by a difference in the physical properties of the micelles which they form and, in the first place, by the different specific areas occupied by the corresponding molecules at the surface of separation of the phases. It has been shown by van Deenen et al. [4] that hydrolysis by phospholipase A_2 (pancreatic) takes place fastest where the PC molecule occupies an area of about 90 $Å^2$, which corresponds to a PC with two caprylic acid residues. It is obvious that the pentanetriol analog (II) occupies an area on the surface of the micelles which is closer to the optimum than the diol analogs (IV) and (V).

EXPERIMENTAL METHOD

The solvents were purified by standard methods. For thin-layer chromatography (TLC) we used type KSK silica gel with 5 % of gypsum. The pentanetriol (II) and hyroxypropionyl (VI) analogs of PC, and also the diol analogs (IV) and (V) were synthesized as described previously [5].

As the preparation of phospholipase A_2 (EC 3.1.1.4) we used lyophilized venom of the Central Asian cobra (Naja naja oxiana). A mixture of 1 mg of phospholipid (the accurate amount was calculated from the phosphorus content [6]), 0.7 ml of borate buffer (pH 7.5), 1 ml of ether, and 0.2 ml of a 0.8 % aqueous solution of sodium deoxycholate was emulsified for 5 min with ultrasound (22 kHz, intensity 50 W) with ice cooling. To the resulting emulsion was added 0.2 ml of a 0.005 M solution of $CaCl_2$ and 1 mg of lyophilized cobra venom, and the mixture was incubated with shaking in a thermostated space (28° C). After the end of the reaction, a solution of the standard for GLC (margaric acid) was added to the mixture, it was evaporated to dryness in vacuum at 20° C, 1 ml of a mixture of chloroform and methanol (1 : 1) was added, and it was treated with an ethereal solution of diazomethane and evaporated. The residue was separated by preparative TLC in benzene (zones visible in UV light after treatment with morin). The zones containing the methyl esters of the fatty acids were identified by comparison with the standard (methyl margarate) and were separated off and eluted with chloroform. In the epxeriments with the hydroxypropionyl analog of PC (VI), a solution of myristyl alcohol was used as standard, the mixture was evaporated to dryness, and the residue was separated by preparative TLC in the benzene–ethyl acetate (3 : 1) system, (the spots being revealed with morin). The zone containing the fatty alcohols was eluted with chloroform containing 5 % of methanol, the eluate was evaporated to dryness, and the fatty acids were converted into their trimethylsilyl derivatives by treatment with chlorotrimethylsilane and hexamethyldisilazane in pyridine [7]. The amount of fatty acids (alcohols) was determined by GLC.

SUMMARY

The hydrolysis by phospholipase A_2 of the following synthetic phospholipids has been investigated: 2-oleoyl-1-palmitoyl-3-sn-glycerophosphorylcholine, 4-stearoyloxybutylphosphorylcholine, 4-oleoyloxy-5-palmitoyloxypentylphosphorylcholine, and 2-(hexadecyloxycarbonyl)ethylphosphorylcholine.

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