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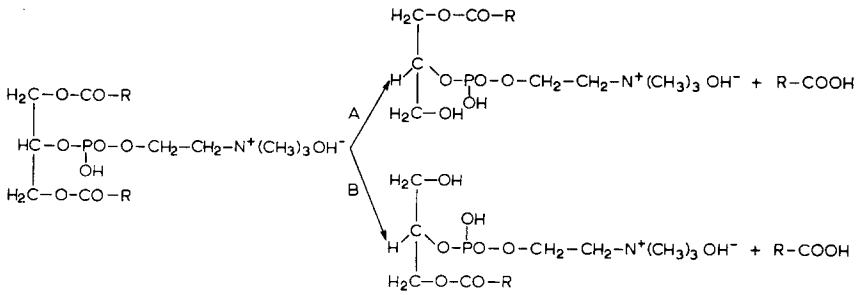
## The site of action of phospholipase A on β-lecithins

Recently we reported[1,2] on the action of phospholipase A (phosphatide acyl-hydrolase EC 3.1.1.4) on so-termed β-lecithins (1,3-diacyl-glycerol-2-phosphorylcholine). This enzyme was shown to catalyse in a stereospecific way the hydrolysis of only one fatty acid from a symmetric β-lecithin. As indicated in Scheme 1, such an asymmetric degradation may occur in two ways (either Pathway A or B).

Although on theoretical grounds Pathway A was believed to function, any definite conclusion about the site of attack of the enzyme could not be reached. Further experiments on a racemic 1,3-diacyl-glycerol-2-phosphorylcholine composed with palmitic and oleic acids confirmed that the enzyme did not possess a preference for a certain fatty acid species. In this case the isolated optically active lysolecithin as well as the freed fatty acids appeared to contain equimolar amounts of both fatty acids, indicating that the one had been released from the D-enantiomer and the other from the L-isomer. The configuration of the enzymically formed lysolecithin might be determined after its conversion into a monoglyceride through the action of phospholipase C (phosphatidylcholine cholinephosphohydrolase, EC 3.1.4.3).

Since it has been reported that this enzyme from *Clostridium welchii* is rather inactive towards lyso-derivatives[3,4], preference was given to a synthesis of a mixed-acid 1,3-diacyl-glycerol-2-phosphorylcholine of defined stereochemical configuration. Determination of the nature of the fatty acid liberated by phospholipase A would elucidate the site of attack of the enzyme. When this synthetic work was in progress, studies in our laboratory revealed, however, that phospholipase C from *Bacillus cereus*

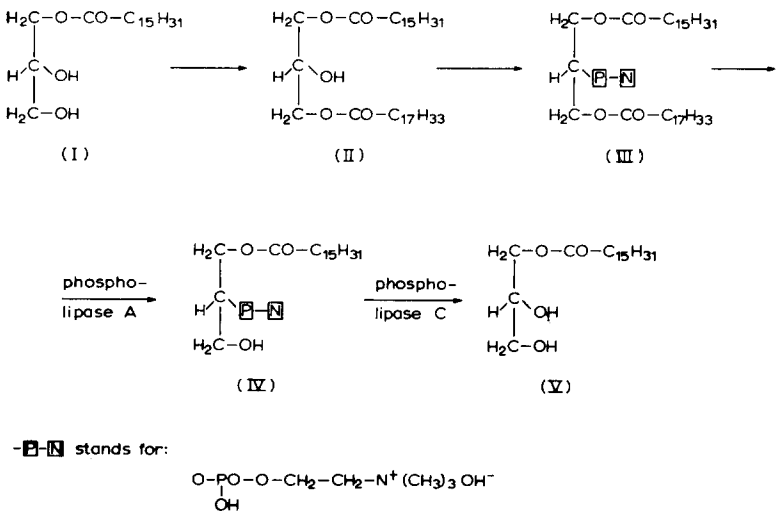


Scheme 1. Theoretical pathways for the asymmetric degradation of 1,3-diacyl-glycerol-2-phosphorylcholine by phospholipase A.

was fairly active towards lysolecithins. Thus it became possible to prove the site of attack of the enzyme by means of two different methods as indicated by the sequence of reactions given in Scheme 2.

L-$\alpha$-Monopalmitin, or according to HIRSCHMANN[5] 3-palmitoylglycerol (I, $[\alpha]_D^{20} = -4.30°$, $c$ 8 in pyridine), was monoacylated with oleoylchloride yielding a mixture of the isomeric 1,3 (II) and 1,2 diglycerides. Purification of II was accomplished by silicachromatography and low-temperature crystallization. The phosphorylcholine moiety was introduced according to the method of HIRT AND BERCHTOLD[6] yielding the optically pure mixed-acid 2-lecithin (III, $[\alpha]_D^{20} = +0.0°$, $c$ 10 in chloroform). Phospholipase C hydrolysis of III quantitatively yielded the 1,3 diglyceride II indicating that the conversion of II into III had proceeded without isomerization.

Hydrolysis of III with phospholipase A (*Crotalus adamanteus*) both in an aqueous and in an ethereal medium resulted in a quantitative degradation of the 2-lecithin with the formation of a 3-acyl-glycerol-2-phosphorylcholine (IV, $[\alpha]_D^{20} = +6.30°$, $c$ 9



Scheme 2. Synthesis and biochemical degradation of 1-oleoyl-3-palmitoyl-glycerol-2-phosphorylcholine (III).

in chloroform–methanol, 9:1, v/v)* composed with palmitic acid only. The enzymically released fatty acids were found to consist almost exclusively of oleic acid. Adhering to the nomenclature of HIRSCHMANN[5] we can conclude that the enzyme exclusively catalyses the hydrolysis of the glycerol-C-1-attached fatty acid linkage from 1,3-diacyl-glycerol-2-phosphorylcholine derivatives.

Finally the structure of the lysolecithin IV obtained was confirmed by degradation with phospholipase C from *B. cereus*. The reactionproduct (V, $[\alpha]_D^{20} = -4.27°$, *c* 8 in pyridine) which was isolated in a yield of about 90% of the theoretical, turned out to be identical to L-α-monopalmitin (I), or 3-palmitoylglycerol.

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## Occurrence and localization of α-linolenic acid containing galactolipids in the photosynthetic apparatus of *Anabaena variabilis*

In many photosynthetic microorganisms and in the green tissues of higher plants, α-linolenic acid is a major constituent of the chloroplast lipids[1,2]. Photosynthetic bacteria, which have a more simply organized photosynthetic apparatus and a primitive type of photosynthesis[3,4] do not contain α-linolenic or other polyenoic acids. The blue-green algae are related morphologically to the photosynthetic bacteria, but functionally and biochemically these algae resemble the higher plants; they evolve oxygen during photosynthesis, and contain α-linolenic acid. These facts have led to the suggestion that α-linolenic acid is required for the operaztion of one or more of the steps leading to the production of oxygen during green plant photosynthesis[5]. Further support for this suggestion is provided by the present report on the intracellular localization of α-linolenic acid in the blue-green alga, *Anabaena variabilis*, and on the occurrence of α-linolenate in the galactolipids of this organism.

* The optical rotations of lysolecithins were found to vary considerably with the solvent system used. The previously reported value[2] of +1.45° has now been shown to be a wrongly interpreted reading.