STERIC ANALYSIS OF GLYCEROPHOSPHOLIPIDS BY CIRCULAR DICHROISM. STEREOSPECIFITY OF PHOSPHOLIPASE D CATALYZED TRANSESTERIFICATION

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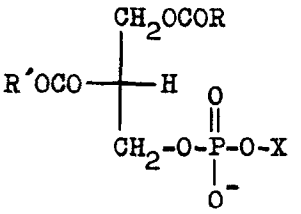
Received May 31,1975

SUMMARY: The circular dichroism spectra of natural glycerophospholipids and synthetic 1-sn-phosphatidic acid were recorded. 3-sn-phosphatidic acid derivatives were found to show a positive Cotton effect, while 1-sn-phosphatidic acid revealed a negative Cotton effect. The results are interpreted in terms of the carboxyl sector rule. By this method phospholipase D was shown to produce stereospecifically 3-sn-phosphatidyl-1-sn-glycerol when incubated with egg yolk lecithin and exess of glycerol.

INTRODUCTION

An important problem in the structural analysis of the glycerophospholipids is the determination of the configuration of the asymmetric carbon (C_2) of the glycerol residue.



I R,R′= alkyl

Usually natural glycerophospholipids are derivatives of

3-sn-glycerophosphate; however certain exeptions are known. Thus from the extreme halophyl Halobacterium cutirubrum 2,3-di-O-phytanyl-sn-glyceryl-1-phosphoglycerol phosphate was isolated [1]. Other lipids containing the unusual sn-glycerol-1-phosphate unit are glycerophosphoryl diglucosyl diglyceride from Streptococci [2] and lyso-bis-phosphatidic acid of cultured BHK-cells [3]. Acylated phosphatidyl glycerols, which may be regarded as derivatives of 1-sn-glycerophosphate [4-8] have been found in a number of other natural sources.

There are two methods for the determination of the absolute configuration of the phosphatidic acid moiety of a glycerophospholipid. One of them involves hydrolysis of the glycerophospholipid by phospholipase A_2. This enzyme which is known for its high steric specifity, splits only those phospholipids which are derivatives of 3-sn-phosphatidic acid but not derivatives of its antipode [9]. The second method is based on enzymatic oxidation of the glycerophosphate obtained by phospholipase D treatment of the phospholipid under investigation and subsequent chemical hydrolysis. When the oxidation is carried out by L-α-glycerophosphate dehydrogenase in the presence of NAD it will afford dihydroxy acetone only in the case of 3-sn-glycerophosphate while the 1-sn-derivative will remain unaffected [10].

Both methods suffer from several drawbacks. They are time consuming and require enzymes which not always are readily available. The lipid under investigation has to be degraded and consequently cannot be used in further investigation. Besides this, not every phospholipid is easily hydrolyzed by phospholipase A_2 [11].

The present communication describes a new method for the

steric analysis of glycerophospholipids which is based on circular dichroism measurements.

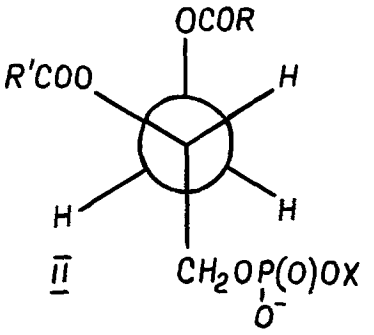
METHODS

Circular dichroism spectra were measured at 25° with a Cary-60 spectropolarimeter equipped with a CD-6002 attachment. Optical path of cells was 0.01-1.0 cm. For calculation of $[\theta]_{max}$ values (expressed in degree.cm^2/decimole) of the lipid fractions under investigation the "average" molecular weight of the latter was taken into account.

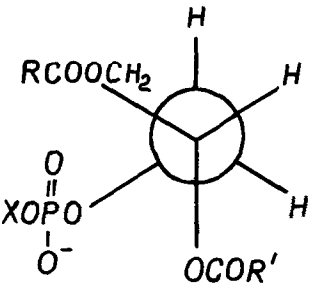
Phospholipase D was obtained from fresh cabbage according to Yang [12]. Enzymatic hydrolysis and transesterification of egg yolk lecithin were carried out as described by Dawson [13,14].

RESULTS AND DISCUSSION

A study of the UV-spectra of a number of glycerophospholipids revealed them to show a selective adsorption near 212 nm, which must be ascribed to the $n \longrightarrow \pi^*$ transition of the ester carbonyls. Since one of these two chromophores is situated in the nearest neighborhood of the asymmetric center of the glycerol moiety lipids of type I should display a Cotton effect in the same wavelength region. NMR studies of dipalmitoyl-lecithin [15,16] have demonstrated that in polar (MeOH) or low-polar ($CHCl_3$) organic solvents the fatty acid chains are arranged gauche to each other (II) and that the phosphate group is predominantly gauche to the C_2-acyl chain (III). The close structural relationship between phosphatidyl



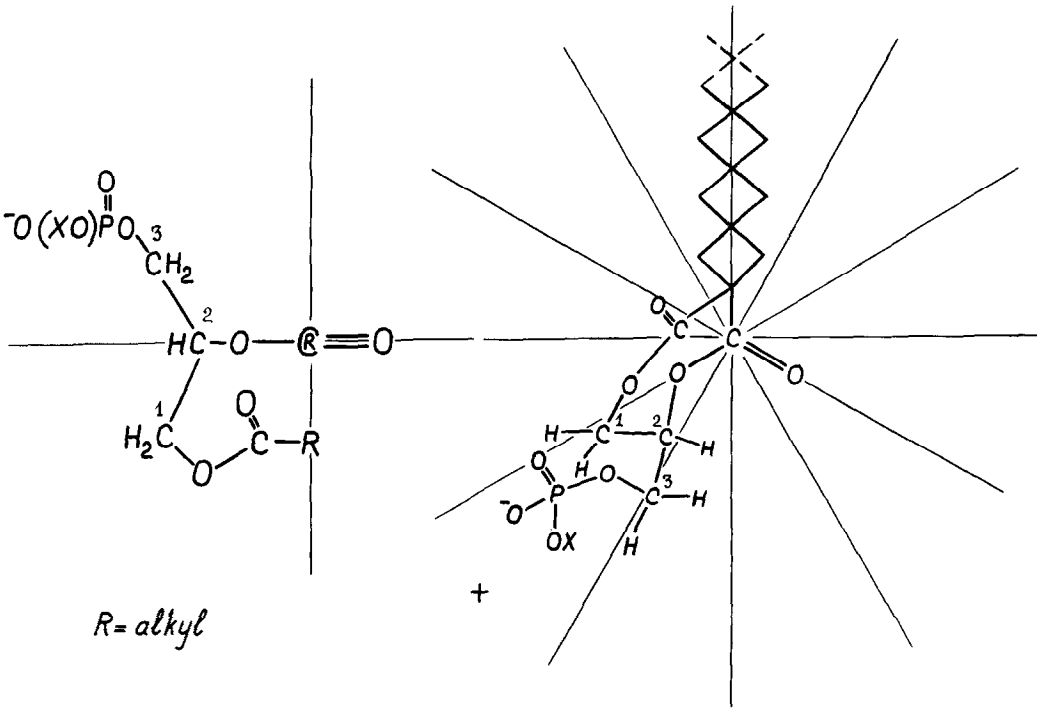
II



III

choline and the other glycerophospholipids makes it likely, that they should contain high populations of the same rotamers. If this be so, application of the carboxyl sector rule [17] leads to the conclusion that those glycerophospholipids which are derivatives of 3-sn-phosphatidic acid should reveal a positive Cotton effect (see Scheme).

Circular dichroism measurements carried out with a number



of natural glycerophospholipids showed them indeed to have a positive Cotton effect at 214-226 nm (Table). The 3-sn-configuration of all these phospholipids was confirmed by phospholipase A_2 hydrolysis [11] using Naja naja oxiana venom. The observed differences in the Cotton effect values are presumably caused by variances in the fatty acid composition and in the structure of the polar head groups. As follows from experiments with egg yolk lecithin and bovine spinal cord phosphatidyl ethanolamine the Cotton effect values are practically independent of the concentration of the lipid solution over the range 0.001-0.1 mmole/ml.

To compare the optical properties of 3-sn-phosphatidic acid with those of its enantiomer, 1-sn-phosphatidic acid was obtained in an optically pure form by chemical synthesis. As anticipated the synthetic specimen showed a strong negative Cotton effect (Table).

The results described suggest a new method for the steric analysis of glycerophospholipids based on measurements of their circular dichroism. A positive Cotton effect of the glycerophospholipid permits to ascribe to it the 3-sn-phosphatidic acid configuration and a negative Cotton effect indicates a 1-sn-glycerophosphate derivative. The method is rapid and has the advantage of requiring not more than 2-3 mg of lipid substance.

We used the above method for an investigation of the stereospecifity of the transesterification of phospholipids by phospholipase D. This enzyme was shown to catalyze the formation of phosphatidyl glycerol when incubated with phosphatidyl choline and exess of glycerol [14]. However the configuration of the glycerol moiety in the newly formed

T a b l e

Circular dichroism spectra of glycerophospholipids

Lipid	Source	Solvent	λ_{max} nm	$[\theta]_{max}$
Phosphatidyl choline	bovine liver [18]	heptane	226	+330
	egg yolk [19]	heptane	226	+429
Phosphatidyl ethanolamine	Actinomyces olivaceus [20]	heptane	216	+363
	Streptomyces griseus *)	heptane	215	+660
	bovine spinal cord [21]	heptane-EtOH, 1:1	222	+490
Phosphatidyl inositol	baker's yeast [22]	MeOH	217	+330
Phosphatidyl butane-2,3-diol	Actinomyces olivaceus [20]	heptane	216	+460
Phosphatidyl glycerol	**)	heptane	218	+210
Cardiolipin	Streptomyces griseus *)	heptane	216	+360
3-sn-Phosphatidic acid	***)	dioxane	214	+1560
1-sn-Phosphatidic acid	Chemical synthesis *)	EtOH	214	-990

*) The isolation and synthesis of these lipids will be described elsewhere.

**) Obtained by transesterification of egg yolk lecithin with phospholipase D [14].

***) Obtained by phospholipase D hydrolysis of egg yolk lecithin [13].

phosphatidyl glycerol is not known and the stereospecifity of the transesterification reaction (if any) remains uncertain.

To clarify this point phosphatidyl glycerol obtained from egg yolk lecithin by cabbage phospholipase D catalyzed transesterification [14] was acylated by palmitoyl chloride in the presence of pyridine. The bis-phosphatidic acid obtained showed no detectable Cotton effect while the $[\theta]_{max}$ value of the starting phosphatidyl glycerol was in the same range as those of the other natural glycerophosphatides (see Table). This shows the transesterification reaction to proceed stereospecifically leading to phosphatidyl glycerol of "natural" (3-sn),(1-sn)-configuration. Otherwise our bis-phosphatidic acid should reveal a positive Cotton effect.

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