The Isomerization and Transesterification of Phosphodiester Groups in Phospholipids

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During an investigation of the phospholipid fraction from a rough strain of *Pneumococcus* type 1 (D. E. Brundish, N. Shaw & J. Baddiley, unpublished work) novel and ready isomerization and transesterification reactions involving phosphodiesters were observed, and, in view of the implication of these observations on the interpretation of phospholipid analysis, this preliminary report is presented.

The components of the phospholipid fraction were identified by deacylation to the water-soluble phosphate esters, which were separated by ion-exchange chromatography on a DEAE-cellulose column. Three fractions were obtained, corresponding to diglycerol phosphate (GPG), glycerylphosphorylglycerol phosphate (GPGP) and bis(glycerylphosphoryl)glycerol (GPGPG). On paper chromatography in the solvent system propan-1-ol–aq. ammonia (sp.gr. 0·88)–water (6:3:1 by vol.) the fraction corresponding to GPG was shown to consist of two isomers, R_F 0·61 and 0·65. On storage of the deep-frozen (about $-14°$) acidic solution of GPG for 8 weeks, the corresponding isomers of bis(glycerylphosphoryl)glycerol, $\alpha\alpha'$-GPGPG and $\alpha\beta'$-GPGPG, together with glycerol, were detected chromatographically in the mixture, indicating that the transesterification reaction:

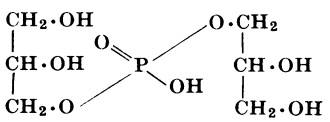
$$2\text{GPG} \rightarrow \text{GPGPG} + \text{G}$$

had occurred.

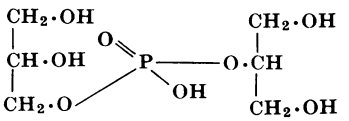
The GPG isomers were shown to have the structures $\alpha\alpha'$-GPG and $\alpha\beta'$-GPG respectively (structures I and II) by application of the sodium periodate–dimethylhydrazine degradative procedure used by LeCocq & Ballou (1964) in their studies on cardiolipin. $\alpha\alpha'$-GPG yielded 2mol.prop. of formaldehyde on oxidation with periodate and removal of the resulting glycolaldehyde groups with dimethylhydrazine yielded inorganic phosphate. $\alpha\beta'$-GPG gave 1mol.prop. of formaldehyde on oxidation with periodate, and treatment of the oxidation product with dimethylhydrazine yielded glycerol 2-phosphate. The structures of the isomers of GPGPG were also elucidated by application of the periodate–dimethylhydrazine degradation. Under the storage and handling conditions 46% of the GPG was converted into GPGPG. However, when acidic conditions were avoided the products obtained by deacylation of the fresh phospholipid fraction were normal in structure. The isomerization is therefore a consequence of the acidic conditions used during isolation of the phosphate esters.

Brown, Magrath, Neilson & Todd (1956) in their studies on the degradation of phosphodiesters in acid or alkali have shown that intermediate cyclic ester formation is obligatory in these reactions if one or more hydroxyl groups occur adjacent to the phosphodiester linkage. Under acidic conditions the reaction can lead to migration of a phosphodiester group to a neighbouring hydroxyl group, presumably through the intermediate formation of a phosphotriester. Thus the dinucleoside phosphate, adenosine 2′-phosphate 5′-uridine, was readily isomerized under acidic conditions to a mixture of adenosine 2′- and 3′-phosphate 5′-uridine. The acid-catalysed isomerization observed in the present case is directly analogous to that of the dinucleoside phosphate and probably occurred during acidification of the alkaline hydrolysate obtained during deacylation, or on storage of the phosphate esters in the free acid form. The intermolecular transesterification leading to the formation of GPGPG from GPG is explained in a similar manner; a mechanism is outlined in Scheme 1.

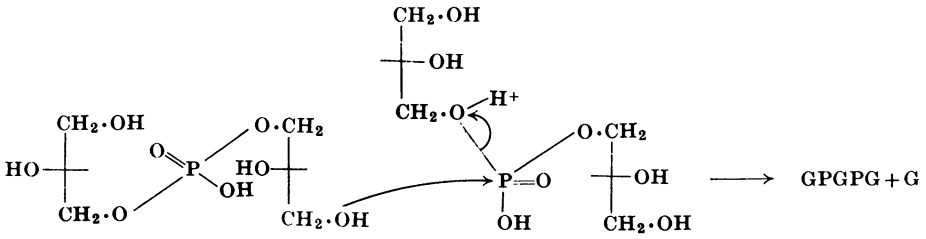
Schwarz, Dreisbach, Polis, Polis & Soffer (1965) in an investigation of the lipids in particulate fractions from rat liver have reported the formation of an unknown isomer of GPG arising during deacylation



(I)



(II)



Scheme 1.

of the phospholipids. In view of our results it is probable that the unknown isomer is $\alpha\beta'$-GPG arising by acid-catalysed migration during drying of the acidic solution after passage through Amberlite IRC-50 (H^+ form) resin. These authors also report that similar treatment of an authentic sample of a GPG derivative yielded, in addition to the unknown isomer, a small amount (3%) of GPGPG, thus confirming our observation of the transesterification reaction.

The formation of GPGPG from GPG under acidic conditions suggests an alternative biosynthetic route to bisphosphatidylglycerol (cf. LeCocq & Ballou, 1964). An analogous reaction between two molecules of phosphatidylglycerol, under enzymic catalysis, would yield bisphosphatidylglycerol and glycerol.

In view of the ease with which these isomerization and transesterification reactions occur, it is necessary to emphasize the dangers of misinterpretation that could arise by the use of mild acidic conditions during structural investigations on phospholipids containing phosphodiester linkages.

Brown, D. M., Magrath, D. I., Neilson, A. H. & Todd, A. R. (1956). *Nature, Lond.*, **177**, 1124.
LeCocq, J. & Ballou, C. E. (1964). *Biochemistry*, **3**, 976.
Schwarz, H. P., Dreisbach, L., Polis, E., Polis, B. D. & Soffer, E. (1965). *Arch. Biochem. Biophys.* **111**, 422.

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The Relationship between the Rates of Conversion of Palmitate into Citrate or Acetoacetate and the Acetyl-Coenzyme A Content of Rat-Liver Mitochondria

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Changes in the acetyl-CoA content of rat livers have been implicated in the control of acetoacetate synthesis (Wieland & Weiss, 1963*a*; Tubbs & Garland, 1964), and increases of the acetyl-CoA content of rat-liver mitochondria during palmitate oxidation have been described (Garland, Shepherd & Yates, 1965). We now describe kinetic relationships between the acetyl-CoA content of rat-liver mitochondria and the rates of conversion of acetyl-CoA into citrate or acetoacetate, and factors controlling this process.

Methods. Reagents, preparation of mitochondria, incubation apparatus for continuous recording of oxygen pressure with rapid sampling facilities, and fluorimetric assays for acetyl-CoA were as described by Garland *et al.* (1965). Oxaloacetate was assayed fluorimetrically. L-Carnitine and acyl-L-carnitine were assayed either by the methods described by Pearson & Tubbs (1964*a*,*b*) or by a fluorimetric modification. All mitochondrial incubations were made at 25° at a protein concentration of 2–4mg./ml. in a medium containing initially KCl (80mM), tris–chloride buffer, pH 7·2 (20mM), $MgCl_2$ (2mM), EDTA (1mM), defatted bovine plasma albumin (5mg./ml.), AMP (1·25mM), P_i (1·25mM) and malonate (10mM), with further additions as indicated.

Experimental design. Rat-liver mitochondria