Isolation of Acylated α-Alkoxy-glycerylphosphoryl Cholines from Normal Human Serum

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Alkoxy-glycerylphosphoryl cholines were recently isolated from a hydrolysate of highly purified serum lecithin [1]. The present report describes the isolation of the corresponding unhydrolyzed lipids which are shown to be acylated α-alkoxy-glycerylphosphoryl cholines.

The sample of serum lecithin which served as starting material was quite similar to that described previously [1]. It contained 3.2 % ether phosphatides [a] and 1.3 % plasmalogens [b].

Acid and alkali stable lipids (0.49 mg P) were prepared from 0.50 mmole of the lecithin as described previously [1]. Their chromatographic fractionation on silicic acid gave a lecithin-like fraction (0.12 mg P) and a lysolecithin-like one (0.33 mg P).

The lecithin-like fraction was shown to be rather pure acylated alkoxy-glycerylphosphoryl choline. It migrated on thin layer chromatography (TLC) [2] like pure lecithin but it contained acyl esters [3], and phosphorus [4] in molar ratios 1.03:1.00. Strong acid hydrolysis [5] of the preparation released only 0.12 mole free glycerol, but in addition the hydrolysate contained a stable lipid which showed the same mobility as batyl alcohol on TLC. This behaviour is typical of ether phosphatides [1]. The phosphatide contained 0.80 mole choline [6], but it was free of phosphatidyl cholines and choline plasmalogens as 96 % of its phosphorus remained "stable" in a renewed mild acid and alkaline hydrolysis.

The lysolecithin-like fraction isolated from the lecithin hydrolysate was identical with the alkoxy-glycerylphosphoryl cholines described previously [1]. A sample of this preparation (4.5 μmoles) was subjected to acetolysis as described by Carter *et al.*[7] Subsequent chromatography on alkali-free aluminium oxide [8] and silicic acid gave a lipid (3.6 μmoles) [c] which on TLC was identical with batyl alcohol diacetate. The diacetyl compound, after deacylation with alkali, released 1.18 moles formaldehyde by oxidation with periodate. The experimental conditions used gave 1.10 moles formaldehyde from deacylated batyl alcohol diacetate. This shows that the "ether-ester lecithins" of serum are probably α-alkoxy compounds.

A second sample (0.50 mmole) of the serum lecithin was subjected to mild alkaline hydrolysis only, and the stable lipids (0.69 mg P) were fractionated as above.

The lecithin-like fraction (0.18 mg P) thus obtained again moved like pure lecithin on TLC, although it contained carboxylic esters and phosphorus in equimolar amounts. The composition of this preparation became evident after mild acid hydrolysis. After chromatographic fractionation the acid hydrolysate yielded "ether-ester lecithins" identical to those described above, and true lysolecithins which were formed from choline plasmalogens. Thus the preparation was a mixture of unhydrolyzed choline plasmalogens (about 30 %) and "ether-ester lecithins" (about 70 %). It did not contain detectable amounts of "lecithins" of divinylether [d], vinylether-alkoxy [e] or dialkoxy [f] type [9].

The alkaline hydrolysate of the lecithin gave also a lysolecithin-like fraction (0.47 mg P) which was a mixture of choline lysoplasmalogens (about 30 %) and alkoxy-glycerylphosphoryl cholines (about 70 %) [1].

Thus the mild alkaline hydrolysis of serum lecithin caused complete deacylation of phosphatidyl cholines, but at the same time it resulted in only about 75 % deacylation of choline plasmalogens and "ether-ester lecithins". The combination of mild acid and mild alkaline hydrolysis accordingly gave pure "ether-ester lecithins" in about 25 % yield although the starting material contained rather small amounts of these lipids.

These observations confirm and extend the recent finding of Hanahan and Watts [10], who obtained pure acylated α-alkoxy-glycerylphosphoryl ethanolamines by selective alkaline deacylation of a mixture containing about 80 % "ether-ester cephalines" and about 20 % phosphatidyl ethanolamines.

We think that also the slow deacylation of plasmalogens indicated in our experiments will eventually prove useful for isolation of pure native plasmalogens from natural sources.

a. Estimated as stable phosphatides after combined acid and alkaline hydrolysis [1].

b. Estimated as difference between alkali stable phosphatides and ether lipids.

c. Assayed by analysis of carboxylic esters; calculated by assuming that the lipid was a diacyl compound.

d. The phosphorus of the sample remained completely "stable" in mild acid hydrolysis.

e. The lysolecithins formed by the mild acid hydrolysis were free of alkoxy-glycerylphosphoryl cholines as shown by their requimolar content of carboxylic ester and phosphorus.

f. The "ether-ester lecithin" obtained from the mild acid hydrolysate gave nearly quantitative yield of "monochain derivatives" of glycerylphosphoryl choline by prolonged mild alkaline hydrolysis.

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Gas Chromatographic Determination of some Volatile Compounds in Urine

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For several years this laboratory has used a chemical as well as an enzymatic method for the determination of alcohol in blood and urine [1]. In some cases large discrepancies were found between the results obtained by the two methods.

The number of cases where these large discrepancies are found as well as the number of cases with smaller but significant differences seem to increase and these findings induced a closer investigation of the cause of the disagreement.

To this purpose a Perkin-Elmer gas fractometer with a flame detector was used. As column filling we used 10 g of Carbowax 1500 (polyethyleneglycol, 10 % by weight on Teflon) in the first part of the column followed by 5 g of Ucon polyglycol LB-550-x (polypropyleneglycol, 20 % by weight on Chromosorb).

This packing ensured complete separation of the volatile compounds which could be expected to be present in the urine samples, without interference of the relatively large amounts of water present. At the same time the water was not, as is the case with polyethyleneglycol alone, unnecessarily retarded which is obviously important as the next sample can not be introduced until all water has left the column.

The temperature was 100°C throughout the procedure and the nitrogen pressure was 1 atm. The urine was injected directly and 2 μl was used. Under these conditions a new sample could be injected every tenth min. At the maximum sensitivity 0.01 % of ethanol in a 2 μl sample gave a deflection of 8 to 10 cm on the recorder.

As could be expected, the chromatographic analysis revealed besides ethanol the presence of methanol, acetone and acetaldehyde which in some but not all cases could account for the differences seen in Table 1. In no cases a higher aliphatic alcohol than ethanol has been found. A typical recording is shown in Fig. 1. Acetone was commonly encountered in diabetic in-