Different Molecular Species of Glycerophosphatides.

Part I. Proposal for a Method of Analysis

OSSI RENKONEN

Department of Serology and Bacteriology, University of Helsinki, Finland

The analysis of individual molecular species within different classes of glycerophosphatides might open interesting possibilities for structural as well as metabolic studies of, for instance, various biomembranes.[1-2] However, at present it seems that the phosphatides are not easily separated into individual molecular species. On the other hand, several types of "neutral lipids" can be separated into fairly homogeneous subgroups with different partition and adsorbtion methods.[3-4] Therefore it appears desirable to attempt the analysis of phosphatides by converting them into some form of "neutral lipids" and by fractionating them in this modified form. Promising conversion methods for this purpose might be, *e.g.*, the enzymic breakdown of phosphatidyl compounds to diglycerides,[5] and possibly the diazomethanolysis of ethanolamine and serine cephalins,[6] but a more general procedure seems to be available in the dephosphorylation of phosphatides with a mixture of acetic acid and acetic anhydride.[7] The present report shows that the products of this reaction really can be used for the analysis of different molecular species of many glycerophosphatides.

Malkin's early observations on acetolysis of phosphatides [7] were first confirmed and extended. Very nearly quantitative yields of diglyceride acetates were obtained by preparative acetolysis of purified egg lecithins. Also phosphatidic acids, cardiolipins, phosphatidylglycerols, phosphatidylinositols, phosphatidylserines and phosphatidylethanolamines gave good yields of diglyceride acetates as judged by TLC analysis of the reaction mixtures.

Further it was established that the reaction products thus obtained can be fractionated into subgroups of decreasing complexity. As expected this was easily achieved with silver nitrate-silicic acid chromatography,[4] whereby the diglyceride acetates were nicely separated into subgroups of zero, one, two, *etc.* double bonds per molecule. This type of fractionation was accomplished both with synthetic model compounds and with diglyceride acetates derived from natural glycerophosphatides.

The problem of possible fatty acid exchange between neighboring phosphatide or diglyceride molecules was studied in special experiments. It is obvious that such exchange would make subsequent analysis of the diglyceride acetates pointless. Therefore, samples of about equal size of dipalmitoyl-phosphatidylethanolamine and dioleoyl-phosphatidylethanolamine were acetolyzed together. If any exchange of acids had occurred the reaction mixture would have contained monoenoic diglyceride acetates in addition to the saturated and dienoic ones. However, when the reaction products were analyzed on silver nitrate-silicic acid TLC, which separates these three types of compounds, no formation of the monoenoic diglyceride acetates was seen. A similar experiment with the same result was carried out also with samples of dipalmitin and diolein.

Another undesirable side reaction would be the possible addition of acetic acid to to double bonds of the phosphatides,[8] but not even this reaction was observed under the conditions used. The diglyceride acetates obtained from egg lecithins showed the correct molar ratio of carboxylic ester to glycerol and GLC analysis of long chain methylesters showed the same relative amounts of different acids both in the diglyceride acetates and in the parent lecithins.

One minor side reaction takes place, however, during the acetolysis. The reaction products invariably contained small amounts of 1,3-diglyceride acetates in addition to the expected, and quite predominating, 1,2-diglyceride isomers. This isomerization does not disturb the analysis of the individual "fatty acid combinations", and it should be possible to purify the 1,2-diglyceride acetates if desired, since the two types of acetates are separated on conventional TLC quite as the corresponding diglycerides.

The procedure suggested for the analysis of individual molecular species of phosphatidyl compounds seems thus quite promising. The dephosphorylation reaction is practically free from disturbing side reactions, and it gives good yields of di-

glyceride acetates from a wide variety of different phosphatidyl lipids. Furthermore, several different methods are already available for the study of the diglyceride acetates. Besides the silver nitrate-silicic acid chromatography also the effective partition methods, for instance those of Kaufmann,[3] should be easily adapted for their fractionation. The oxidation methods of Privett and Blank [9] could be used for analysis of fatty acid localization in these molecules, and pancreatic lipase [10] might also be usable. In short, it seems to me that a rather complete picture of the different molecular species of many phosphatidyl lipids might be obtained when all these procedures as well as GLC are successively applied to the study of the acetolysis products. It is a great advantage that all different phosphatidyl compounds can be studied through the same procedures of diglyceride acetate analysis.

Acknowledgements. I wish to thank Mrs. Satu Liusvaara and Miss Anneli Miettinen for skilled technical assistance. The samples of dipalmitoyl- and dioleoyl-phosphatidylethanolamine were gifts from Fluka AG, Switzerland, and from Dr. G. V. Marinetti, Rochester, USA. The support of grants from *Sigrid Jusélius Foundation* and from the *Finnish State Committee for Science* is gratefully acknowledged.

1. Vandenheuvel, F. A. *J. Am. Oil Chem. Soc.* **40** (1963) 455.
2. Collins, F. D. *Biochem. J.* **88** (1963) 319.
3. Kaufmann, H. P., Makus, Z. and Das, B. *Fette Seifen Anstrichmittel* **63** (1961) 807.
4. De Vries, B. *Chem. Ind. (London)* **1962** 1049.
5. Macfarlane, M. G. and Knight, B. C. J. G. *Biochem. J.* **35** (1941) 884.
6. Baer, E. and Maurukas, J. *J. Biol. Chem.* **212** (1955) 39.
7. Bevan, T. H., Brown, D. A., Gregory, G. I. and Malkin, T. *J. Chem. Soc.* **1953** 127.
8. Meade, E. M. and Walder, D. M. *J. Am. Oil Chem. Soc.* **39** (1962) 1.
9. Privett, O. S. and Blank, M. L. *J. Lipid Res.* **2** (1961) 37.
10. Desnuelle, P. and Savary, P. *J. Lipid Res.* **4** (1963) 369.

Received December 13, 1963.

A Comparison of the Solid Behaviour of Stearic Acid, 17-Bromoheptadecanoic Acid and 17-Iodoheptadecanoic Acid

KÅRE LARSSON

Crystallography Group, Institute of Medical Biochemistry, University of Göteborg, Sweden

Isomorphous replacement of a terminating methyl group in long-chain compounds by an ω-bromine atom has been investigated at this Institute,[1] and the present study concerns this as well as replacement by an ω-iodine atom.

The experimental technique used has been described earlier.[1] 17-Bromoheptadecanoic acid was synthesized according to Hunsdiecker and Hunsdiecker,[2] and its methyl ester was boiled with sodium iodide in acetone and hydrolysed to give 17-iodoheptadecanoic acid. The samples used were prepared by Miss G. Ställberg in 1948. Thermal and X-ray data on stearic acid given in the text have been taken from von Sydow [3] and from Stenhagen and von Sydow.[4]

Data for the crystal forms observed and the phase behaviour are collected in Table 1. It is remarkable that at high temperatures the B-forms of the bromo- and iodo-acids are much more stable than that of stearic acid. A new crystal form of fatty acids called B_1 has been found for 11-bromoundecanoic acid.[1] It showed an X-ray diffraction pattern very similar to that of the B-form but the infrared absorption spectra of the two forms were clearly different. The B-forms of the acids studied here were identified on the basis of their infrared absorption[1] (according to the OH-out-of-plane band). It was accidentally found that the B-form of 17-iodoheptadecanoic acid may transform into the B_1-form. An infrared absorption spectrum was recorded seven years ago and the same pellet (pressed of the sample mixed with potassium bromide) was used for a new recording. The spectra showed that a transition from the B-form into the B_1-form had taken place during storage at + 7°C. The rest of the sample stored at room temperature was still in the B-form. The lattice parameters of the B-forms of the three acids are given in Table 2, showing small