SC 2272

The fatty acid components of polyphosphoinositide prepared from calf brain

A preparation of the mixed di- and triphosphoinositides of calf brain[1], essentially free from nitrogenous contaminants, served for the study here reported on the nature of the fatty acid components.

The procedure for the preparation of polyphosphoinositides, described in detail by KERR, KFOURY AND DJIBELIAN[1], may be summarized as follows. Calf-brain cephalin was prepared by the method of DEBUCH[2] and from this the material designated "Fractions I and II" by FOLCH[3] was obtained. This was partitioned in the solvent system C described by COLE *et al.*[4] and the non-polar phase was then passed through a column of Dowex-50W (H^+ form) which removed cations, inorganic phosphate, and most of the nitrogenous impurities. The filtrate was taken to dryness *in vacuo*, dissolved in chloroform–methanol (8:1, v/v), and treated with 7 vol. of methanol which precipitated the remaining nitrogenous impurities, together with some carbohydrate. The supernatant fluid contained the free acids of mixed diphosphoinositide (15 % of the total P) and triphosphoinositide (85 % of the P). Determination of the ester:P ratio[5] (0.70) showed these to be diacyl esters.

The solvent was removed *in vacuo*, the residue was taken up in anhydrous methanol, and the methyl esters of the component fatty acids were produced and purified by the methods described by STOFFEL *et al.*[6]. The mixed methyl esters were separated both on 4-ft columns of Apiezon L and on ethylene glycol adipate polyester at 197° on a Pye argon chromatograph. An argon detector was used. Estimation of

TABLE I

THE FATTY ACID COMPONENTS OF POLYPHOSPHOINOSITIDE FROM CALF BRAIN

Trivial name	*Short-hand designation*	*Molecular percentage*
Lauric acid	12:0	0.01
Myristic acid	14:0	0.05
	15:0	trace
Palmitic acid	16:0	5.40
	br* 16:0	0.17
Margaric acid	17:0	1.00
	h.br** 17:0	0.14
	br 17:0	0.13
Stearic acid	18:0	48.14
	h.br 18:0	0.11
	br 18:0	0.11
Oleic acid	18:1	9.81
	19:0	4.53
	19:?	0.46
	20:1	1.22
	20:2	4.55
	20:3	0.98
Archidonic acid	20:4	20.64
	20:?	0.37
	22:5	1.76
	22:6	0.41

* br, branched.
** h.br, highly branched.

the relative amounts of individual components was made from both records by the method of CARROLL[7]. Components were identified by the method of JAMES[8] and with use of his tables.

The results of these calculations are presented in Table I. Use of pure methyl margarate as internal standard showed that 97.5 % of the total material applied to the column was recovered. 21 component acids were found, of which 6 (16:0, 18:0, 18:1, 19:0, 20:4 and 20:2) accounted for 93 % of the total. Saturated acids formed 59.1 % of the total (48.1 % stearic, 11.0 % other acids). Only 6.9 % of the acids had less than 18 carbon atoms. Odd-numbered acids represented 5.5 % of the total. No poly-unsaturated acids below C_{20} were detected.

The percentage of stearic acid (48.1) suggests that it is a constant component of one of the ester positions in the molecule, the other position being occupied by a wide variety of acids, the greater part of these (40.9 %) being unsaturated.

Studies reported on the monophosphoinositide of heart muscle[9] and of liver[10] indicate that in each approx. 50 % of the fatty acid is stearic, the remainder being a variety of unsaturated acids. The equivalent weight reported for the mixed fatty acids of heart monophosphoinositide was 291, and for liver monophosphoinositide 287, these values being similar to the 290.9 calculated for the mean molecular weight of fatty acids in our brain polyphosphoinositides. This corresponds to a mean chain-length of 18.55:0, and a molecular weight of 1040 for the free acid of triphosphoinositide.

This work was supported by grants from the National Multiple Sclerosis Society (No. 296), and from the National Institutes of Health (No. AM-05285-02 to the Institute of Nutritional Sciences, Columbia University and the American University of Beirut).

Department of Biochemistry, American University of Beirut, Beirut (Lebanon) STANLEY E. KERR

Nutritional Research Program, American University of Beirut, Beirut (Lebanon) W. W. C. READ

[1] S. E. KERR, G. A. KFOURY AND L. G. DJIBELIAN, *Biochim. Biophys. Acta*, 70 (1963) 474.
[2] H. DEBUCH, *Z. Physiol. Chem.*, 304 (1956) 109.
[3] J. FOLCH, *J. Biol. Chem.*, 177 (1949) 497.
[4] P. G. COLE, G. H. LATHE AND C. R. J. RUTHVEN, *Biochem. J.*, 54 (1953) 449.
[5] M. M. RAPPORT AND N. ALONZO, *J. Biol. Chem.*, 217 (1955) 193.
[6] W. STOFFEL, F. CHU AND E. H. AHRENS, JR., *Anal. Chem.*, 31 (1959) 307.
[7] K. K. CARROLL, *Nature*, 191 (1961) 377.
[8] A. T. JAMES, *J. Chromatog.*, 2 (1959) 552.
[9] M. J. MORELEC-COULON AND M. FAURE, *Bull. Soc. Chim. Biol.*, 40 (1958) 1071.
[10] M. FAURE, M. J. MORELEC-COULON, J. MARECHAL AND L. LEBORGNE, *Bull. Soc. Chim. Biol.*, 41 (1959) 101.

Received February 8th, 1963