The isolation of a new complex lipid: triphosphoinositide from ox brain

By solvent fractionation FOLCH[1] prepared from ox brain a phospholipid which he designated diphosphoinositide and which consisted in its simplest composition of one molecule each of inositol, glycerol and fatty acid together with two molecules of phosphoric acid. After short acid hydrolysis its main phosphorus-containing product was found to be inositol-*m*-diphosphate. Recently a similar diphosphoinositide fraction from brain was shown to yield three spots on a paper chromatogram[2].

FOLCH AND LEBARON[3–5] also described a complex inositol-containing phosphatido-peptide which was extracted from lipid-free trypsin-digested brain material with acidified solvent. This contained phosphorus and inositol in the molar ratio 2.7:1, a sphingosine-like substance, fatty acid, and abundant nitrogen, mainly in the form of peptide-linked amino acids; it yielded inositol diphosphate on acid hydrolysis.

Recently a new hydrolytic technique[6] has been developed for the quantitative examination of the phospholipids in a complex lipid fraction isolated from tissues. When this method was applied to lipid extracted from brain tissue by chloroform–methanol (2:1, v/v) it failed unexpectedly to give evidence for the presence of any inositol-containing phospholipid apart from monophosphoinositide. The reverse was true, however, if the tissue was pre-treated with acetone prior to the chloroform-methanol extraction. This observation has been made the basis of a method for isolating the complex inositide fraction from ox brain, the purification being followed by preparing acid and alkaline hydrolysates of the fractions and examining these by high-voltage ionophoresis and chromatography.

The brain tissue is exhaustively extracted with chloroform–methanol to remove all other lipids, and the residue then extracted with slightly acidified chloroform–methanol solvent[4]. When the latter extract is shaken with aq. 0.9 % NaCl and centrifuged, the inositide is largely found in the interfacial protein layer. After heating this with acetone and ethanol, the protein is all removed and the chloroform-soluble inositide is obtained. On adding methanol, a methanol-insoluble fraction is precipitated, designated triphosphoinositide A. If the supernatant is now neutralized with methanolic NaOH, the main component of the fraction, triphosphoinositide B, is precipitated as its sodium salt.

The sodium salt of triphosphoinositide B is soluble in water and chloroform but insoluble in methanol. It is virtually nitrogen-free. Its simplest composition is found to be $(\text{fatty acid})_6(\text{phosphate})_6(\text{glycerol})_3(\text{inositol})_2$ and this accounts for all of the components of the molecule. After brief acid hydrolysis it yields considerable diglyceride together with free glycerol, fatty acids, and a trace of free inositol. Of the water-soluble phosphorus-containing components formed, inositol triphosphate has been isolated in a yield of 75–80 %; a compound containing $(\text{inositol})_1\text{-}(\text{phosphate})_2$, a few per cent of glycerophosphoric acid and traces of inorganic phosphorus comprise the rest of the hydrolysate. The fatty acids on examination by gas chromatography have been shown to be a complex mixture with stearic acid, arachidonic acid and a C_{22} polyunsaturated acid predominating; palmitic, oleic, linoleic acids and another C_{20} unsaturated acid are also present.

A fraction equivalent in composition to the triphosphoinositide A fraction is

obtained in much better yield, by working up the lipids obtained by chloroform–methanol extraction of acetone-pretreated brain tissue. The Folch I fraction[7] prepared from such an extract is run through an Amberlite IRC 120 column which removes cations and most of the contaminating inorganic phosphate. The lipid is then distributed in a biphasic system formed from chloroform, ether, ethanol and water. The product, thus separated from contaminating phosphatidyl serine and monophosphoinositide, is insoluble in methanol. Analysis indicates the simplest composition as $(\text{glycerol})_1$-$(\text{inositol})_1$ $(\text{phosphate})_3(\text{acyl ester})_{2?}$. On brief acid hydrolysis, mono- and diglycerides can be isolated from the hydrolysate. The phosphorus-containing hydrolysis products are very similar to those from triphosphoinositide B except that more inorganic P and less inositol triphosphate are formed. After mild alkaline hydrolysis[6] the predominant phosphorus-containing product analyses as $(\text{glycerol})_1(\text{inositol})_1(\text{phosphate})_3$.

By means of alumina and silicic acid columns[8,9], monophosphoinositide giving the correct analysis has also been obtained from brain lipid extracts. This confirms the recent evidence, obtained by chromatography, for the existence of this lipid in brain tissue[2,6,9].

The present results indicate that as well as monophosphoinositide, brain tissue contains two triphosphoinositides which are probably very closely related in structure, and which are tightly attached to brain proteins. No information has yet been obtained regarding the relation of these to diphosphoinositide or the relative amounts of the various inositol-containing lipids.

Dr. D. RHODES is thanked for the fatty acid analysis of triphosphoinositide B. One of us, J. C. DITTMER, is a U.S. Public Health Service Research Fellow of the National Heart Institute.

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Received March 25th, 1960

Biochim. Biophys. Acta, 40 (1960) 379–380

A mechanism of *ortho*-hydroxylation of aromatic amines *in vivo*

Several groups[1–3] have obtained data with various species which suggest that separate enzyme systems catalyze the *p*-hydroxylation and *o*-hydroxylation of aromatic amines and amides *in vivo*. It was recently found in this laboratory that N-hydroxy-