Composition of the Phospholipid Fraction of *Corynebacterium diphtheriae*

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Corynebacterium diphtheriae gravis previously washed with acetone was extracted with chloroform–methanol–water. Lipids were dried, washed, converted into the sodium form and freeze-dried (Brennan, 1968). This phospholipid fraction was then subjected to the following treatments.

Hydrolysis and chromatography in several solvent systems showed that the lipids were composed of inositol, mannose, glucose, glycerol and five amino acids. Ethanolamine and serine were absent from the ninhydrin-positive products. Deacylation of the lipid fraction and chromatography in two solvents showed the presence of glucose, trehalose, glycerophosphorylinositol, glycerophosphorylinositol dimannoside and a major unidentified product.

About 1·2g. of the phospholipid fraction was applied to a column of DEAE-cellulose and eluted first with chloroform–methanol–water. This removed acylglucoses (Brennan & Lehane, 1969) and acyltrehaloses (Senn, Ioneda, Pudles & Lederer, 1967). Further elution of the column with ammonium acetate removed the partially purified phospholipids. These were subjected to preparative t.l.c. for complete purification.

Phosphatidylinositol was readily identified as one of the major phospholipids. Most of the lipids that yielded glycerophosphorylinositol dimannoside on deacylation were found to be identical with the triacylated dimannophosphoinositide B from mycobacteria (Brennan & Ballou, 1967) with smaller amounts corresponding to the diacylated dimannophosphoinositide C. None of the tetra-acylated dimannophosphoinositide A was evident. A major phospholipid in these fractions has not been fully identified. It contains glycerol and an amino acid and no monosaccharide. Surprisingly, no phosphatidylethanolamine or the higher oligomannophosphoinositides prominent in mycobacteria have been found in *C. diphtheriae*.

The phospholipids of the corynebacteria have been the subject of a number of studies. Chargaff (1931) recognized some of the similarities between the phospholipids of *C. diphtheriae* and those of the mycobacteria. Asselineau (1961) tentatively identified phosphatidylinositol dimannoside in *C. diphtheriae*. However, in *Corynebacterium ovis* the mannophosphoinositides are apparently replaced by arabinophosphoinositides (Lacave, Asselineau & Toubiana, 1967). Brennan (1968) isolated and characterized a phosphoinositide and a dimannophosphoinositide from *Corynebacterium xerosis*. The present work shows that some members of the family of dimannophosphoinositides found in mycobacteria are also present in *C. diphtheriae* and describes other components of the phospholipid fraction.

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The Oxidation of Acetate, Ethanol and Pyruvate by Baker's Yeast

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Commercial baker's yeast oxidizes acetate linearly after a short lag period. The duration of this lag can be extended by starvation of the yeast and by the addition of 1mM-ammonium chloride (Gosling & Duggan, 1968).

Cycloheximide (1μg./ml.) completely inhibits adaptation to acetate oxidation such that the initial low rate of oxidation does not increase. Thus cytoplasmic protein synthesis is required for adaptation (Siegel & Sisler, 1965). Chloramphenicol, which inhibits yeast mitochondrial protein synthesis (Roodyn & Wilkie, 1968), has much less effect (at 4mg./ml.) and causes a 5–10% decrease in the final rate.

The activities of a number of enzymes involved in acetate metabolism were measured during adaptation. These were citrate synthase, aconitate hydratase, isocitrate lyase, malate synthase, fumarate hydratase, malate dehydrogenase and fructose diphosphatase. All activities increased at pH4·4 in starved yeast on incubation with 10mM-acetate. At pH7·5 and 20mM-acetate similar though smaller increases were found. Most of these increases occurred at the same time as the increase in oxidation rate.

In contrast, starved baker's yeast oxidizes 10mM-pyruvate at pH4·4 without an adaptation period, though the activities of the above enzymes also increase under these conditions. However, these activities level off or even decrease after 2hr. The rate of pyruvate oxidation is about one-third of the maximal rate of acetate oxidation.

Incubation with ethanol, which is readily oxidized, also causes increases in these enzyme activities. These increases occur more quickly than with acetate or pyruvate, probably because ethanol can, in being oxidized to acetate, serve as an immediate source of energy (Maitra & Estabrook, 1967).

Thus this yeast has an active tricarboxylic acid cycle and oxidative pathways. However, there is a lag before acetate is oxidized linearly. Inhibition of cytoplasmic protein synthesis completely prevents adaptation, and the activities of a number of relevant enzymes, especially isocitrate lyase, increase during adaptation. Consequently it is probable that the activity of some enzyme or enzymes, such as those above, is directly or indirectly limiting for acetate oxidation but not for the oxidation of ethanol or pyruvate.

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Glycopeptide Linkages in a Phosphomannan Peptide from Yeast Cell Walls

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Glycopeptides containing mannose have been extracted from yeast cell walls by anhydrous ethylenediamine (Korn & Northcote, 1960). Subsequent purification (Sentandreu & Northcote, 1968) by treatment with Pronase and gel filtration yielded a high-molecular-weight mannan–peptide rich in serine and threonine. Sentandreu & Northcote (1968) were able to demonstrate, from their investigations of the β-elimination reaction of the glycopeptide in dilute alkali, that one of the linkages between carbohydrate and amino acids is an *O*-mannosyl bond to serine and threonine. The present report deals with similar investigations on the glycopeptide linkages of a mannan–peptide that was liberated directly from isolated yeast cell walls by Pronase digestion (Cawley & Letters, 1968).

Amino acid analyses of yeast cell wall, and of the phosphoglycopeptide released from it by Pronase treatment, show that Pronase treatment results in an enrichment of serine and threonine in the phosphoglycopeptide. Treatment of the phosphoglycopeptide (70mg.) with 0·1M-KOH (10ml.) at room temperature for 24hr. results in a loss of 75% of the serine and 79% of the threonine initially present. These losses are due to a β-elimination reaction that produces dehydro derivatives of serine and threonine with a characteristic u.v. absorption at 240nm. During the elimination reaction a steady increase in the extinction at this wavelength was observed. When the phosphoglycopeptide (100mg.) was treated with sodium borohydride (30mg.) in 0·1M-KOH (10ml.) for 24hr. at room temperature, and then hydrolysed to liberate amino acids, we were able to detect a new amino acid, identified as α-aminobutyric acid by high-voltage paper electrophoresis. This provides confirmatory evidence for formation of dehydrothreonine during the β-elimination. The decrease in the proportion of serine and threonine in the glycopeptide after treatment with alkaline borohydride and the simultaneous increase in the proportion of alanine together with the appearance of α-aminobutyric acid indicate that serine and threonine are involved in linkages through their hydroxyl groups. The alkoxide moiety eliminated from the phosphoglycopeptide by 0·1M-KOH was found to be a mixture of free mannose and mannose oligosaccharides, thus showing that the serine and threonine hydroxyl groups are involved in glycosidic linkages. Yeast mannan, which can be obtained by alkali treatment of cell wall, is found to be rich in glucosamine. However, Pronase digestion of the cell wall does not solubilize glucosamine but concentrates it in the insoluble residue. The residue obtained after continuous digestion with Pronase for 7 days contained 0·2% nitrogen. An amino acid analysis showed that alanine and glucosamine each accounted for approx. 10% of the total α-amino nitrogen, and aspartic acid, glutamic acid and serine each accounted for a further 8%.

These results show that the phosphomannan–peptide released by direct treatment of yeast cell walls with Pronase contains glycopeptide linkages that are similar to those found in the glycopeptide investigated by Sentandreu & Northcote (1968). Therefore the direct treatment of isolated yeast cell walls with Pronase provides a simpler and quicker method for obtaining large amounts of phosphomannan–peptide for chemical studies.

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