Sargeant, K., East, D. N., Whitaker, A. R. & Elsworth, R. (1971) *J. Gen. Microbiol.* **65**, iii

Phosphatidylinositol Kinase Activity in *Saccharomyces cerevisiae*

By G. E. WHEELER, R. H. MICHELL and A. H. ROSE
(Microbiological Laboratories, School of Biological Sciences, University of Bath, Bath, U.K., and Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.)

Phosphatidylinositol kinase catalyses the reaction:

The enzyme has been detected in a variety of animal tissues, and is localized predominantly in the plasma membrane. The function of phosphatidylinositol kinase in animal cell membranes is obscure, but it is possible that diphosphoinositide is involved in the binding of bivalent cations. Phosphatidylinositol kinase has not so far been reported in microorganisms, although its activity has been implicated (Cerbón, 1970) to explain arsenate transport into yeast. In addition, small amounts of diphosphoinositide and triphosphoinositide have been detected in lipid extracts of *Saccharomyces cerevisiae* (Lester & Steiner, 1968). The present communication reports the detection of phosphatidylinositol kinase in a strain of *Sacch. cerevisiae*.

Sacch. cerevisiae N.C.Y.C. 366 was grown aerobically in a glucose-salts-vitamins medium, pH 4.5, and sphaeroplasts were obtained from exponential-phase cells by digestion with a basidiomycete glucanase preparation, in citrate-phosphate buffer, pH 6.0, containing 10 mM-MgCl₂ and 1 M-sorbitol. Phosphatidylinositol kinase activity was assayed by using a reaction mixture containing phosphatidylinositol and [γ -³²P]ATP and measuring the radioactivity of the diphosphoinositide formed after extraction in chloroform-methanol and separation by paper chromatography. Phosphatidylinositol kinase activity was not detected in the supernatant left after sphaeroplasts had been removed from the glucanase digest, which suggests that the enzyme is not located in the periplasm in this organism. Sphaeroplasts were lysed by suspension in ice-cold 10 mM-MgCl₂, pH 6.0, and crude plasma membranes were separated by centrifugation at 45000g-min. Phosphatidylinositol kinase activity was detected in the membranes, but not in the particle-free supernatant obtained by centrifuging the lysate at 6.8 $\times$ 10⁶g-min. Separation of membrane fractions from the yeast on a discontinuous gradient of sucrose has

shown a preferential location of the enzyme in a plasma-membrane-rich fraction. The distribution of phosphatidylinositol kinase in the subcellular fractions is similar to that of the (Na⁺+K⁺)-Mg²⁺-stimulated adenosine triphosphatase. The yeast enzyme, like the mammalian enzyme, is stimulated by Mg²⁺, but differs from mammalian phosphatidylinositol kinase in showing a greater linearity on time-activity plots. Also, it may require a higher concentration of phosphatidylinositol in the reaction mixture.

Cerbón, J. (1970) *J. Bacteriol.* **102**, 97

Lester, R. L. & Steiner, M. R. (1968) *J. Biol. Chem.* **243**, 4889

The Hexose Phosphate Synthetase of *Methylococcus capsulatus*

By M. B. KEMP (Borden Microbiological Laboratory, Shell Research Ltd., Sittingbourne, Kent, U.K.)

Previous work (Kemp & Quayle, 1966; Lawrence *et al.*, 1970) established the existence in crude extracts of *Methylococcus capsulatus* of an enzyme system catalysing the condensation of [¹⁴C]formaldehyde with D-ribose 5-phosphate to give a hexose phosphate, tentatively identified as allulose phosphate.

The enzyme system has now been fractionated by high-speed centrifugation of ultrasonic extracts. When the soluble fraction was incubated with D-ribose 5-phosphate, a product was formed that could condense with formaldehyde in the presence of the particulate fraction. Various lines of evidence support the identification of the intermediate as D-ribulose 5-phosphate, although the commercial product inhibited the condensation. For instance, the soluble fraction could be replaced by a phosphoriboisomerase preparation purified from spinach.

The nature of the condensation product has been re-examined. The previous work (Kemp & Quayle, 1966) suggested that the sugar moiety was allulose. Further, reduction of the sugar with borohydride gave the two sugar alcohols expected from allulose, allitol and altritol. However, g.l.c. of the trimethylsilyl derivative has clearly differentiated the compound from allulose (kindly given by Dr. F. J. Simpson). Also, neither the sugar phosphate formed in the condensation nor the free sugar isolated by chromatography after treatment with phosphatase gave the colour reactions expected for a 2-hexulose (phosphate).

The possible nature of the product will be discussed and analogy drawn to the condensation of formaldehyde with dihydroxyacetone phosphate catalysed by rat liver (Charalampous & Mueller, 1953).