Hexose-phosphate as an Intermediate in the Assimilation of Methanol by *Candida* sp

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A number of studies on the assimilation of methane, methanol and formate by bacteria have so far revealed the following pathway; the ribulose diphosphate cycle,¹⁾ the glycine-serine pathway^{2,3)} and the allulose phosphate pathway.⁴⁾ However, the assimilative pathway of methanol in yeast has not been reported. The previous paper⁵⁾ has shown that *Candida* N-16 capable of growing on methanol can oxidize methanol, formaldehyde and formate to carbon dioxide. In the present paper it is reported that most of the radioactivity of ¹⁴C-methanol fixed by the yeast at the earliest time of incubation appeared in hexose-phosphate, presumably fructose or its-related compound.

The yeast was grown in a medium containing 1%(w/v) methanol at 30°C as described previously.⁵⁾ Cells in a logarithmic growth phase were harvested by centrifugation, washed twice with 10 mM phosphate buffer (pH 7.3), and then suspended in the same buffer. The incorporation of ¹⁴C-methanol was carried out in a 50 ml Erlenmeyer flask containing 10 ml of a reaction mixture. The reaction mixture consisted of 19 mg (dry wt.) of cells, ¹⁴C-methanol (100 μ Ci, 20 μ moles) and 40 μ moles of phosphate buffer (pH 7.3). At time intervals 1 ml of the mixture was transferred to 4 ml of hot ethanol, boiled for 20 min and then centrifuged. The resultant precipitates were extracted with 5 ml of 20% ethanol and centrifuged. The combined supernatant was evaporated to dryness under a reduced pressure at 45°C. The residue was dissolved in 100 μ l of 20% ethanol, 50 μ l of which was applied on filter paper (Toyo No. 50) for

two-dimensional and descending paper chromatography using the following solvents:⁶⁾ phenol-water (100:40, v/v) and *n*-butanol-propionic acid-water (142:71:100, v/v). After chromatography the paper was exposed to X-ray film (Sakura X-ray film; non-screen type N) to prepare radioautogram. Radioactive spots were cut out from chromatogram and the radioactivity was measured by a liquid scintillation spectrometer (Packard 'Tricab' model 3320). A scintillation mixture contained 4% naphthalene, 0.4% 2,5-diphenyl-oxazole and 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene in dioxane-cellosolve-toluene (3:3:10, v/v). The radioactivity of a phosphorylated compound was measured after the compound was eluted from chromatogram, hydrolyzed by phosphatase and subjected to two-dimensional paper chromatography using the following solvents:⁷⁾ phenol-water (5:1, v/v) and *n*-butanol-pyridine-water (6:4:3, v/v). For dephosphorylation the compound was incubated with 100 μ g of acid phosphatase (wheat germ, Seikagaku Kogyo Co.) in 100 μ l of 2 mM acetate buffer (pH 5.0) at 30°C for 12 hr. The identification of a radioactive compound was carried out by co-chromatography with each authentic sample. The two-dimensional paper chromatography of sugar was carried out by the following solvents:⁸⁾ phenol-90%(w/v) formic acid-water (500:13:167, w/v/v) and *n*-butanol-propionic acid-water (47:22:31, v/v). For the paper chromatography of amino acid, ethanol-water (7:3, v/v) was used as solvent, and for carboxylic acid *n*-butanol-acetic acid-water (4:1:2, v/v). The radioactive spot was revealed by radioautography and the authentic compound was detected by spraying with the following reagent: for ketose⁹⁾ 0.2% naphthoresorcinol in ethanol-phosphoric acid (10:1, v/v), for aldose¹⁰⁾ a reagent containing 2 g of diphenyl amine, 2 ml of aniline and 10 ml of 80% phosphoric acid in 100 ml of acetone, for amino acid 0.02% ninhydrine in butanol and for carboxylic acid 0.1% bromocresol green (pH 5.0) in ethanol.

The radioactivity of ¹⁴C-methanol rapidly

- 3) P. J. Large and J. R. Quayle, *Biochem. J.*, **87**, 386 (1963).
 - 4) M. B. Kemp and J. R. Quayle, *ibid.*, **102**, 94 (1967).
 - 5) T. Fujii and K. Tonomura, *Agr. Biol. Chem.*, **36**, 2297 (1972).
 - 6) N. Ogasawara and S. Miyachi, *Plant and Cell Physiol.*, **11**, 1 (1970).
 - 7) A. Jeanes, C. S. Wise and R. J. Dimler, *Anal. Chem.*, **23**, 415 (1951).
 - 8) M. B. Kemp and J. R. Quayle, *Biochem. J.*, **99**, 41 (1966).
 - 9) V. Prey, H. Berbalk and M. Kausz, *Mikrochim. Acta*, **1961**, 968.
 - 10) R. W. Bailey and E. J. Bourne, *J. Chromatog.*, **4**, 206 (1960).
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