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A NEW ENZYMATIC SYNTHESIS OF HEXOSE PHOSPHATES[1]

Sir:

An enzyme which catalyses the formation of hexose-6-phosphate and ammonia from potassium phosphoramidate (PNH_2) and hexose (reaction 1) has been obtained from extracts of succinate grown *E. coli*. The enzyme, presently named phosphoramidic hexose transphosphorylase (PHT), has been purified about 60-fold by fractionation with protamine sulfate, ammonium sulfate and diethylaminoethyl cellulose. The formation of P^{32} labeled organic phosphate esters from the free sugar and labeled PNH_2 was used as an enzyme rate assay. The results shown in Table I indicate that PHT catalyzed a phosphoryl transfer from PNH_2 to several hexoses, although the rate differed considerably with different hexoses. Neither pentoses nor nucleosides were phosphorylated by partially purified PHT.

TABLE I

FORMATION OF HEXOSE PHOSPHATES FROM PHOSPHORAMIDATE

The reaction mixture contained in 1 ml. 2-amino-2-methyl-1,3-propanediol buffer, *p*H 8.0, 100 μmoles; PNH_2, 9 μmoles (2160 c.p.m. per μmole); carbohydrate, as shown, 10 μmoles; enzyme *ca.* 0.9 mg. protein (obtained from a protamine treated extract of *E. coli* by precipitation with $(NH_4)_2SO_4$ at 68 to 78% saturation). Reaction was incubated at 37°, and stopped by addition of 0.5 ml. of 12% trichloroacetic acid and boiled for two minutes to hydrolyze remaining PNH_2.

Organic substrate	Total incorporation[a] c.p.m./15 min.	PNH_2 utilization[b] μmoles/15 min.
D-Fructose	3810	1.85
D-Fructose[c]	3030	1.40
L-Sorbose	2480	1.15
D-Glucose	1800	0.80
D-Glucosamine	980	0.45
D-Galactose	370	0.20

[a] Organic and inorganic phosphates separated by method of S. O. Nielson and A. L. Lehninger, *J. Biol. Chem.*, **215**, 555 (1955). [b] Measured as inorganic phosphate by method of C. H. Fiske and Y. SubbarRow, *ibid.*, **66**, 375 (1925). [c] Enzyme pretreated with charcoal at *p*H 5.5, 0.7 mg. of protein in assay.

The PHT reaction could be coupled to the reaction catalyzed by glucose-6-phosphate dehydrogenase, when glucose was used as the phosphate acceptor. Thus, the rate of the over-all reaction could be followed by reduced triphosphopyridine nucleotide (TPNH) formation and furthermore glucose-6-phosphate could be assumed as the product of transphosphorylation reaction. When individual hexoses were used as phosphate acceptors, with PHT and PNH_2, the corresponding hexose-6-phosphate was isolated and identified chromatographically using the solvent systems of Mortimer.[2]

PHT showed neither a divalent metal requirement nor a dependence on added nucleoside diphosphates and treatment of the partially purified enzyme with charcoal (*p*H 5.5), or with Dowex-I or with Versene (*p*H 7.5) failed to remove any cofactors participating in the reaction. Furthermore, when PHT was coupled with glucose-6-phosphate dehydrogenase no TPNH formation was observed if PNH_2 was replaced by adenosine triphosphate (ATP). When crystalline yeast hexokinase replaced PHT in the coupled system TPNH formation was observed only if ATP was added.

The enzyme preparation used in these studies was contaminated with PNH_2 hydrolase activity,[3] making stoichiometric measurements of NH_3 in reaction 1 unreliable. Further work on the purification and characterization of PHT is in progress.

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(2) D. C. Mortimer, *Can. J. Chem.*, **30**, 653 (1952).

(3) R. A. Smith and D. J. Burrow, *Biochim. et Biophys. Acta*, **34**, 274 (1959).

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THE EFFECT OF PRESSURE ON SEDIMENTATION RATE

Sir:

Recently, Fujita[1] has derived relationships relating the boundary position to time for a monodisperse species in a sector-shaped cell, when the sedimentation coefficient depends on both pressure and concentration.

The boundary position $y_* = (r/r_0)^2$, the dilution (or concentration) factor $\theta_* = C/C_0$ and the reduced time, $\tau = 2\omega^2 S_0 t$, are related as shown in eq. (1).

$$\frac{dy_*}{d\tau} = \frac{y_*}{1 + \alpha\theta_*}(1 - m(y_* - 1)) \qquad (1)$$

where m is a pressure dependence parameter[1] and α is a concentration dependence parameter.[1]

We have solved Fujita's system of Equations (69) through (72), p. 3603 of reference (1), by the use of Runge-Kutta integration combined with trial and error iteration, using a Bendix G-15D digital computer programmed in pseudocode. A range of α from 0.1 to 1.0 was covered, and of m from 0.1 to 0.9. For flotation, negative values of τ were used. In the case of flotation, the reference pressure is the pressure at the cell bottom (see reference (1)) for definition of symbols.

It is the purpose of this communication to show that a simple relation between boundary position and reduced time can be developed, which fits the exact solution of this system of equations to a very good approximation. Oth and Desreux[2] developed a relationship which is essentially equivalent to letting $\theta_* = 1/y_*$ in Equation (1).

(1) H. Fujita, THIS JOURNAL, **78**, 3598 (1956).

(2) J. Oth and V. Desreux, *Bull. soc. Chim. Belges*, **63**, 133 (1954).