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A SOLUBLE ENZYME SYSTEM FROM YEAST WHICH CATALYZES THE BIOSYNTHESIS OF INOSITOL FROM GLUCOSE*

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Previous studies in our laboratory on the biosynthesis of inositol from glucose had shown that in yeast glucose undergoes extensive biochemical transformations resulting in redistribution of its carbon atoms prior to its ultimate conversion to inositol. Preferential utilization of certain carbon atoms of glucose was also demonstrated (Charalampous, 1957). Similar studies in higher plants (Loewus and Kelley, 1962) and in the rat (Imai, 1963) suggested that glucose was converted to inositol after relatively little redistribution of its carbon atoms. More recently Hauser and Finelli (1963) reported that mammalian kidney slices could synthesize radioactive inositol from C^{14} labeled glucose, and that carbon atom 6 of glucose was incorporated into inositol less efficiently than carbon atoms 1 and 2.

In order to obtain unequivocal evidence of the mechanism of conversion of glucose to inositol it was necessary to obtain cell-free systems which would enable one to study this mechanism in greater detail. The present communication describes a soluble enzyme system from yeast which catalyzes the synthesis of inositol from glucose.

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Materials and Methods. Uniformly labeled C^{14}-glucose was a crystalline product purchased from Volk Radiochemical Company. Nucleotides were purchased from Sigma Chemical Company. The yeast used in these studies was the Candida utilis (Torulopsis utilis) strain Y-900 obtained from the American Type Culture Collection. It was grown in the medium described by Lewis (1944) at 28°C with constant shaking. The cell-free extract was prepared by suspending the washed cells in 0.02 M phosphate buffer, pH 7.2 containing glutathione at 5×10^{-4} M, and pressing in a French pressure cell at 6000 lbs. per square inch. The suspension was centrifuged at 900 x g for 10 minutes in the International refrigerated centrifuge. The resulting supernatant fluid was centrifuged at 100,000 x g for 50 minutes in the Spinco ultracentrifuge. The clear supernatant fluid was dialyzed overnight at 2°C against 0.02 M phosphate buffer, pH 7.2 containing 0.1 M KCl. This dialyzed, water clear, pale-yellow solution was used as the source of enzyme. The standard conditions for assaying of enzymatic activity are given in Table 1. At the end of the incubation the reaction was terminated by heating in a boiling water bath for 2 minutes followed by centrifugation of precipitated proteins. To the supernatant fluid containing the radioactive inositol was added equal volume of 0.3 N $Ba(OH)_2$, and the mixture was heated at 100°C for 15 minutes. The resulting precipitate was removed by centrifugation and the supernatant fluid was deionized by passing through short columns of Dowex-1 (acetate) and IR-120 (H^+) resins. The radioactive inositol was isolated by paper chromatography using isopropanol:pyridine:water (3:1:1 v/v) as the solvent system. The inositol area was eluted and an aliquot was plated in stainless steel planchets and counted in a windowless flow counter. The purity of the isolated inositol was confirmed by: a) sequential paper chromatography in two additional solvent systems: acetone:water (85:15); and phenol:water (4:1 w/w). It was found that when the radioactivity eluted from the inositol area of the first chromatogram was rechromatographed sequentially in the other two solvent systems all the radioactivity was recovered in the inositol area; b) crystallization of the radioactive inositol to constant specific activity using carrier inositol. The crystals melted at 227° in the Fisher Johns melting point apparatus; c) conversion of the isolated radioactive inositol to its hexaacetate and crystallization to constant specific activity. The hexaacetate had a m.p. of 218° as compared to 216° (Griffin and Nelson, 1915), and a specific activity which was greater than 97% of that of the radioactive inositol used in its preparation.

Results. Table 1 shows that the conversion of glucose to inositol proceeds at a constant rate for at least two hours.

TABLE 1

Biosynthesis of inositol-C^{14} from glucose-C^{14}

Incubation Time (min.)	Total c.p.m. in Inositol
15	1,640
30	3,100
45	5,180
60	6,360
120	14,220

The incubation mixture contained in a final volume of 1.5 ml. (in μmoles): ATP 15.0; nicotinamide 15.0; DPN 1.2; $MgCl_2$ 8.0; carrier inositol 0.27; uniformly labeled glucose-C^{14} 23.5 with a specific activity of 63,000 c.p.m. per μmole; Tris buffer pH 8.0 160; enzyme solution (6.0 - 6.2 mg. of protein). Incubation temp. 29°C.

Experiments were performed to determine the cofactor requirements of this system. Table 2 summarizes the data. It is seen that the enzyme system has an absolute requirement for DPN, $MgCl_2$ and a nucleoside triphosphate as an energy source. Of the compounds tested, ATP is the best energy source with UTP and ITP exhibiting 80% of the activity of ATP. GTP is only 28% as active as ATP. TPN cannot replace DPN, and addition of TPN to the system containing DPN causes slight inhibition. The optimum concentration of $MgCl_2$ under standard conditions of assay described in Table 1 was found to be 0.003 M. Above this concentration $MgCl_2$ inhibited the reaction, and at 0.009 M it caused 38% inhibition.

TABLE 2

Cofactor Requirements

Conditions	Total c.p.m. in Inositol
Standard*	4,740
" - ATP	147
" - ATP + UTP	3,792
" - ATP + ITP	3,839
" - ATP + GTP	1,327
" - DPN	891
" - DPN + TPN	957
" + TPN	4,027
" - $MgCl_2$	123
" but with boiled enzyme solution	42

*Standard conditions are those described in Table 1. Each of the nucleoside triphosphates was present at a concentration of 0.01 M; TPN at 8 x 10^{-4} M. Incubation time 40 minutes.

The effect of varying amounts of DPN on the enzymatic activity is shown in Fig. 1.

The dependence of the rate of biosynthesis of inositol on the amount of enzyme solution added is shown in Table 3.

Discussion. The present studies represent the first demonstration of a soluble enzyme system which catalyzes the biosynthesis of inositol from glucose. Under conditions of our assay the rate of

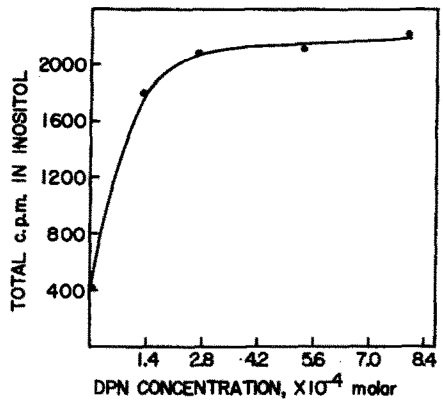

Fig. 1. Effect of DPN concentration on the biosynthesis of inositol from glucose-C^{14}. The incubation mixture was similar to that described in Table 1 except that 4.0 μmoles of $MgCl_2$ were used instead of 8.0, and the protein content was 5.4 mg. Incubation time was 40 minutes

TABLE 3

Enzyme solution in ml.	Total c. p. m. in Inositol
0.1	380
0.2	910
0.4	2,200
0.6	3,280

Conditions were the same as those mentioned in Fig. 1, with DPN at 8 x 10^{-4} M. The enzyme solution contained 13.0 mg. of protein per ml.

the reaction is linear over two hours of incubation, and it can be calculated that at least 220 mμmoles of inositol were synthesized at the end of this period. This figure was calculated from the total radioactivity of the isolated inositol and the specific activity of the radioactive glucose. It represents a minimal figure since it was assumed that the radioactivity of glucose was not diluted by endogenous substances, and that the radioactive inositol was uniformly labeled.

The availability of a soluble enzyme system that can synthesize inositol from glucose makes it now possible to study in detail the enzymatic mechanism.

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