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Phosphofructokinase determination

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Abstract

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Phosphofructokinase (PFK) activity is assayed by a modification of the method of Ling, et al. (1966 p. 425. In Colowick and Kaplan, (eds) Methods in Enzymology Vol. 9, Academic Press, N. Y.). Aldolase, triosephosphate isomerase (TPI) and a-glycerophosphate dehydrogenore (a-GPDH) are coupled with the PFK-catalyzed reaction ond the resulting oxidation of DPNH is recorded as a decrease in optical density at 340 mμ.

$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow[Mg^{++}]{\text{PFK}} \text{Fructose-1,6-diphosphate} + \text{ADP} + H^+$$

$$\text{Fructose-1,6-diphosphate} \xrightarrow{\text{Aldolase}} \text{Triose phosphates}$$

$$\text{Glyceraldehyde-3-phosphate} \xrightarrow{\text{TPI}} \text{Dihydroxyacetone phosphate}$$

$$\text{Dihydroxyacetone phosphate} + \text{DPNH} + H^+ \longrightarrow \text{o-Glycerophorpho'e} + \text{DPN.}$$

Preparation of crude extract: The washed mycelial mat is lyophilized and milled (Wiley) to pars 60 mesh sieve. One gram of powder is homogenized with 10 ml of 0.03 hl KF, 0.001 M EDTAn an ice bath. 0.5 ml of 1 M $MnCl_2$ is added to precipitate the nucleic acids. The homogenate is centrifuged to 60 min at 15,000 X g ond the precipitate is discorded.

Assay Procedure: The assay mixture has a final volume of 1 ml and contains 50 mM Tris-HCl, pH 8.4, 25 mM fructose-6-phosphate, 5 mM ATP, 4 mM $MgCl_2$, 6.6 mM mercaptoethanol, 0. 16 mM DPNH, and 0.05 ml auxiliary enzyme solution (0.2 mg/ml aldolase, 0.04 mg/ml triosephosphate isomerase, 0.04 mg/ml a-glycerophorphote dehydrogenore, and 0.2 mg/ml bovine serum albumin in 0.01 M Tris-HCl, pH 8.0). The reaction is initiated by the addition of 0.002 ml of extract and the OD change at 340mμ immediately recorded. The reaction velocity normally will not remain linear with time, and it is therefore important to use the initial velocity to determine PFK activity. Background DPNH oxidation is also occasionally encountered before the addition of extract. This must be subtracted from the DPNH oxidation rote after the addition of extract.

One unit of PFK activity is defined as that amount catalyzing the formation of 1 μmole of fructose-!,6-diphorphote per min. at 25°C under the conditions of the standard assay. Specific activity is expressed as units per mg of protein. The value for crude extracts is co. 0. 1. PFK from N. crassa is very ilabile and activity will be alost rapidly in crude extract. delayed b y adding an equal volume of glycerol to the extract ond storing at -20°C. - - - School of Medicine, University of California, Davis, Davis, California 95616.