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Phosphofructokinase determination

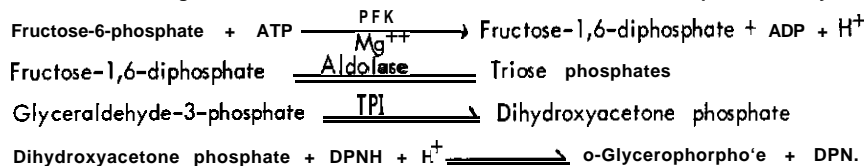
Abstract

Phosphofructokinase determination

Tsao, M. U. and T. 1. Madley. Assay of phosphofructokinase from Neurospora crassa.

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 α -glycerophosphate dehydrogenase (α -GPDH) are coupled with the PFK-catalyzed

reaction and the resulting oxidation of DPNH is recorded as a decrease in optical density at 340 m μ .



Preparation of crude extract: The washed mycelial mat is lyophilized and milled (Wiley) to pass 60 mesh sieve. One gram of powder is homogenized with 10 ml of 0.03 M KF, 0.001 M EDTA on 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 and the precipitate is discarded.

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 α -glycerophosphate dehydrogenase, 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 340 m μ 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 rate after the addition of extract.

One unit of PFK activity is defined as that amount catalyzing the formation of 1 μ mole of fructose-1,6-diphosphate 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 ca. 0.1. PFK from N. crassa is very labile and activity will be lost rapidly in crude extract. delayed by adding an equal volume of glycerol to the extract and storing at -20°C. - - - School of Medicine, University of California, Davis, Davis, California 95616.