Phosphofructokinase determination

M. U. Tsao
T. I. Madley

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Abstract
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This enzyme methodology is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol14/iss1/23
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 o-glycerophosphate dehydrogenase (a-GPDH) are coupled with the PFK-catalyzed reaction and the resulting oxidation of DPNH is recorded as a decrease in optical density at 340 nm.

\[
\text{Fructose-6-phosphate + ATP} \xrightarrow{\text{PFK}} \text{Fructose-1,6-diphosphate + ADP + H}^+ \\
\text{Fructose-1,6-diphosphate} \xrightarrow{\text{Aldolase}} \text{Triose phosphates} \\
\text{Glyceraldehyde-3-phosphate} \xrightarrow{\text{TPI}} \text{Dihydroxyacetone phosphate} \\
\text{Dihydroxyacetone phosphate + DPNH + H}^+ \xrightarrow{\text{o-Glycerophosphate dehydrogenase}} \text{o-Glycerophosphate + DPN.}
\]

Preparation of crude extract: The 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 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 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 o-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 nm is 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 pmole 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, California 95616.

Fass, D. N. Isolation of γ-amylase (glucoamylase) from the culture filtrate of N. crassa. An amylase-super-producer strain, e.g., inas (89601) α (FGSC#498) is grown 84-96 hours in 1% sucrose Vogel's medium. Good yields may be expected from 1 liter cultures in 2.8 I Fernbach flasks incubated at 25°C on a shaker. Adequate aeration is essential for production of the enzyme. At the end of this time, the mycelia are removed by filtration and the medium is chilled to 4°C and cold ethanol is added to 40%. The solution is allowed to stand overnight at 4°C and the resulting precipitate is removed by centrifugation at 25,000 x g for 10 min. To the alcoholic supernatant is added a water solution of 2% glycogen in the proportion of 25 ml/l of original medium. The white precipitate which forms immediately at 4000 X g for 10 min ond renaturated in a small volume of Vogel's salts. This mixture enzyme solution is incubated for 1 hr at 37°C and then dialyzed twice at 4°C against 50 vols of citterate buffer 0.01 M in \(\text{NaNO}_2\), pH 5.0, for 4 hrs and 8 hr. The sample is applied to a 2 x 15 cm column of Amberlite CG-50 equilibrated with the same buffer. Elution is carried out at 4°C with a 500 ml linear gradient from 0.01 to 1.1 N Na\(_2\)PO\(_4\) at approximately 40-50 ml/hr. Citrate is the counter-ion.

The amylolytic activity recovered at about 0.4 N Na\(_2\)PO\(_4\) shows an E-fold increase in specific activity, no invertase or α-amylase activity, and a single bond in acrylamide gel electrophoresis. This work was supported in part by the NSF and NIH Training Grant in Genetics (TOI-GMO1316) to Florida State University. - Genetics Laboratories, Department of Biological Science, Florida State University, Tallahassee, Florida 32306.

Fass, D. N. Agar gel electrophoresis of Neurospora amylases.

The procedure of Kikkawa (1963 Ann. Rep. Sci. Works. Fac. Sci. Osaka Univ. 11: 41) previously used for the production of zymograms of Drosophila amylase has been modified for use with Neurospora crassa. A glass plate ca. 13 x 10 cm is frosted on one side by grinding with household cleaner (Comet). The electrophoretic medium consists of 2% Difco Purified Agar in 0.01 M citric acid buffered to pH 5.0 with NaOH. This some buffer is used in the electrode reservoirs. A layer of reg agar 1 mm thick is pipetted onto the frosted side of the prewarmed plate and is evenly with the ride of pipette. Whatman #2 filter paper strips 1 x 0.15 cm soaked with enzyme are applied too line cm from a long edge of the plate. The enzyme is applied for 10 min, after which the strips are removed. The plate is connected to the reservoirs by the thicknesses of Whatman #1 filter paper. A potential gradient of 40 V/cm is applied and maintained for 2 hours. The amylases will migrate to the cathode.

The plate is then immersed in a 4 mg/ml soluble starch solution for 15 min, followed by a brief water rinse. Digestion of the starch is allowed to occur for 20 min in a 37°C incubator, after which the agar is stained in a solution of 0.3% KI - 0.03% 12. Two types of bonds will be visible: clear (against dork blue) and faint pink. The former ore the Y-amylases (gluco-amylases) and the latter are the α-amylaser.