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Fructosediphosphatase of N. crassa

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Abstract

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Fructosediphosphatase (FDPase) occupies a key step in gluconeogenesis and is a point of regulation in many organisms. In yeast this enzyme is also rapidly inactivated upon glucose repression. We have examined FDPase in Neurospora to determine whether or not this enzyme is regulated by turnover.

Conidia of the *Emerson* a wild type strain (FGSC#352) were inoculated and grown with shaking in Vogel's medium containing 1.5% sucrose for 24 hr. The cells were collected, washed thoroughly and resuspended in fresh medium containing various carbon sources and then shaken for an additional 24 hr. The mycelial pods were collected and homogenized with an equal weight of sand in 3- to 5-fold volumes of 0.05 M Tris-HCl buffer, pH7.5; after centrifugation, the supernatant fluid was passed through a Sephadex G-25 column to remove small molecular weight metabolites which otherwise interferred with the enzyme assay. FDPase activity was *assayed* by coupling it to NADP reduction via glucose-&phosphate dehydrogenase and determining the increase in absorbance at 340 nm. The 2 ml assay mixture contained 1 unit each of hexose isomerase and glucose-6-phosphate dehydrogenase plus the indicated amount of the following compounds in μmoles: Tris-HCl, pH7.5, 80; KCl, 200; MgC12, 20; EDTA, 2; fructose-1,6-diphosphate, 0.4; and NADP, 0.4. The enzyme activity was linear with time and completely dependent upon the addition of fructose-1,6-diphosphate, demonstrating that the assay was specific for FDPase. A single pH optimum of about 7.5 was found. The crude enzyme is quite labile ond significant activity is lost in a few hours at 0-2°C or when it is frozen for even a few days. After partial purification, however, the FDPase activity doer appear to be reasonably stable when frozen.

The level of FDPase possessed by Neurospora is regulated and depends upon the carbon source in which the mycelial pads were incubated during the second 24-hr period. The results of Table 1 show that growth on ethanol maximally derepressed synthesis of the enzyme, whereas sucrose repressed FDPase synthesis. The activity of the enzyme is also sensitive to inhibition by AMP, a likely allosteric effector; 90 μM and 400 μM AMP caused 50% and 85% inhibition of FDPase activity, respectively. To determine whether or not FDPase was subject to turnover, mycelia which hod been, incubated with ethanol and had a derepressed level of the enzyme were transferred to sucrose-containing medium in which new enzyme synthesis was repressed. The fate of the preexisting enzyme was then followed for O-6 hrs; FDPase was found to be a fully stable enzyme in vivo. Our experience with FDPase and similar enzymes indicates that turnover doer not constitute a general mechanism for regulation of the levels of cytoplosmic enzymes in Neurospora.

Table 1. Levels of FDPase after growth on various carbon sources.

Derepressing C source (1.5%)	Activity (milli-units/mg protein)
Sucrose	1.07
Pyruvate	2.20
Succinate	2.32
Acetate	2.79
Ethanol	5.36

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