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Evidence for a single hexokinase in *Coprinus cinereus*

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1. INTRODUCTION

Wild-type monokaryotic mycelia of the basidiomycete *Coprinus cinereus* grow equally well whether glucose or fructose is given as sole source of carbon + energy [1]. Growth is inhibited by a number of sugar analogues [2,3], and many mutants have been isolated which are resistant to such inhibitions [4,5]. The mutants so far described are alleles of a single cistron (called *ftr*) [6] and share, among other phenotypic features, an inability to grow on fructose as sole carbon + energy source (Fig. 1). Although it is assumed that the *ftr* mutants are defective in a transport process, the possibility of their having a defect in a specific fructokinase has not been properly excluded. We report here an analysis of hexose phosphorylating activity in wild type and mutant alike which indicates that *Coprinus* normally possesses a single hexokinase of broad substrate specificity and that *ftr* mutants show an unimpaired activity of this enzyme.

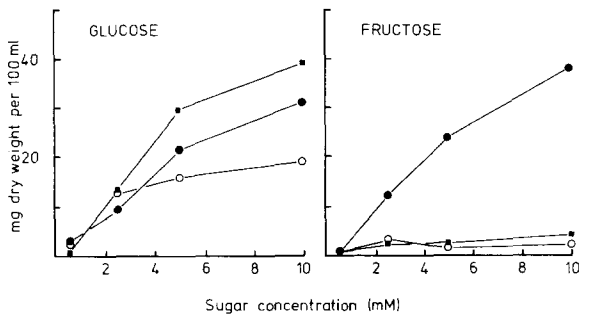

Fig. 1. Dry weight yield of the wild type BC9/6,6 (●), and *ftr* mutants Z492/6,6 and ZR215/40,40 (○ and ■, respectively) grown as still cultures at 37°C for 8 days in medium containing either glucose or fructose as sole carbon source.

3. MATERIALS AND METHODS

2.1. Organism

The strains of *Coprinus cinereus* (Schaeff. ex Fr.) S.F. Gray used in this work were the wild type monokaryon, stock number BC9/6,6, and the *ftr* mutants numbered Z492/6,6 and ZR215/40,40. Most culture techniques and media have been described before [1–6].

2.2. Enzyme extraction

Mycelia harvested from liquid medium were homogenized with 50 mM Tris–HCl buffer pH 7.5. Debris was removed by centrifuging and hexokinase separated by fractional precipitation with ammonium sulphate using a modification of the procedure of Womack et al. [7]. All hexokinase activity precipitated in the range 45–75% saturation. After washing the precipitated enzyme was dissolved in 50 mM Tris–HCl pH 8 containing 2 mM mercaptoethanol and was then used without further purification.

2.3. Assay procedures

Glucose phosphorylation activity was measured by following the appearance of NADPH in a

coupled system containing commercial glucose-6-phosphate dehydrogenase. When other hexoses were tested as substrate for hexokinase the alternative assay measuring the rate of ADP formation by coupling with pyruvate kinase and lactate dehydrogenase was used. The two methods were modified from the assays described by Maitra [8].

3. RESULTS AND DISCUSSION

Adomako et al. [9], working with *Chaetomium globosum*, found separate glucokinase and fructokinase activities on the basis of stability and response to K^+ ions, the fructokinase being especially unstable in the absence of potassium. On the other hand, Mazon et al. [10] distinguished a very specific glucokinase activity from a hexokinase with broad substrate specificity on the basis of the greater thermostability of the latter enzyme.

The optimum extraction method for both glucose- and fructose-phosphorylating activity from *Coprinus* does not include K^+ ions and we have not observed any dependence of either enzyme activity on this metal. Furthermore, both activities were found to be stable for some days when stored in Tris–HCl buffer (pH 8) containing 2 mM mercaptoethanol. Similarly, the two enzyme activities revealed similar responses to high-temperature

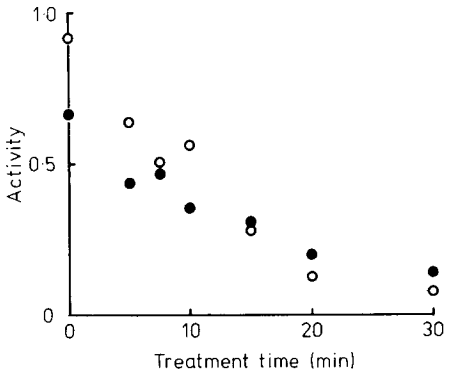

Fig. 2. Heat inactivation of sugar phosphorylating activity. Samples of the same enzyme preparation were assayed with fructose as substrate (fructokinase, ●) or with glucose as substrate (glucokinase, ○) after various times of treatment at 55°C. Enzyme activity expressed in arbitrary units.

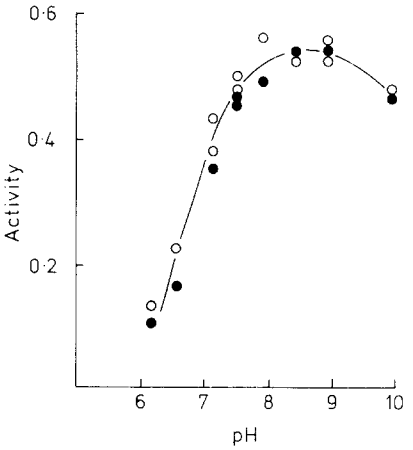

Fig. 3. Dependence of sugar phosphorylating activity on the pH of the assay mixture. Comparison of 'fructokinase' (●) and 'glucokinase' (○). Enzyme activity expressed in arbitrary units.

treatment (Fig. 2) and had identical pH-activity profiles (Fig. 3). Determination of substrate affinities revealed the following K_m values: glucose 0.1 mM, glucosamine 0.1 mM, 2-deoxy-D-glucose 0.4 mM, fructose 2.9 mM, 3-*O*-methylglucose 7.5 mM, and ATP 0.15 mM. Sorbose was not used as a

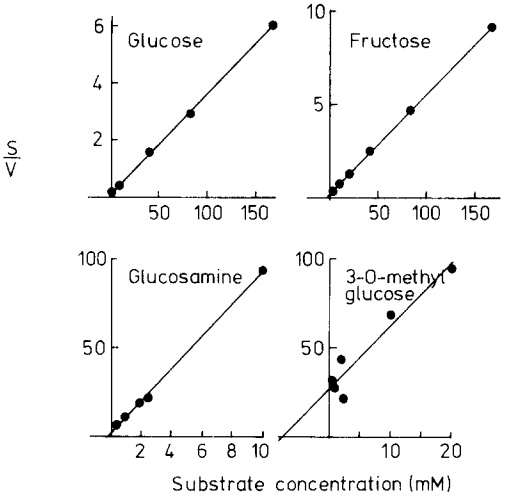

Fig. 4. Kinetic plots ([S]/V×[S]) showing relationship of hexokinase with different substrates. K_m values were calculated from these plots by regression analysis and are given in the text. The plots are presented to compare results obtained with 3-*O*-methylglucose with those obtained with the other sugars. For glucose and fructose the vertical scales are contracted by a factor of 100.

Table 1

Comparison of enzyme activities in mycelium grown with either glucose or fructose as sole carbon source

Mycelium	Carbon source in medium	Hexokinase activity	
		With glucose as substrate	With fructose as substrate
Wild type BC9/6,6	glucose	223 [a]	135
Wild type BC9/6,6	fructose	273	155
ftr strain Z492/6,6	glucose	253	165
ftr strain Z492/6,6	fructose	291	195

[a] Specific activity in units of nmol substrate used/min/(mg protein).

substrate. We were able to detect activity in vitro with 3-*O*-methylglucose (indeed the V_{max} was about the same as that observed with glucose) although the data obtained were unusually erratic at low substrate concentrations (Fig. 4). The material was found to be chromatographically homogeneous but no phosphorylated sugars could be detected in vivo after exposure to radioactively labelled 3-*O*-methylglucose. This sugar analogue is usually considered to be metabolically inert; the in vitro activity observed here suggests that some caution should be applied to its use.

The substrate affinities listed above do not markedly differ from those reported for hexokinases from many other sources. Another similarity between *Coprinus* and other organisms is that we observed no difference in activity between material grown on glucose and that grown on fructose as sole carbon sources (Table 1). Table 1 also shows that the *ftr* mutant, Z492/6,6, had much the same enzyme activities as did the wild type. We therefore conclude that hexose phosphorylation in *C. cinereus* is carried out by an hexokinase of broad substrate specificity and that the enzyme is unimpaired in *ftr* mutants.

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