SHORT COMMUNICATION

Glycerol Utilization by *Schizosaccharomyces pombe*: Dehydrogenation as the Initial Step

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Strains NCYC 132 and 972h⁻ of the fission yeast *Schizosaccharomyces pombe* both lacked glycerol kinase, but produced an NAD⁺-linked glycerol dehydrogenase. In both strains, production of the dehydrogenase was subject to catabolite repression, although in strain 972h⁻ glycerol also appeared to exert some inductive effect.

INTRODUCTION

Although glycerol is used by many yeasts as a growth substrate (Barnett, 1976), the mechanism of its utilization has been investigated in very few species. Following the detection of glycerol kinase in *Saccharomyces cerevisiae* (Wieland & Suyter, 1957) and *Candida mycoderma* (Bergmeyer *et al.*, 1961), Gancedo *et al.* (1968) proposed that in *Candida utilis* glycerol was metabolized by a pathway which involved phosphorylation of the glycerol to yield *sn*-glycerol 3-phosphate (G3P), followed by dehydrogenation of the G3P to yield dihydroxyacetone phosphate. However, although Hunter & Rose (1971) and Sols *et al.* (1971) inferred that this was the only pathway of glycerol metabolism in yeasts, only in *Saccharomyces cerevisiae* has the role of the pathway been confirmed (Sprague & Cronan, 1977). Regulation of glycerol kinase and G3P dehydrogenase has been studied in *Candida utilis* and *Saccharomyces cerevisiae*: in the former, the enzymes were inducible (Gancedo *et al.*, 1968); whereas in the latter, catabolite repression was the principal controlling factor (Sprague & Cronan, 1977).

This report describes an investigation of the nature and regulation of the primary enzyme involved in the utilization of glycerol by the fission yeast *Schizosaccharomyces pombe*.

METHODS

Maintenance of strains. *Schizosaccharomyces pombe* NCYC 132 and 972h⁻ were maintained as described by Mitchison (1970), on slopes of malt extract agar (Oxoid) for long-term storage and in Edinburgh minimal medium no. 1 for short-term stocks.

Growth and harvesting of cells. For each starter culture, 100 ml of Edinburgh minimal medium no. 2 (Mitchison, 1970), modified by reducing the glucose to 0.5% (w/v), was inoculated with 1 ml of stock culture and incubated until well into the stationary phase (72 h). Cells for determination of enzyme activities were grown in 500 ml batches of Edinburgh minimal medium no. 2 containing either glucose (the normal carbon source) or glycerol (3%, w/v), each batch being inoculated with 4 ml of starter culture. These cultures were grown aerobically at 30 °C in Erlenmeyer flasks (100 ml in 500 ml flasks and 500 ml in 2 l flasks) on an orbital shaker (Gallenkamp) rotating at about 200 rev. min⁻¹. Absorbance values of samples of cultures were measured at a wavelength of 450 nm using a single-beam spectrophotometer (Spectronic 20; Bausch & Lomb). Cells were harvested from chilled cultures by centrifuging (700 g, 20 min, 4 °C) and then were washed twice in ice-cold Tris/HCl buffer (0.1 M, pH 7.6). When required for estimation of glucose, the cell-free supernatant fluid obtained by centrifuging a culture was removed and stored at -20 °C.

Preparation of cell-free extracts. Immediately after harvesting and washing, cells (4 to 8 g wet wt) were resuspended in 5 ml of ice-cold Tris/HCl buffer (0.1 M, pH 7.6) containing dithiothreitol (4 mM) and then disrupted by passing three times through a pre-cooled French pressure cell (Aminco) operated at approximately 138 MPa. Debris was removed by centrifuging (27 000 g, 30 min, 4 °C) and the resulting clear supernatant fluid was used for enzyme assays.

Enzyme assays. All assays were carried out in triplicate at 25 °C. A double-beam recording spectrophotometer (model 552, Perkin Elmer) was used to follow changes in absorbance at 340 nm. Radioactivity was measured in a liquid scintillation spectrometer (model 2211, Packard).

Glycerol kinase (EC 2.7.1.30) was assayed by two procedures, both depending upon the amount of G3P formed from glycerol and ATP. In the first, the rate of formation of G3P was ascertained by coupling with G3P dehydrogenase and measuring the reduction of NAD⁺ spectrophotometrically as described by Wieland & Suyter (1957). In the second, the [¹⁴C]G3P formed from [¹⁴C]glycerol was adsorbed to a disc of DEAE-cellulose (Whatman DE81) as described by Thorner & Paulus (1973), except that the reaction mixture also contained 90 mM-hydrazine. After drying, each disc was placed in a separate vial together with 1 ml of 0.1 M-HCl and 10 ml of scintillation fluid [10 g 2,5-diphenyloxazole and 0.2 g 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in 2 l toluene plus 1 l Triton X-100].

Glycerol dehydrogenase was assayed by following the reduction of NAD⁺ spectrophotometrically as described by Ruch *et al.* (1974).

The molar absorption coefficient of NADH at 340 nm was taken to be $6.22 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$. Specific activities of enzymes are expressed as nmol substrate transformed min⁻¹ (mg protein)⁻¹.

Analytical procedures. Protein was estimated by the Lowry method using bovine serum albumin as the standard. Glucose was estimated using the glucose oxidase method (GOD-Perid; Boehringer).

RESULTS AND DISCUSSION

Using either method of measuring G3P, we could not detect any glycerol kinase activity in cell-free extracts of either strain of *S. pombe* when harvested during exponential growth in glycerol medium. However in the same extracts we found significant activities of an NAD⁺-linked glycerol dehydrogenase.

The effect of different conditions of growth upon the activity of the NAD⁺-linked glycerol dehydrogenase in cells of *S. pombe* is shown in Table 1. In both strains the activity of the enzyme in cells growing on glycerol was higher than in cells growing on glucose (12-fold in strain 132 and 29-fold in strain 972h⁻). The activity of the enzyme in stationary phase (i.e. glucose-depleted) cells was also increased in relation to the activity in cells growing on glucose (30-fold in 132 and 19-fold in 972h⁻). Thus the activity of the enzyme in glucose-depleted cells relative to that in cells growing on glycerol was higher (250%) in 132, but lower (64%) in 972h⁻.

The different patterns of production of the NAD⁺-linked glycerol dehydrogenase shown by the two strains of *S. pombe* suggest that they differ in their regulatory systems for the enzyme. Regulation of production of the dehydrogenase in 132 can be explained solely in terms of catabolite repression, the substrate exerting a limited repressive effect rather than acting as a specific inducer. On the other hand, while catabolite repression clearly operates in 972h⁻, the substrate also appears to exert some inductive effect. Nevertheless, this difference between the two strains might not involve a direct inductive role for the substrate in 972h⁻, but rather be a consequence of either (a) the relative levels of the metabolic effector in 132 under the two derepressive conditions (i.e. depletion of glucose and growth on glycerol) being reversed in 972h⁻, or (b) the nature of the metabolic effector in 132 being different from that in 972h⁻. Indeed, whereas 972h⁻ shows an inverse relationship between the intracellular concentration of cyclic AMP and the concentration of glucose in the medium (Schlanderer & Dellweg, 1974), in 132 the intracellular concentration of cyclic AMP is constant over the crucial range of concentrations of medium glucose (Mahadi, 1977). It is interesting to note that Mitchison *et al.* (1973) found that production of maltase in 132 was not induced by substrate, being only repressed by glucose; whereas Schlanderer & Dellweg (1974) reported that in 972h⁻ maltase was induced by substrate as well as being repressed by glucose.

Table 1. Activity of NAD⁺-linked glycerol dehydrogenase in cell-free extracts of *S. pombe*

Specific activities are expressed as nmol substrate transformed min⁻¹ (mg protein)⁻¹. Each value is the mean of measurements made on at least four extracts, each prepared from an independently grown and harvested batch of cells.

Strain	Specific enzyme activity when cells harvested during:		
	Exponential phase in glycerol medium	Exponential phase in glucose medium*	Stationary phase in glucose medium†
132	9.6	0.8	24.3
972h-	17.6	0.6	11.3

* Medium contained >2 mg glucose ml⁻¹ at time of harvesting.

† Medium contained <0.1 mg glucose ml⁻¹ at time of harvesting.

The presence of the NAD⁺-linked glycerol dehydrogenase in the absence of glycerol kinase indicates a primary role for the former enzyme in the utilization of glycerol by *S. pombe*. NAD⁺-linked dehydrogenation of glycerol rather than its phosphorylation, although not previously reported in yeasts, does occur in bacteria where the product – dihydroxyacetone – is phosphorylated to yield dihydroxyacetone phosphate (see Lin, 1976). The nature of the dehydrogenation product from glycerol and its subsequent metabolism in *S. pombe* are currently being investigated.

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