Glycerol Utilization by *Schizosaccharomyces pombe*: Phosphorylation of Dihydroxyacetone by a Specific Kinase as the Second Step

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(Received 11 January 1982)

Crude cell extracts of the fission yeast *Schizosaccharomyces pombe* (strains NCYC 132 and 972h⁻) can phosphorylate dihydroxyacetone but not glycerol; activity for DL-glyceraldehyde is very low. This suggests that a specific dihydroxyacetone kinase is present and catalyses the second step in a pathway for glycerol utilization, in which the initial step is an oxidation by an NAD⁺-linked glycerol dehydrogenase. In support of this pathway (glycerol→dihydroxyacetone→dihydroxyacetone phosphate), both strains can utilize glycerol or dihydroxyacetone, but not DL-glyceraldehyde, as growth substrates. Both enzymes are subject to catabolite repression and may also be inducible but are not co-ordinately regulated.

INTRODUCTION

Most studies on glycerol utilization have been done with bacteria (Lin, 1976) or mammals (Lin, 1977), and in most cases the pathway was found to be:

Gancedo *et al.* (1968) presented evidence that glycerol utilization by *Candida utilis* involves the same pathway; indeed, Hunter & Rose (1971) and Sols *et al.* (1971) inferred this to be the only pathway of glycerol utilization in yeasts. However, we have recently reported that the fission yeast *Schizosaccharomyces pombe* lacks glycerol kinase but possesses an NAD⁺-linked glycerol dehydrogenase, suggesting that it must use an alternative pathway (May & Sloan, 1981).

We now report that *S. pombe* also possesses glucose-repressible kinase activity for dihydroxyacetone but only negligible activity for glyceraldehyde (the other possible product of glycerol dehydrogenation). This is consistent with the pathway: glycerol→dihydroxyacetone→dihydroxyacetone phosphate, reported for some bacteria (see Lin, 1976).

METHODS

Maintenance of strains. *Schizosaccharomyces pombe* NCYC 132 and 972h⁻ were maintained as described previously (May & Sloan, 1981).

Growth and harvesting of cells. Cells were grown in batch culture and harvested as described previously (May & Sloan, 1981), except that flasks were incubated on an orbital shaker (model G-52, New Brunswick Scientific) rotating at 150 rev. min⁻¹.

Preparation of cell extracts. Extracts of washed cells were prepared as described previously (May & Sloan, 1981).

Enzyme assays. All assays were carried out at 20 °C. Reduction of NAD⁺ or oxidation of NADH was measured by following changes in *A*₃₄₀ with a double-beam recording spectrophotometer (model 552, Perkin Elmer).

Glycerol kinase (EC 2.7.1.30) was assayed by the method of Wieland & Suyter (1957), in which the rate of formation of glycerol 3-phosphate from glycerol and ATP was measured by following the rate of reduction of NAD^+ in the presence of glycerol-3-phosphate dehydrogenase.

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

Dihydroxyacetone kinase was assayed by a modification of the method of van Dijken *et al.* (1978) in which the rate of formation of dihydroxyacetone phosphate from dihydroxyacetone and ATP was measured by following the rate of oxidation of NADH in the presence of glycerol-3-phosphate dehydrogenase. Both test and reference cuvettes contained 2.4 ml 0.08 M-hydrazine hydrate in 0.04 M-glycine buffer (pH 9.5), 0.2 ml 0.1 M-ATP and 0.1 ml each of 0.2 M- MgCl_2 , 0.1 M-dihydroxyacetone, 0.02 M-NADH and cell extract. The reaction was started by adding 10 μl (17 units) of glycerol-3-phosphate dehydrogenase to the test cuvette. When dihydroxyacetone was omitted from both cuvettes, no significant oxidation of NADH occurred on adding glycerol-3-phosphate dehydrogenase.

Triose phosphate isomerase (EC 5.3.1.1) was assayed by a method based on that of Bergmeyer *et al.* (1974), in which the rate of formation of dihydroxyacetone phosphate from glyceraldehyde 3-phosphate was measured by following the rate of oxidation of NADH in the presence of glycerol-3-phosphate dehydrogenase. The test and reference cuvettes contained 2.7 ml 0.08 M-hydrazine hydrate in 0.04 M-glycine buffer (pH 9.5) and 0.1 ml each of 0.1 M-DL-glyceraldehyde 3-phosphate, 0.02 M-NADH and cell extract. The reaction was started by adding 10 μl (17 units) of glycerol-3-phosphate dehydrogenase to the test cuvette.

A value of $6.22 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ was used for the molar absorption coefficient of NADH at 340 nm. Specific activities of enzymes are expressed as nmol substrate transformed $\text{min}^{-1} (\text{mg protein})^{-1}$.

Analytical methods. Protein was estimated by the biuret method as described by Koch & Putnam (1971) using bovine serum albumin as the standard. Glucose was estimated using the glucose oxidase method (GOD-Perid; Boehringer).

Chemicals. Dihydroxyacetone and DL-glyceraldehyde were obtained from Sigma (the latter was further recrystallized from ethanol/ether). Glycerol-3-phosphate dehydrogenase, DL-glyceraldehyde 3-phosphate diethyl-acetal (dicyclohexylammonium salt), ATP, NAD^+ and NADH were obtained from Boehringer. All other chemicals were of the highest purity commercially available.

RESULTS AND DISCUSSION

As previously reported (May & Sloan, 1981) cell extracts of both strains of *S. pombe* grown on glycerol medium contained NAD^+ -linked glycerol dehydrogenase activity, but no detectable glycerol kinase. On further examination such extracts were found to possess significant dihydroxyacetone kinase activity.

The effect of different conditions of growth upon the activity of the NAD^+ -linked glycerol dehydrogenase and the dihydroxyacetone kinase in cell extracts of *S. pombe* is shown in Table 1. The levels of glycerol dehydrogenase were similar to those reported by May & Sloan (1981): a low basal level in both strains when grown on glucose and increased levels following exhaustion of glucose or when grown on glycerol. The highest level for strain 132 was found in cells following exhaustion of glucose (27-fold increase over basal level), while for strain 972h⁻ the highest level was found in cells grown on glycerol (35-fold increase over basal level). Similarly, the levels of dihydroxyacetone kinase were low in cells grown on glucose and increased following exhaustion of glucose or when grown on glycerol. However, in each strain the greater increase in the level of dihydroxyacetone kinase was observed in cells grown on glycerol (7-fold in 132 and 6-fold in 972h⁻), with a slightly lesser increase in cells following exhaustion of glucose (6-fold in 132 and 4-fold in 972h⁻). For both strains, in relation to the enzyme levels in glucose-grown cells, the increases in the levels of glycerol dehydrogenase following exhaustion of glucose or when growing on glycerol were 18- to 35-fold, whereas the increases in the levels of dihydroxyacetone kinase under the same conditions were only 4- to 7-fold; this indicates that regulation of the two enzymes is not co-ordinated. Nevertheless, it is clear that both enzymes are subject to catabolite repression, although the glycerol dehydrogenase in 972h⁻ and the dihydroxyacetone kinase in 132 and 972h⁻ may also be subject to induction by glycerol or some related metabolite.

In the dihydroxyacetone kinase assay, the replacement of dihydroxyacetone by DL-glyceraldehyde reduced the rate of formation of dihydroxyacetone phosphate (as judged

Table 1. Activity of NAD^+ -linked glycerol dehydrogenase and dihydroxyacetone kinase in cell extracts of *S. pombe*

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

Strain	Enzyme	Specific enzyme activity when cells harvested during:		
		Exponential phase in glucose medium*	Stationary phase in glucose medium†	Exponential phase in glycerol medium
132	Glycerol dehydrogenase	0.8	21.6	14.2
	Dihydroxyacetone kinase	1.9	11.3	14.1
972h ⁻	Glycerol dehydrogenase	0.6	13.3	21.1
	Dihydroxyacetone kinase	2.1	8.6	12.2

* Medium contained >2 mg glucose ml^{-1} at time of harvesting.

† Medium contained <0.1 mg glucose ml^{-1} at time of harvesting.

by the oxidation of NADH in the presence of glycerol-3-phosphate dehydrogenase) to only 1% for 132 and 6% for 972h⁻. This low activity with glyceraldehyde could be due to: (a) the presence of a specific glyceraldehyde kinase (cell extracts of the yeasts contained sufficient triose phosphate isomerase to convert glyceraldehyde 3-phosphate to dihydroxyacetone phosphate at a rate at least 60 times greater than the observed rate of formation of dihydroxyacetone phosphate from glyceraldehyde); (b) phosphorylation of glyceraldehyde by dihydroxyacetone kinase; (c) the presence of a triose isomerase converting glyceraldehyde to dihydroxyacetone. However, even if dihydroxyacetone phosphate is formed from glyceraldehyde 3-phosphate following phosphorylation of glyceraldehyde by a specific kinase, the low rate of its formation by such a route compared with its rate of formation by phosphorylation of dihydroxyacetone suggests that, of the two activities, phosphorylation of dihydroxyacetone plays the major role in the utilization of glycerol in these two strains of *S. pombe*. Furthermore, whereas both strains were able to grow equally well in Edinburgh minimal medium no. 2 in which the sole carbon source was either glycerol (1%, w/v) or dihydroxyacetone (1%, w/v), neither was able to grow in the medium when the sole carbon source was DL-glyceraldehyde (1%, w/v), indicating that the major if not the only pathway of glycerol utilization in these two strains of *S. pombe* involves dehydrogenation of glycerol to dihydroxyacetone which is then phosphorylated to yield dihydroxyacetone phosphate.

Several enzymes have been reported which can phosphorylate dihydroxyacetone, and their specificity varies widely. The glycerol kinases (EC 2.7.1.30) of *Escherichia coli* and rat liver phosphorylate dihydroxyacetone more rapidly than glycerol (1.87 and 2.0 times more rapidly, respectively) while L-glyceraldehyde is phosphorylated at 43% and 75% of the rates for dihydroxyacetone (Thorner & Paulus, 1973). The glycerol kinase of *Candida mycoderma*, however, phosphorylates dihydroxyacetone and L-glyceraldehyde at approximately equal rates, which are only 10% of the rate for glycerol (Gancedo *et al.*, 1968). Triokinases (EC 2.7.1.28) which have no activity against glycerol and approximately equal activities against dihydroxyacetone and D-glyceraldehyde have been reported in guinea pig liver (Hers, 1962) and human erythrocytes (Beutler & Guinto, 1973). Phosphorylation of dihydroxyacetone, but not glycerol, has been reported also in the methanol-utilizing yeasts *Hansenula polymorpha* and *Candida boidinii* (van Dijken *et al.*, 1978) and *C. methylica* (Hofmann & Babel, 1981). Although the function proposed for these enzymes is to phosphorylate dihydroxyacetone, an intermediate in methanol metabolism, this activity is still accompanied by an appreciable capacity (22–25%) to phosphorylate DL-glyceraldehyde. Indeed, no kinase phosphorylating only dihydroxyacetone and not glyceraldehyde or glycerol is listed by the

International Union of Biochemistry Nomenclature Committee (1979). However, such a specific kinase has been reported recently in *Dunaliella parva* (Lerner & Avron, 1977; Lerner *et al.*, 1980); the suggested function of this enzyme is not catabolic, as presumed for the dihydroxyacetone kinase of *S. pombe*, but to remove dihydroxyacetone produced from glycerol, the osmoregulatory solute of this halophilic green alga (Lerner & Avron, 1977). The enzyme activity reported here in each of the two strains of *S. pombe* would seem to be also due to a dihydroxyacetone kinase, similar to that in *D. parva* in terms of specificity, but functionally quite different.

Further investigations are being carried out to purify and characterize this novel dihydroxyacetone kinase of *S. pombe*.

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