COLD-INDUCED INCREASE OF GLYCEROL KINASE IN *NEUROSPORA CRASSA*

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1. Introduction

A number of fungal enzymes are known which can be induced or repressed by changes in the composition of the growth medium [1,2]. In this communication a novel phenomenon is described in which large changes in an intracellular enzyme activity occur in response to changes in temperature. When the growth temperature of *N. crassa* is lowered a large increase in glycerol kinase (ATP: glycerol phosphotransferase, EC 2.7.1.30) activity occurs which is dependent on protein synthesis. This is believed to be the first report of such a cold-induced increase in an enzyme activity.

2. Materials and methods

Cultures were prepared in Erlenmeyer flasks by inoculating conidia of a wild type St. Lawrence strain, mating type A, into Fries' minimal medium [3] with 2% sucrose as sole carbon source (to give approximately 2×10^4 conidia/ml). They were shaken orbitally in air at 26°C, and reciprocally in water baths at lower temperatures (100 rpm).

Cultures were harvested by filtration onto Whatman filter paper in a Buchner funnel, washed with water and then with Tris buffer (83 mM, pH 8.0). The mycelial mat was finally resuspended in 5 ml of buffer, blended in an M.S.E. homogenizer (Measuring and Scientific Equipment Ltd., London, England) for 1 min, and subjected to 2 min sonication in an M.S.E. ultrasonic disintegrator. The resulting suspension was centrifuged for 20 min at 25 000 *g* in an M.S.E. High Speed 18 and the pellet discarded. Throughout preparation the extract was kept at 0°–4°C and was stored frozen until required. Freezing had no effect on glycerol kinase activity.

Glycerol kinase was assayed radiochemically by a method adapted from that of Newsholme et al. [4]. The final assay medium contained the following, normally in a final volume of 0.225 ml: extract (up to 0.195 ml), Tris–HCl, pH 8.0 (83 mM), EDTA (1.6 mM), mercaptoethanol (31 mM), NaF (22 mM), $MgSO_4$ (5 mM), ATP (5 mM), [1-^{14}C]glycerol (1 mM, 0.84 mCi/mmole) supplied by the Radiochemical Centre, Amersham, England. The mixture was incubated at 25°C, samples were withdrawn into 2 vol ethanol, diluted with unlabelled glycerol and filtered onto Whatman DE 81 paper. The filters were washed with 4% glycerol, followed by a large volume of water, then dried at 90°C for 30 min, and the radioactivity counted in a Beckman scintillation counter. The radioactivity remaining bound to the filter has been shown by chromatography to be due almost entirely to L-α-glycerol phosphate. Glycerol kinase activity is given in units of nmoles glycerol phosphate bound/hr/mg protein.

Protein was estimated by the method of Lowry et al. [5], using bovine serum albumin as the standard.

Cycloheximide and chloramphenicol were supplied by Sigma Chemical Co., London, U.K.

3. Results and discussion

Cultures of *Neurospora* growing on sucrose medium at 26°C possess a very low level of glycerol kinase activity (2–4 units). When cultures are transferred to medium containing glycerol in place of sucrose there

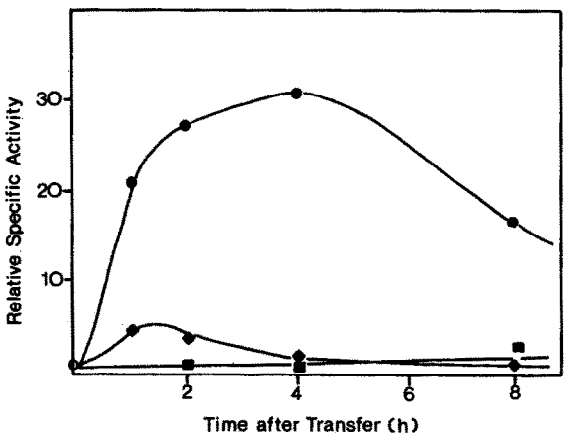

Fig. 1. Effect of glycerol on the glycerol kinase activity in *Neurospora* cultures at 26°C. 100 ml cultures were grown on sucrose medium in 250 ml flasks at 26°C for 48 hr. The cultures were filtered, washed with distilled water and resuspended in 100 ml fresh medium containing: sucrose (■—■—■), glycerol (●—●—●) and no carbon source (♦—♦—♦). Incubation was continued at 26°C and 10 ml samples were taken periodically and extracted. Glycerol kinase specific activity is given relative to the basal level.

is a 20–30-fold increase in this activity (fig. 1). This is much greater than the 4-fold increase achieved by simply removing sucrose. However, with sucrose medium and no added glycerol a marked increase in the specific activity also occurs on lowering the growth temperature.

Fig. 2 shows the effect of temperature change on the glycerol kinase activity. With the possible exception of the effect at 0°C, the changes show the same characteristic pattern independent of the new temperature. Following a lag, which is longer at lower temperatures, the activity rises to a maximum and then decreases slowly. The maximum activity attained increases with decreasing temperature, and varies from approximately six times the basal level at 12°C to nearly 50 times at 4°C, greater than the increase induced by glycerol at 26°C (fig. 1). The difference in the kinetics at 0°C may be due only to the longer time required for the effects to take place. At later times of incubation at 0°C (not shown in fig. 2) the activity levels off but no decrease occurs during at least the first 4 days after the temperature shift.

The increase in activity can be inhibited by cycloheximide, but chloramphenicol, an inhibitor of mitochondrial protein synthesis [6,7], has no effect (table 1). Almost complete inhibition of the cold-induced increase is achieved with cycloheximide at a concentration of 5 μM, which is sufficient to inhibit protein synthesis in *Neurospora* by over 90% [8]. The increase is therefore dependent on cytoplasmic protein synthesis

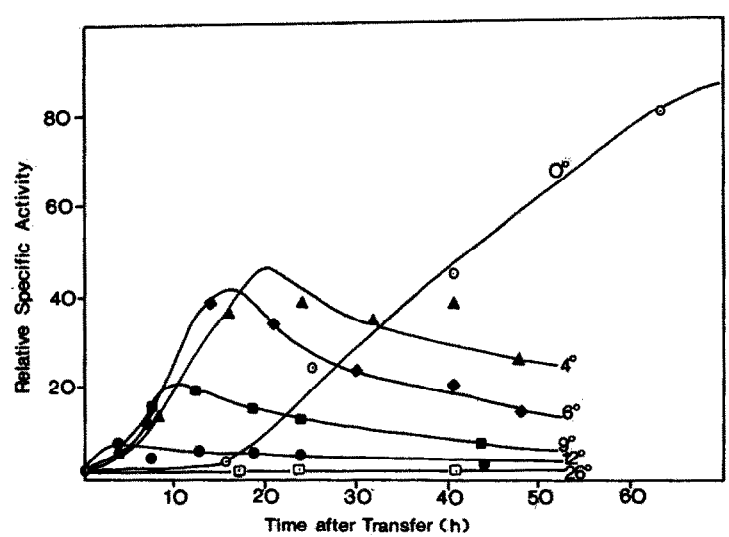

Fig. 2. Effect of change of growth temperature on the glycerol kinase activity in *Neurospora* cultures. 20 ml cultures were grown on sucrose medium in 50 ml flasks at 26°C for 48 hr before transfer to new temperatures. Extracts were prepared from whole cultures. Glycerol kinase specific activity is given relative to the basal level.

Table 1
Effect of inhibitors of protein synthesis on the increase in glycerol kinase activity at 4°C.

	Specific activity (units)	% Inhibition
2 Days, 26°C	2.40	
2 Days, 26°C; 2 days, 4°C	85.4	
+ cycloheximide, 0.5 μM	57.9	33.0
5.0 μM	3.64	98.5
+ chloramphenicol, 0.1 mg/ml	95.0	–
4.0 mg/ml	93.0	–

20 ml cultures were grown in sucrose medium as indicated. The inhibitors were added at the time of transfer to 4°C. Extracts were prepared from whole cultures.

The changes in activity need not necessarily represent actual changes in the enzyme protein level and other factors present in the extracts could be responsible. The presence of small molecular weight effectors, or larger activators or inhibitors such as those reported for trehalase [9] and nucleases [10, 11] in certain *Neurospora* extracts, could alter the activity of the enzyme. In addition, the observed activity could differ from the true activity through the presence of phosphatases or glycerol which would reduce the amount of [1-^{14}C]glycerol phosphate formed, the former by hydrolysis and the latter by lowering the specific activity of the labelled glycerol. If diffusible factors are responsible they should be detected on mixing extracts. Fig. 3 shows the typical result of mixing a 26°C extract (basal activity, A) with a 4°C extract (high activity, B). The observed activity is that expected solely from the contribution of the component extracts and so neither extract contains a factor which affects the activity of the other. Dialysis, which would remove small effectors, also fails to alter the glycerol kinase activity of any extracts.

The results are consistent with the cold-induced increase in glycerol kinase activity being due to an increase in enzyme protein. Further investigations are being made to verify this and to determine whether, as suggested by its sensitivity to cyclohexamide, the increase is the result of de novo synthesis.

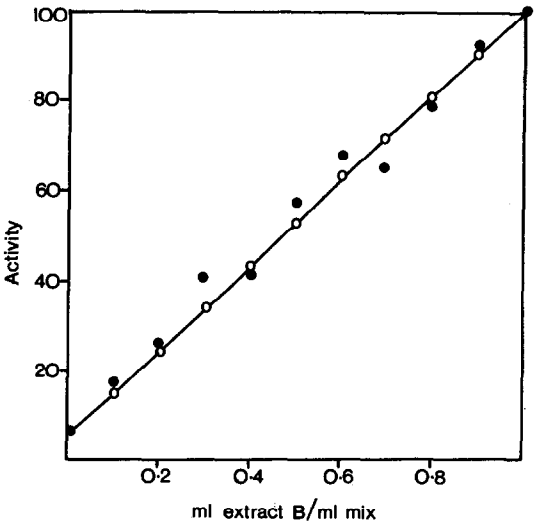

Fig. 3. Effect of mixing extracts on their glycerol kinase activity. Extracts were prepared from 96 hr cultures incubated, entirely at 26°C (A), or for 48 hr at 26°C and 48 hr at 4°C (B). (Extract A contained approx. 1.5 times more protein than extract B.) They were mixed in different proportions and the mixes and original extracts assayed for glycerol kinase activity. Activity is given as a percentage of that in extract B; observed activity (•), expected activity (o—o—o).

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