FEMS Microbiology Letters 23 (1984) 249–252
Published by Elsevier

FEM 01809

Evidence for synthesis de novo of NADP-linked glutamate dehydrogenase in *Coprinus* mycelia grown in nitrogen-free medium

(*Coprinus*; enzyme induction; derepression; nitrogen metabolism)

Falih N. Jabor and David Moore

Department of Botany, The University, Manchester M13 9PL, U.K.

Received 5 March 1984
Accepted 30 March 1984

1. SUMMARY

NADP-linked glutamate dehydrogenase is developmentally regulated in the mushroom fruit bodies of *Coprinus cinereus*. The enzyme can also be specifically induced by transfer of mycelium of the monokaryon BC9/6,6 to an ammonium-free medium containing an excess of pyruvate. Tests involving differential labelling show that the enzyme is synthesised de novo in this latter type of experiment.

2. INTRODUCTION

Maturation of the cap of *Coprinus cinereus* (Schaeff. ex Fr.) S.F. Gray is accompanied by a specific pattern of changes in enzyme activity and metabolite levels. The most significant changes result in amplification of activity in the tricarboxylic acid cycle and the urea cycle, with the enzymes succinate dehydrogenase, NADP-linked glutamate dehydrogenase (NADP-GDH), glutamine synthetase (GS), ornithine-acetyl transferase (OAT) and ornithine-carbamyl transferase (OCT) being elevated to levels in excess (in some cases greatly in excess) of those found in the stipe; while activity of the enzyme urease is absent from the cap though present in both stipe and mycelium [1,2].

The net result of the shift in metabolism is accumulation of urea, and probably other nitrogenous metabolites, as osmotic solutes which drive water into the cells of the gill hymenium. The cell expansion which results is central to the whole morphogenesis of the developing cap of the mushroom fruit body of this basidiomycete fungus. The gill hymenia are largely made up of paraphyses—the cells which increase most dramatically in size. Expansion of these cells therefore increases the area of the gill plate but since this is bounded on its outer edge (i.e. the edge furthest from the stipe) by the inextensible, but flexible, layer of outer cap tissue the increase in gill area is accommodated by a curling of the gill away from the stipe [3].

Regulation of NADP-glutamate dehydrogenase depends on a circuit involving accumulation of acetyl-CoA in tissues where ammonium is limiting [4]. This enzyme, together with glutamine synthetase, probably contributes to an ammonium scavenging system. We have a monokaryotic strain in which the same enzymic events as occur endogenously in the developing cap can be induced ex-

perimentally. This is unusual among strains of this fungus [4]. The experiment involves transfer of mycelium to medium lacking ammonium but containing 100 mM pyruvate. The result of this treatment is that the mycelium produces high activities of NADP-GDH, GS, OAT and OCT, and shows diminished activities of NAD-GDH and urease. In this paper we present evidence which indicates that the NADP-GDH enzyme is synthesised de novo after transfer to the pyruvate medium.

3. MATERIALS AND METHODS

The monokaryon strain BC9/6,6 (= ATCC42725) was used throughout. For the medium-transfer technique mycelium was first grown in a medium containing 1% Bacto Casamino Acids (hydrolysed casein), 10 mM glucose, 30 mM ammonium chloride, 10 mM disodium hydrogen phosphate, 10 mM potassium dihydrogen phosphate, 2 mM sodium sulphate, 0.5 mM magnesium sulphate and 3 μM thiamine hydrochloride. Incubation for 4–5 days on an orbital shaker operating at 120 strokes/min at 37 °C was sufficient to produce a useful yield of mycelium.

Mycelium was harvested by centrifugation, washed, recentrifuged and then resuspended for further incubation in the experimental medium. This consisted of a basal solution containing 10 mM disodium hydrogen phosphate, 10 mM potassium dihydrogen phosphate, 2 mM sodium sulphate, 0.5 mM magnesium sulphate and 3 μM thiamine hydrochloride to which different supplements were added.

For the basic NADP-GDH induction experiment 100 mM sodium pyruvate was added to this solution and the mycelium was incubated for a further 9 to 22 h without added nitrogen source. In other experiments metabolic inhibitors (noted below) were also included in this transfer medium. For experiments involving differential labelling, radio-labelled leucine was added to the media (see below).

Final harvesting was done by filtration through Whatman GF/A filters. For enzyme assay crude extracts were prepared by grinding tissue with 100 mM sodium phosphate buffer, pH 7.5. After filtration through Whatman GF/A extracts were used without further treatment. The procedure described by Al-Gharawi and Moore [5] was used to assay glutamate dehydrogenase enzymes in the amination direction. Protein contents were determined by the Lowry method.

Electrophoresis was carried out in a Shandon 'disc' PAGE apparatus using 5% polyacrylamide gels following the methods of Moore and Jirjis [6]. To measure the radioactivity of samples labelled with ^{3}H and ^{14}C prior to being electrophoretically separated, bands stained for enzyme activity were excised and solubilized in 0.5 ml of 30% hydrogen peroxide before being mixed with a xylene based scintillation fluid [7].

4. RESULTS AND DISCUSSION

Induction of NADP-GDH activity did not occur when cycloheximide was included in the

Table 1

Effects of some metabolic inhibitors on glutamate dehydrogenase activities in mycelium of *Coprinus cinereus* transferred to medium containing 100 mM pyruvate and no nitrogen source

Enzyme activity is shown as nmol substrate used/min/mg protein. Entries are the means of at least three replicates.

Medium	Glutamate dehydrogenase specific activity	
	NAD-GDH	NADP-GDH
Initial control (untransferred)	693	14
transferred to:		
100 mM pyruvate	445	581
100 mM pyruvate + 1 mM methionine sulphoximine	578	382
100 mM pyruvate + 1 mM methionine sulphoximine + 5 mM NHCl	417	95
100 mM pyruvate + 20 μM actinomycin D	346	255
100 mM pyruvate + 1 μg/ml thiolutin	167	339
100 mM pyruvate + 8 μg/ml thiolutin	171	274
100 mM pyruvate + 95 μM cycloheximide	1054	41

Table 2

Radioactivity in total protein and in specific enzyme proteins separated by electrophoresis and extracted from mycelium grown first in ^{14}C-labelled medium and then transferred to ^{3}H-labelled medium

Radioactivity is shown as dpm/mg protein applied to the polyacrylamide gel. Enzyme activity is shown as nmol substrate used/min/mg protein. Entries are the means of at least two replicates.

Medium	sample	dpm/mg protein		Enzyme activity
		^{14}C	^{3}H	
Experiment 1: 9 h incubation in pyruvate medium				
Casamino acids				
(before transfer)	total protein	21838	0	
	NAD-GDH	648	0	217
	NADP-GDH	1097	0	1
Pyruvate medium				
(after transfer)	total protein	14430	78099	
	NAD-GDH	361	0	165
	NADP-GDH	903	714	22
Experiment 2: 22 h incubation in pyruvate medium				
Casamino acids				
(before transfer)	total protein	65144	0	
	NAD-GDH	3610	0	486
	NADP-GDH	3770	0	1
Pyruvate medium				
(after transfer)	total protein	22599	167968	
	NAD-GDH	105	3379	272
	NADP-GDH	471	5680	50

pyruvate transfer medium (Table 1) implying that protein synthesis was necessary for production of the enzyme activity. However, inhibitors of RNA synthesis had no effect (Table 1) although this could be due to their inability to penetrate the wall or membrane of the cell. The specific inhibitor of glutamine synthetase, methionine sulphoximine, did not change the response of the mycelium to the transfer medium. This suggests that glutamine synthetase activity was not involved in regulation of NADP-GDH.

Results presented in Table 2 suggest that NADP-GDH was synthesised de novo when the mycelium was transferred to the pyruvate induction medium. In these experiments the mycelium was first grown in a 'casamino acids' medium which was supplemented with L-[U-^{14}C]leucine at 62.5 nCi/ml so that all proteins formed prior to transfer were labelled with ^{14}C. The pyruvate transfer medium was supplemented with L-[4,5-^{3}H]leucine at 625 nCi/ml so that proteins formed after transfer would be labelled with tritium. After 9 h incubation in the transfer medium the NADP-GDH shows tritium labelling whereas the NAD-GDH shows only C-labelling. Only when incubation was continued to 22 h did the NAD-GDH protein show tritium labelling, implying some turnover of this protein during this extended incubation period. We thus conclude that the NADP-GDH enzyme protein is newly synthesised in the mycelium as the result of a derepression event caused by transfer to the pyruvate medium.

ACKNOWLEDGEMENTS

We wish to thank Miss Ann Farnworth for assistance in developing the differential labelling method. FNJ thanks the Government of the Republic of Iraq for the award of a scholarship which enabled this work to be carried out.

REFERENCES

[1] Moore, D. and Ewaze, J.O. (1976) J. Gen. Microbiol. 97, 313–322.
[2] Ewaze, J.O., Moore, D. and Stewart, G.R. (1978) J. Gen. Microbiol. 107, 343–357.
[3] Moore, D., Elhiti, M.M.Y. and Butler, R.D. (1979) New Phytol. 83, 695–722.
[4] Moore, D. (1981) Biochim. Biophys. Acta 661, 247–254.
[5] Al-Gharawi, A. and Moore, D. (1974) J. Gen. Microbiol. 85, 274–282.
[6] Moore, D. and Jirjis, R.I. (1981) New Phytol. 87, 101–113.
[7] Tishler, P.V. and Epstein, C.J. (1968) Anal. Biochem. 22, 89–98.