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COMPARISON OF SPECTROPHOTOMETRIC AND ELECTROPHORETIC MEASUREMENTS OF GLUTAMATE DEHYDROGENASE ACTIVITY DURING MYCELIAL GROWTH AND CARPOPHORE DEVELOPMENT IN THE BASIDIOMYCETE *COPRINUS CINEREUS*

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1. Introduction

Like many fungi *Coprinus cinereus* possesses two glutamate dehydrogenase enzymes; one linked to the coenzyme NAD (NAD-GDH) and the other linked to NADP (NADP-GDH) [1–3]. The NAD-GDH is found at moderate to high levels of activity in mycelia whatever the nature of the growth medium, and occurs in both cap and stipe tissues of the mushroom carpophore. In sharp contrast the NADP-GDH is found only in mycelium grown on very particular synthetic media and during normal development occurs exclusively in the maturing carpophore cap; stipe tissue showing barely detectable levels of activity [3]. The appearance of NADP-GDH in cap tissues is thought to represent a developmentally related derepression with its regulatory origin at the genetic level, and with a function connected with cap morphogenesis [4]. However, irrespective of the nature and function of the regulation, comparison of NADP-GDH activities in cap and stipe shows that quite different regulatory programmes operate in these two adjacent tissues.

These data have been obtained by spectrophotometric analyses of cell-free tissue homogenates. Recent observations using light microscope cytochemistry of frozen sections have shown that adjacent cells may exhibit these same regulatory contrasts, but they have also shown that the cytochemical technique can reveal considerable enzyme activity in young carpophore primordia in which only minimal activity can be detected by spectrophotometric analysis of cell-free homogenates [5]. Since only a minority of cells of the primordial cap show NADP-GDH activity it has been argued that the difference between the techniques is due to an overwhelming dilution of that activity during extraction for spectrophotometry. However, it is also feasible that the integrity of the intact cell is required for the expression of NADP-GDH activity at these very early developmental stages.

Electrophoretic analysis offers a procedure which correlates the two already used. Electrophoresis employs cell-free homogenates, but the concentrative effects of the process coupled with the specificity of enzyme staining methods provide a highly sensitive procedure for detection of low levels of enzyme activity. In this work we present a comparison of electrophoretic and spectrophotometric measurements of enzyme activity in mycelia and developing carpophores. The results corroborate and expand those of earlier reports.

2. Materials and Methods

2.1. Organism and growth conditions

The organism used was *Coprinus cinereus* (Schaeff. ex Fr.) S.F. Gray *sensu* Konr. It has previously been called *C. lagopus* [2,3]. The compatible monokaryons H1 and BC9/6,6 and their joint dikaryon were grown as vegetative mycelia in unstirred liquid cultures using the yeast extract-malt extract-glucose (YMG) medium described by Gooday [6]. Cultures were incubated at 37°C without illumination. Carpophores were ob-

tained from dikaryon cultures grown on sterilised horse dung using the cultivation conditions described by Moore and Ewaze [7].

2.2. Extraction and assay

Extracts were made by grinding with sand in ice-cold buffer (100 mM Tris–HCl, pH 9). The suspension was filtered through Whatman GF/A filters and the filtrate desalted on a small column of Sephadex G-25. Samples of the desalted extract were subjected to spectrophotometric assay for NADP-GDH using the deamination assay mixture described by Al-Gharawi and Moore [8]. Electrophoresis was done at 2°C in polyacrylamide gel columns, prepared using 5% (w/v) Cyanogum-41 and mounted in a Shandon-Southern Analytical PAGE apparatus. Samples of 100 to 150 μl of the tissue extract were applied (after a 30 min pre-run) together with 50 μl of 40% (w/v) sucrose and 50 μl of 0.001% (w/v) bromophenol blue. Electrophoresis was carried out with a current density of 3 mA per tube and was usually completed in about 60 min. At this time the gels were extruded into cold extraction buffer, allowed to equilibrate, and then transferred to a reaction mixture containing (dissolved in extraction buffer) 0.653 mM $NADP^+$, 0.163 mM nitro blue tetrazolium salt, 0.027 mM phenazine methosulphate, and 49.3 mM monosodium glutamate. The gels were incubated in this mixture at 37°C in the dark. One replicate was drained, washed and scanned densitometrically after 20 min incubation; another sample gel was scanned after 40 min.

2.3. Data acquisiton and interpretation

Protein contents of the extracts were measured by the method of Lowry et al. [9] and specific activities calculated in the usual way for spectrophotometric experiments. As noted previously [3,10] *Coprinus* NADP-GDH activity migrates electrophoretically as a single band. The intensity of formazan staining on the polyacrylamide gels was taken as a measure of total NADP-GDH activity and was measured with a Pye-Unicam SP1809 Densitometer accessory fitted to an SP1800B Spectrophotometer operating at 550 nm. The scan being recorded with an AR25 chart recorder. A gel loaded with the same sample but

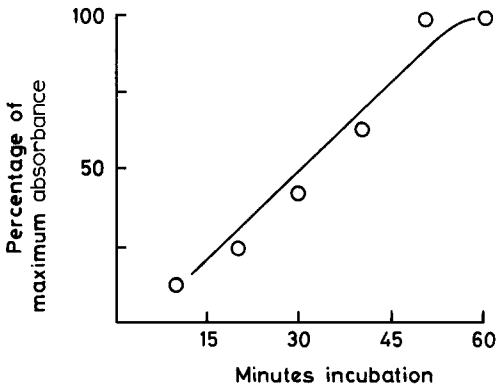

Fig. 1. Course of the deamination reaction catalysed by NADP-linked glutamate dehydrogenase after electrophoresis. The polyacrylamide gel was incubated in the reaction mixture at 37°C and at intervals was transferred to the densitometer where the absorbance due to formazan staining was measured (at 550 nm) as the height of the peak in the record of the densitometric scan of the stained band.

incubated in reaction mixture lacking glutamate was used as control in each case. Stain intensity was proportional to the time of incubation (Fig. 1), a feature common to other similar systems [11,12], so the rate of enzyme activity could be estimated by using the densitometer to measure stain intensity at sequential times during incubation; the period between 20 and 40 min was chosen as being representative of the typical reaction depicted in Fig. 1. Since the total amount of protein applied to the acrylamide gel was determined separately, the measured rates of change in staining intensity in different gels were adjusted to a common (notional) protein loading to give a specific activity for the enzyme analogues to that obtained spectrophotometrically.

3. Results and Discussion

Vegetative cultures of the monokaryons and the dikaryon were harvested at intervals during a 14-day incubation period and analysed both electrophoretically and spectrophotometrically. The results shown in Table 1 are essentially similar to those published before for BC9/6,6 [4,13] in that they show an increasing activity of the enzyme over the first 3 to 5 days followed by a rapid decline. The other mono-

TABLE 1

Specific activity of NADP-GDH during mycelial growth of *Coprinus cinereus* on liquid YMG medium

Days incubated	Identity of mycelium					
	Monokaryon BC9/6, 6		Monokaryon H1		Dikaryon H1 × BC9/6, 6	
	a	b	a	b	a	b
3	0.706	60	1.120	not done	0.133	10.9
5	1.417	78	2.320	104	0.058	9.0
7	0.357	14	1.520	55	0.090	12.0
9	0.200	11	0.760	42	0.001	zero
11	0.137	zero	0.640	34	zero	zero
14	zero	zero	1.160	58	zero	zero

[a] Enzyme activity determined electrophoretically and expressed as rate of change of absorbance due to formazan staining/mg protein applied to the gel/10 min incubation.
[b] Enzyme activity determined spectrophotometrically and expressed as nmol product formed/mg protein/min.

karyon, H1, showed a much less rapid and less complete decline. The differences between the patterns of activity in the two monokaryons must reflect regulatory polymorphisms and it is interesting that the behaviour of the dikaryon is more similar to that of BC9/6,6 than to H1, implying that the regulatory characters of the former may be genetically dominant. The considerable difference in absolute activity between dikaryon and monokaryons emphasises the distinction between the two mycelial types. Significantly, the two techniques revealed the same pattern of behaviour in each mycelium, but the electrophoretic approach provided enhanced sensitivity. In the dikaryon and in BC9/6,6 the activity detected electrophoretically on days 9 and 11 respectively still amounted to 10% of the maximum observed, even though no activity could be detected spectrophotometrically in the same extracts.

Analysis of carpophore samples of different stages of development (Table 2) also gave data similar to those already published. Both methods of activity measurement reveal the enormous difference in specific activity between cap and stipe tissues of the same carpophore. But more particularly, the electrophoretic measurements clearly corroborate the histochemical observations [5]. The specific activity in the primordial cap measured spectrophotometrically is less than 4% of that found in the caps of mature carpophores. On the other hand, the electrophoretic determination shows that the primordial cap contains about 30% of the activity detected in the mature cap. Clearly, the concentrative effects of the electrophoretic process so improve sensitivity that they allow a more realistic picture of the extent of derepression of the enzyme activity to be obtained. The spectrophotometric measurements suggests an exponential increase in activity as development proceeds [4], but this now seems likely to be due to the insensitivity of the method at low levels of enzyme activity. The observations reported here show that there is a much more rapid initial increase in activity in the cap. It appears to be virtually a linear increase, but the

TABLE 2

Specific activity of NADP-GDH in carpophore tissues of *Coprinus cinereus* during development

Stage of development of material examined	NADP-GDH specific activity			
	Determined electrophoretically [a]		Determined Spectrophotometrically [a]	
	cap	stipe	cap	stipe
Primordia	0.333	0.043	30	10
Immature carpophores	0.805	0.013	420	10
Mature carpophores	1.100	zero	780	20

[a] Specific activities are expressed as described in the footnote to Table 1.

time scale is a rather subjective one (although quite closely related to "real" time [7]) and too few samples have yet been examined for this to be stated with confidence. Nevertheless, electrophoretic data show the increase in activity to be rapid, rather than gradual, over the early stages of development; while cytochemical microscopic observations show that this reflects an increase in the number of cells which possess NADP-GDH activity, rather than an activity increase common to all members of the cell population [5].

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