The Enzymes of Ammonia Assimilation in *Trichosporon cutaneum*

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INTRODUCTION

Two major routes of ammonia assimilation have been implicated in yeasts. The first involves anabolic (aminating) NADP-linked glutamate dehydrogenase, the highest activities of which are usually observed in growth in environments containing low concentrations of ammonia (Brown & Johnson, 1970) or where nitrate is the nitrogen source (Burn, Turner & Brown, 1974). The second is a dual enzyme system comprising glutamine synthetase and glutamate synthase (Brown, Burn & Johnson, 1973; Roon, Even & Larimore, 1974; Johnson & Brown, 1974), which is believed to be of major significance when ammonia concentrations are low.

The role of NAD-linked glutamate dehydrogenase has been assumed to be primarily a catabolic (deaminating) one, since highest activities of this enzyme are observed during growth on organic nitrogen sources (Polakis & Bartley, 1965; Thomulka & Moat, 1972; Ferguson & Sims, 1974).

The present communication reports upon the activities of possible ammonia-assimilating enzymes in *Trichosporon cutaneum*, grown in batch and continuous cultures.

METHODS

The organism employed in this study, *Trichosporon cutaneum* (NCYC444) was maintained at 4 °C on yeast extract (1 %, w/v)-peptone (2 %, w/v)-glucose (2 %, w/v) agar and subcultured every two months.

Organisms were grown in batch and continuous cultures in media containing: KH_2PO_4 , 7.35×10^{-3} M; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4.06×10^{-3} M; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.70×10^{-3} M; trisodium citrate. $2\text{H}_2\text{O}$, 4.45×10^{-3} M; citric acid. H_2O , 3.52×10^{-3} M; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3.48×10^{-8} M; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 1.62×10^{-8} M; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.80×10^{-7} M; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 8.99×10^{-7} M; meso-inositol, 1.11×10^{-4} M; pyridoxine-HCl, 2.43×10^{-6} M; calcium pantothenate, 4.20×10^{-6} M; nicotinic acid, 3.25×10^{-5} M; thiamine-HCl, 1.19×10^{-6} M; D-biotin, 4.09×10^{-9} M. Glucose and the nitrogen source (either ammonium sulphate or monosodium glutamate) were added as required. The media employed were either 'low nitrogen' [$(\text{NH}_4)_2\text{SO}_4$, 1.80×10^{-3} M or monosodium glutamate, 4.0×10^{-2} M, with glucose, 5.55×10^{-2} M] or 'high nitrogen' [$(\text{NH}_4)_2\text{SO}_4$, 1.51×10^{-2} M or monosodium glutamate, 3.0×10^{-2} M, with glucose 1.11×10^{-2} M]. The final pH of the medium was buffered by the trisodium citrate-citric acid at 4.5.

Batch cultures were grown in 750 ml portions in 2 l capacity flasks at 25 °C, in an orbital shaker incubator (A. Gallenkamp & Co. Ltd, Stockton-on-Tees, Teesside) using the 'low nitrogen' medium. Chemostat cultures were grown in a 0.5 l capacity vessel of the type described by Brown & Rose (1969) at 25 °C, employing a dilution rate of 0.05 h^{-1} . Batch cultures were harvested in the late exponential phase of growth, and chemostat cultures directly from the culture vessel.

Table 1. *Influence of the nature and concentration of medium nitrogen source on the activities of ammonia assimilating enzymes in chemostat cultures of T. cutaneum*

Cultural condition*	Enzyme activity (nmol/min/mg protein)				
	Glutamine synthetase	Glutamate synthase		Glutamate dehydrogenase	
		NAD	NADP	NAD	NADP
Ammonia limitation	10.8	8.8	ND	131.8	ND
Ammonia excess	6.0	8.0	ND	307.3	ND
Glutamate limitation	45.7	10.5	ND	262.4	ND
Glutamate excess	32.4	12.1	ND	256.7	ND

ND, Not detectable (i.e. less than 0.5 nmol/min/mg protein) although assayed at both pH 6.5 and 7.5.

* For composition of media, see Methods.

Enzyme extracts were prepared as described by Brown & Johnson (1970) using 100 mM-phosphate buffer pH 7.0. The activities of NADP- and NAD-linked glutamate dehydrogenase, NADP- and NAD-linked glutamate synthase and glutamine synthetase were assayed as described by Johnson & Brown (1974). NADP- and NAD-linked alanine dehydrogenase was assayed by the method used for glutamate dehydrogenase, except that pyruvate was substituted for 2-oxoglutarate. The protein contents of the extracts were determined by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Extracts prepared from ammonia-grown batch cultures were initially assayed in a pH range of 5.8 to 9.2 for the enzymes described under Methods. NADP-linked glutamate dehydrogenase was not detectable, and no alanine dehydrogenase activity, either NAD- or NADP-linked, was found. The organism did contain NAD-linked glutamate dehydrogenase activity (pH optimum 8.3), NAD-linked glutamate synthase activity (pH optimum 7.6) and glutamine synthetase activity (pH optimum 6.5).

The apparent absence of NADP-linked glutamate dehydrogenase in this organism was rather surprising, since in most yeasts studied to date it is present (Burn *et al.* 1974); this prompted a closer examination of the activities of ammonia-assimilating enzymes when *T. cutaneum* was grown under different conditions. Chemostat cultures were established and the organisms grown under conditions of ammonia limitation, ammonia excess (glucose limited), glutamate limitation and glutamate excess (glucose limited). The activities of NAD-linked glutamate dehydrogenase, NAD-linked glutamate synthase and glutamine synthetase were assayed at their pH optima (see above). NADP-linked glutamate dehydrogenase, NADP-linked glutamate synthase, and NAD- and NADP-linked alanine dehydrogenase were assayed at pH 6.5 and 7.5.

The results of these experiments are shown in Table 1. The only enzymes detectable were those which had been present in batch cultures. Glutamine synthetase activity was found under all conditions tested, although at a markedly higher level in the presence of glutamate. This finding differs from results obtained previously with *Schizosaccharomyces* sp. and *Saccharomycodes ludwigii* (Johnson & Brown, 1974), but agrees with results obtained in studies involving *Candida utilis* by Ferguson & Sims (1974). NAD-linked glutamate dehydrogenase was detectable in substantial quantities under all conditions, although its level was somewhat reduced at limiting concentrations of ammonia. Previous studies (Hierholzer & Holzer, 1963; Westphal & Holzer, 1964; Thomulka & Moat, 1972; Burn *et al.* 1974;

Ferguson & Sims, 1974), involving a variety of yeasts, have indicated that the presence of glutamate in the growth medium normally increases the level of NAD-linked glutamate dehydrogenase. *Trichosporon cutaneum* is unusual in that even in the absence of glutamate, the level of the enzyme remains high. NAD-linked glutamate synthase activity was present at a consistently low level, similar to results obtained previously with different organisms (Brown *et al.* 1973; Roon *et al.* 1974; Johnson & Brown, 1974). In the present case, the possibility that the observed activity may have been due to the combined activity of a glutaminase and NAD-linked glutamate dehydrogenase cannot be totally precluded.

Thus, so far as nitrogen assimilation is concerned, *T. cutaneum* appears to differ from the majority of yeasts examined in two ways: NADP-linked glutamate dehydrogenase is absent, and NAD-linked glutamate dehydrogenase is present at a consistently high level. It is therefore possible that the latter enzyme plays both an anabolic and catabolic role in this organism, rather than the solely catabolic role which it is presumed to have in organisms possessing the NADP-linked enzyme. When assayed in crude extracts at pH 8.3, NAD-linked glutamate dehydrogenase from *T. cutaneum* showed K_m values for ammonia of 1.33×10^{-2} M, for 2-oxoglutarate of 8.0×10^{-4} M, for NADH of 2.4×10^{-5} M, for L-glutamate of 2.0×10^{-3} M, and for NAD of 4.8×10^{-4} M. These values, particularly that for ammonia, are of the same order as those previously reported for the enzyme in *Saccharomyces cerevisiae* (Holzer & Schneider, 1957) and in other fungi (Sanwal, 1961; Sanwal & Lata, 1961). Thus, the enzyme from *T. cutaneum* does not show an unusually high affinity for ammonia, but if the enzyme does function anabolically this may be compensated for by its continued synthesis at a high level in the presence of ammonia. Such 'compensatory synthesis' is believed to operate in the case of NADP-linked glutamate dehydrogenase in many yeasts (Brown *et al.* 1973).

It is tempting, although perhaps premature, to speculate that the apparently constitutive synthesis of NAD-linked glutamate dehydrogenase may reflect the 'natural' habitat of this organism, which is frequently found in association with warm-blooded animals (Do Carmo-Sousa, 1970). Under such conditions, the predominant available nitrogen sources would probably be organic, and it is possible that the ability of *T. cutaneum* to utilize ammonia efficiently represents an evolutionary modification of those mechanisms controlling the synthesis of NAD-linked glutamate dehydrogenase.

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