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The ornithine pathway in the yeast *Candida utilis*

The pathway of the ornithine synthesis in the yeast *Candida utilis* ((Henneberg) Lodder et Kreger-Van Rij (syn. *Torulopsis utilis* (Henneberg) Lodder) was partly elucidated by ABELSON AND VOGEL[1]. Experiments with tracer techniques revealed that L-ornithine was synthesized in *C. utilis* from L-glutamic acid, most probably *via* L-glutamic γ-semialdehyde[1].

Since cell-free extracts of *C. utilis* showed only minor activities of L-ornithine δ-transaminase (L-ornithine: 2-oxoacid amino transferase, EC 2.6.1.13), the enzyme that converts L-glutamic γ-semialdehyde to L-ornithine, a major role for a pathway involving non-acetylated intermediates is not likely. For this reason the role of acetylated intermediates in the synthesis of L-ornithine from L-glutamate by *C. utilis* was checked in the present investigation.

The production of L-ornithine *via* acetylated intermediates was demonstrated by VOGEL[2] in *Escherichia coli* and by UDAKA AND KINOSHITA[3] in *Micrococcus glutamicus*. In both organisms N-α-acetyl-L-ornithine is formed from L-glutamate *via* N-acetyl-L-glutamate, N-acetyl-L-glutamic γ-phosphate, and N-acetyl-L-glutamic γ-semialdehyde. In *E. coli* the N-α-acetyl-L-ornithine is split by the enzyme acetylornithinase to L-ornithine and acetate. In *M. glutamicus*, however, a transacetyla-

tion reaction occurs between *N*-α-acetyl-L-ornithine and L-glutamate, resulting in the formation of *N*-acetyl-L-glutamate and L-ornithine. Thus in *M. glutamicus* L-ornithine is synthesized in a cyclic process.

In the present investigation three enzymes were assayed in cell-free extracts of *C. utilis*, each being characteristic of one of the known ornithine pathways: L-ornithine δ-transaminase, acetylornithinase and the transacetylating enzyme.

C. utilis (strain CBS 621*) was grown overnight in aerated Kluyver-flasks in a medium containing per liter: glucose, 40 g; $(NH_4)_2SO_4$, 3.0 g; KH_2PO_4, 1.36 g; sodium acetate·3 H_2O, 5.80 g; $MgCl_2 \cdot 6H_2O$, 0.40 g; $CaCl_2$, 0.10 g; NaCl, 0.10 g; $FeCl_3 \cdot 6H_2O$, 2 mg; H_3BO_3, 0.5 mg; $CuSO_4 \cdot 5\ H_2O$, 0.1 mg; KI, 0.1 mg; $MnSO_4 \cdot 5\ H_2O$, 0.4 mg; Na_2MoO_4, 0.2 mg; $ZnSO_4 \cdot 6\ H_2O$, 0.4 mg. For repression studies, cells grown in the above medium were compared with those of a similar growth medium, supplemented with $5 \cdot 10^{-3}$ M L-arginine. The cells were always harvested in the logarithmic growth phase.

Cell-free extracts were prepared in a "Zellhomogenisator"** by shaking cells with glass beads in a 0.1 M phosphate buffer pH 7.5 (see ref. 4). During this procedure temperature did not rise above 20°. The extracts were freed from low-molecular substances by gel filtration on a column of Sephadex G 25***, equilibrated with 0.001 M phosphate (pH 7.5). Protein estimations were made according to LOWRY *et al.*[5], using crystalline bovine serum albumin as a standard.

The assay mixture for L-ornithine δ-transaminase contained per 2.0 ml (μmoles): L-ornithine·HCl, 20; disodium α-oxoglutarate, 20; pyridoxal 5-phosphate, 1.0; $MgCl_2$, 10; potassium phosphate (pH 7.4), 200; cell-free extract, approx. 10 mg protein. The tubes were incubated for 8 h at 28°. Glutamic acid was detected by spraying with ninhydrin after paper chromatography with phenol–water (3:1, v/v) on Whatman No. 1. The formation of L-glutamic γ-semialdehyde was demonstrated with *o*-aminobenzaldehyde[6]. A yellow pigment was formed. A blanc and incubations with omission of either L-ornithine or α-oxoglutarate were included. In these incubation mixtures neither L-glutamate nor L-glutamic γ-semialdehyde were formed.

The activity of L-ornithine δ-transaminase in cell-free extracts of *C. utilis* was very small. Under the conditions used, approx. 0.2 μmole of glutamate was formed after 8 h of incubation at 28°. Since the equilibrium of the transaminase reaction is on the side of glutamate[8], it is not likely that L-ornithine is synthesized in *C. utilis* *via* non-acetylated intermediates.

Acetylornithinase was assayed according to VOGEL AND BONNER[7]. The assay mixture contained per 0.5 ml (μmoles): *N*-α-acetyl-L-ornithine, 3.0; glutathione, 0.5; $CoCl_2$, 0.1; potassium phosphate (pH 7.0), 50; cell-free extract, approx. 2.0 mg protein. After 30 min of incubation at 28° the tubes were assayed for ornithine[7].

The assay mixture for the transacetylating enzyme contained per ml (μmoles): L-ornithine·HCl, 20; *N*-acetyl-L-glutamate (pH 7.0), 50; potassium phosphate (pH 7.0), 100; cell-free extract, 1.5 mg protein. After 2 h of incubation at 28° the tubes were assayed for ornithine[7]. The disappearance of ornithine was linear with time and enzyme concentration, until 5 μmoles of ornithine had disappeared. If

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N-acetyl-L-glutamate was omitted from the incubation mixtures, no ornithine disappeared.

The measured activities of acetylornithinase and of the transacetylating enzyme are given in Table I. Both enzymes occur in *C. utilis* in significant amounts. Their

TABLE I

ACTIVITIES OF ACETYLORNITHINASE AND OF THE TRANSACETYLATING ENZYME IN *C. utilis* GROWN IN A GLUCOSE MEDIUM WITH AND WITHOUT $5 \cdot 10^{-3}$ M L-ARGININE

Enzyme activities are given as μmoles of L-ornithine formed or converted per hour per mg protein. The values represented are the averages of three assays.

Medium	*Enzyme activity (μmoles/h/mg protein)*	
	Without L-arginine	*With 5mM L-arginine*
Acetylornithinase	0.14	0.13
Transacetylating enzyme	1.35	1.37

activities exceed that of L-ornithine δ-transaminase. The synthesis of these enzymes was not repressed by L-arginine.

The results lead to the conclusion that in *C. utilis* L-ornithine is synthesized from L-glutamate *via* acetylated intermediates, in a pathway that was demonstrated for the first time by UDAKA AND KINOSHITA[3] in *Micrococcus glutamicus*. Besides the transacetylating enzyme, however, acetylornithinase is present. These results confirm those obtained by DEDEKEN[9] in a research on *Saccharomyces cerevisiae*.

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