PATHWAY OF ARGININE BIOSYNTHESIS IN YEAST

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Received July 11, 1962

That ornithine and proline could have a common precursor was suggested by the existence of mutants of penicillium (Bonner, 1946) and Neurospora (Srb, Fincham and Bonner, 1950) requiring either proline or ornithine. Glutamic γ-semialdehyde was the supposed precursor and the presence in Neurospora (Fincham, 1953) of a transaminase that interconverts ornithine and glutamic γ-semialdehyde seemed to confirm Scheme I:

Glutamate → glutamic γ-semialdehyde → proline
→ ornithine → → arginine

However, experimental results obtained by Fincham (1953) practically ruled out this scheme as pointed out by Davis (1955) in an excellent discussion of this problem.

In the present work evidence is given about the existence of a pathway in yeast closely related or similar to the one found in Escherichia coli (Vogel, 1953; Maas, Novelli and Lipmann, 1953) i. e. Scheme II: Glutamate → N^{α}-acetylglutamate → N^{α}-acetylglutamic γ-semialdehyde → N^{α}-acetyl-ornithine → → arginine.

1) Reduction of N^{α}-acetylglutamic acid. Exponentially growing cells of Saccharomyces cerevisiae were disrupted by sonic oscillation in a buffer composed of 0.25 M potassium phosphate, pH 7.5, and 0.05 M β-mercaptoethanol. To the 100,000 g supernatant solution, nine volumes of a saturated ammonium sulfate solution were added, in the presence of 0.05 M β-mercaptoethanol. Precipitated proteins were collected and redissolved in a 0.1 M Tris buffer with 0.05 M β-mercaptoethanol. Proteins precipitated at 50 per cent ammonium sulfate saturation were used for the enzymatic test. Conditions were essentially the same as described by Udaka and Kinoshita (1958). The oxidation of reduced TPN was followed in the presence of N^{α}-acetylglutamate and ATP (Figure 1). No reaction was observed with glutamate alone as substrate.

2) Negative feedback control of the reaction. It can be seen from Figure 1 that 8.3×10^{-4} M L-arginine strongly inhibits the reduction of

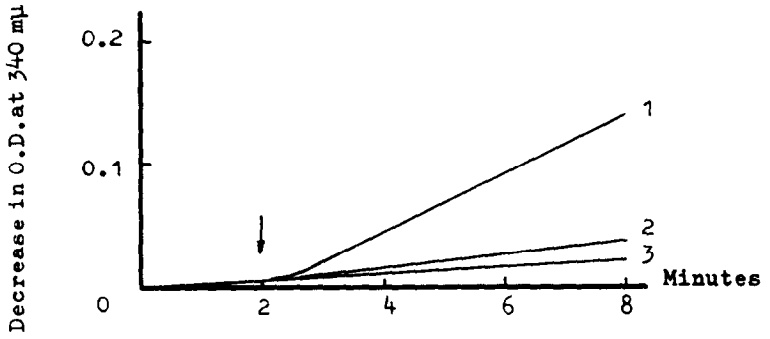

Figure 1. Test conditions : 200 μM TRIS, pH 7.5, 0.5 μM TPNH, 15 μM ATP, 30 μM Mg^{++} and 2 mg proteins. Volume : 3 ml

Addition of the substrates (80 μM) at the arrow :
Curve 1 : N-acetylglutamate,
Curve 2 : N-acetylglutamate + 2.5 μM Arginine,
Curve 3 : Glutamate.

N^{a}-acetylglutamate, as is the case for Micrococcus glutamicus (Udaka and Kinoshita, 1958). Figure 2 shows that the inhibition is of the competitive type and that the affinity of the enzyme for the inhibitor is greater than for its substrate.

3) Specificity of the negative feedback control mechanism. The inhibition is specific for L-arginine. Other basic amino-acids or intermediates in arginine biosynthesis have no affinity for the enzyme (TABLE I).

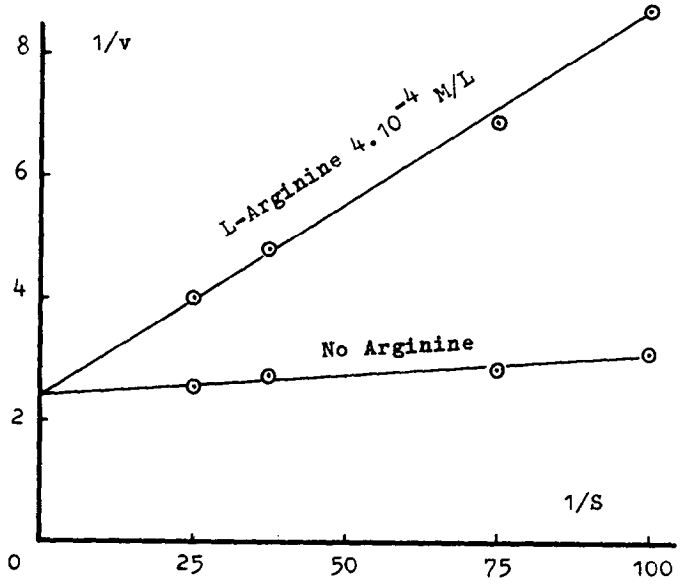

Figure 2. $K_M = 3.1 \cdot 10^{-3}$ $K_I = 5.2 \cdot 10^{-5}$

TABLE I

SPECIFICITY OF THE FEEDBACK CONTROL MECHANISM

Inhibitor	Concentration : M/L	Reaction rate : µM/hour		% inhibition
No		1.55		0
L-arginine	4.16×10^{-4}	0.65		58
L-arginine	8.33×10^{-4}	0.54		65
N^{a}-acetylornithine	8.33×10^{-4}	1.50		
L-ornithine	8.33×10^{-4}	1.50		
L-histidine	8.33×10^{-4}	1.61	Mean	
L-lysine	8.33×10^{-4}	1.57	value :	0
L-proline	8.33×10^{-4}	1.53	1.55	
DL-citrulline	8.33×10^{-4}	1.58		
L-canavanine	8.33×10^{-4}	1.57		

The substrate concentration was 2.66×10^{-2} M/L

4) Repression of the synthesis of N^{a}-acetylglutamate reductase by L-arginine. Repression of the synthesis of ornithine-transcarbamylase was recently shown for an arginine requiring strain of Saccharomyces cerevisiae (D 20) (Bechet, Wiame and De Deken-Grenson, 1962). Variations in the specific activity of this enzyme in response to presence or absence of arginine of 20 fold were observed.

An arginine requiring mutant of S. cerevisiae (D 83) was cultivated under conditions of arginine limitation in a continuous culture apparatus. The rate of medium renewal was 1/7 per hour. The same mutant was cultivated without limiting factor in the presence of an excess of arginine. Cell treatment and measurements of enzyme activities were performed as described above. The results, reported in Table II, show a tenfold variation in the specific activity under these two different culture conditions.

5) N^{a}-acetylornithinase activity. A culture of S. cerevisiae (wild type) was treated as described under 1 except that β-mercaptoethanol was omitted. Proteins were precipitated once at 90 per cent ammonium sulfate saturation and redissolved in a 0.1 M potassium phosphate buffer, pH 7.0. Test conditions and ornithine estimation were those described by Vogel and Bonner (1956). Enzymatic activity was proportional to protein concentration and Co^{++} 2×10^{-4} M/L an important cofactor of the reaction (last line of TABLE III).

TABLE II

REPRESSION OF N^{a}-ACETYLGLUTAMATE REDUCTASE BIOSYNTHESIS

Specific activity : μM/hour/mg total soluble proteins

	Arginine limiting	Excess arginine
Fraction I: 0 - 50% A.S. Saturation	1.544	0.164*
Fraction II: 0 - 90% A.S. Saturation	0.231	>0.02
Total	1.775	0.164

*Most of the activity is present in Fraction I. In Fraction II, activity was difficult to measure, due to the presence of a TPNH oxidase.

TABLE III

N^{a}- ACETYLORNITHINASE ACTIVITY

Protein in μg	N^{a}-acetylornithine in μM (1)	μM ornithine formed in 1 hour at 37°C
222	3.0	0.142
333	3.0	0.204
444	3.0	0.286
444*	0	0.001
333*	3.0	0.034

* Without Co^{++}

(1) Thanks are due to J. Daliers for synthesizing N^{a}-acetylornithine

Conclusions.

The presence in yeast of an N^{a}-acetylglutamate reductase and of an N^{a}-acetylornithinase indicate that the intermediates in ornithine biosynthesis might be acetylated as is the case in Escherichia coli (Maas, Novelli and Lipmann, 1953; Vogel, 1953). The occurence of a feedback control of N^{a}-acetylglutamate reductase by L-arginine as well as the repression of the biosynthesis of this enzyme by L-arginine make it very probable that this biosynthetic chain is the functional one.

The presence of a feedback control on the second enzyme of the pathway is rather surprising, but the existence in yeast of a transacetylation activity between acetylornithine and glutamate (unpublished observations) as is the case for Micrococcus glutamicus (Udaka and Kinoshita, 1958) could

explain this situation. In this case indeed, the function of the first enzyme would be only to feed the cycle formed by steps 2, 3 and 4.

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