ARGININE METABOLISM IN CHLAMYDOMONAS REINHARDI
Arginine deiminase: the first enzyme of the catabolic pathway

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1. Introduction

During a study on the regulation of the arginine biosynthesis in Chlamydomonas [1,2] we observed that an arginine-requiring mutant arg C1, lacking the enzyme acetylglutamylphosphate reductase, contained citrulline. Since this strain is unable to synthesize this compound via the normal biosynthetic pathway of arginine, it must be produced by degradation of arginine. This led us to investigate the catabolic pathway of arginine in Chlamydomonas. We have found that the first step in the degradation is catalyzed by the enzyme arginine deiminase (L-arginine iminohydrolase EC 3.5.3.6.). This enzyme causes the degradation of arginine to citrulline and NH_3, as was shown by radioactive and colorimetric analysis. The pH optimum is at 8.3. The enzyme is inducible by arginine and is inhibited by ornithine.

2. Experimental procedures

The organisms used throughout this study were *Chlamydomonas reinhardi* strain 137C wild type and mutant arg C1 which were kindly supplied by Dr. R.P.Levine.

Conditions of growth and the preparation of cell-free extracts have been described earlier [1]. The cell cultures contained 200 μg/ml arginine as sole nitrogen source unless otherwise stated. The crude cell extracts were dialyzed against 0.25 M glycinate buffer pH 8.3 for 18 hours at 4°C.

Citrulline was determined according to the method of Archibald [3] and NH_3 according to the Sigma Technical Bulletin 108. Protein was measured by the method of Lowry [4].

3. Results and Discussion

The most frequently occurring enzymes involved in catabolic pathways of arginine are arginase which leads to the formation of ornithine and urea, and arginine deiminase which leads to formation of citrulline and NH_3 [5–8]. In order to identify the first enzyme in the catabolic pathway of Chlamydomonas, arginine was incubated with cell extract of wild type, grown on arginine as sole nitrogen source. The cell extract was prepared as described in Experimental Procedures. The reaction mixture contained in a final volume of 1 ml: cell extract (about 3 mg protein), 50 μmoles arginine and 125 μmoles glycinate buffer pH 8.3. The mixture was incubated for 1 hour at 37°C after which the reaction was stopped by addition of trichloroacetic acid to a final concentration of 5%. After treatment of the reaction mixture by Archibald's method [3], the absorption spectrum of the reaction product was measured. This absorption spectrum showed a maximum at 492 nm, while citrulline and urea give maxima at respectively 492 and 480 nm. The shape of the spectrum was similar to that of citrulline. This indicates that arginine was converted into citrulline and that Chlamydomonas possesses an arginine deiminase.

This conclusion was affirmed by thin layer chromatography of the reaction mixture of cell extract incubated with uniformly labelled ^{14}C-arginine. Chromatography on silica gel plates with chloroform-

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methanol-17% NH_4OH (2:2:1) and methanol-pyridine-H_2O (20:1:5) showed one radioactive reaction product with the same Rf values as citrulline. No ornithine or urea could be detected. Since the cell extract was dialyzed apparently no readily dissociable low molecular weight compound is required for enzyme activity. In this respect, the arginine deiminase of Chlamydomonas is similar to that of bacteria and Tetrahymena [6,7,9]. The degradation of arginine by arginine deiminase leads to the formation of citrulline and NH_3. The formation of the latter product by Chlamydomonas enzyme was ascertained by an ammonia determination. We observed that citrulline and NH_3 are formed in equimolar amounts.

The pH optimum curve of this enzyme was determined in glycinate buffers of different pH values (fig. 1). The curve shows a maximum plateau from 8.0–8.6. The enzyme is considerably inhibited by Tris buffer.

Ornithine too, has an inhibiting action on this enzyme. In view of the physiological importance of ornithine, this inhibition was examined more extensively. Lineweaver-Burke plots of the deiminase are presented in fig. 2. The K_m for arginine is 8 mM, while the K_i for ornithine at the higher arginine concentrations has a value of 5.5 mM. However, the competitive inhibition by ornithine shows complicated kinetics at relatively low arginine concentrations. We have not further investigated whether this is due to the use of crude extracts or whether it is a real property of this enzyme.

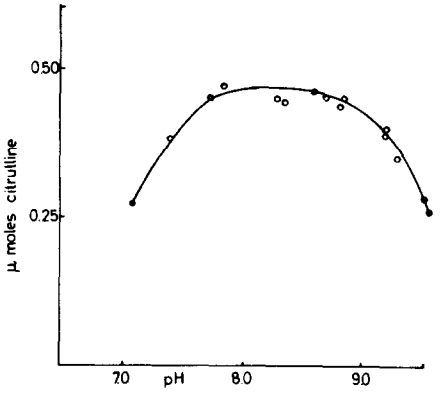

Fig. 1. pH optimum of arginine deiminase. The reaction mixture contained in a final volume of 1 ml: cell extract, 50 µmoles arginine and 125 µmoles glycinate buffer. The mixture was incubated for 1 hour at 37°C.

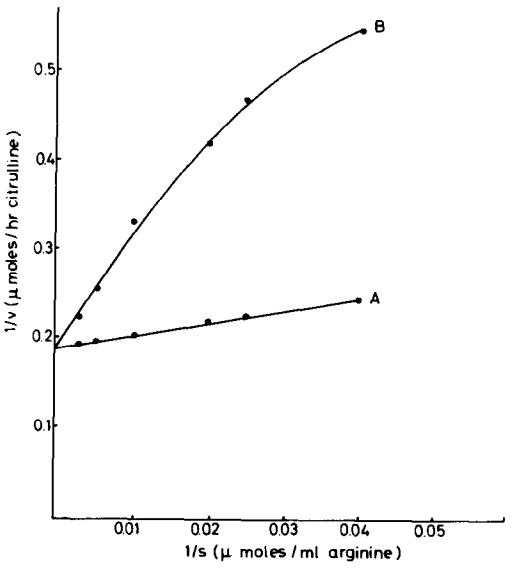

Fig. 2. Line weaver-Burke plot of arginine deiminase activity VS concentration of arginine in the absence (A) and presence (B) of 50 mM ornithine.

The inhibition of arginine deiminase by ornithine provides a means of control over the catabolic pathway. It may be useful if arginine synthesis is desired and arginine deiminase is still present. Nevertheless, the physiological importance has yet to be proven.

Arginine deiminase has been detected in arginine requiring mutant arg C1, grown on minimal medium plus 200 µg/ml arginine. It was also found in wild type and arg C1 grown on 200 µg/ml arginine as sole nitrogen source and in wild type grown on 40 mg/ NH_4Cl (1/10 of the normal amount of NH_4Cl). It was scarcely detectable in wild type grown on minimal medium alone. This indicates that arginine deiminase is inducible by arginine and that NH_4^+ may give product repression. Derepression can occur in nitrogen starved cultures. Similar phenomena have been observed in yeast [10]. The further degradation of citrulline is under investigation.

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