ARGININE METABOLISM IN *CHLAMYDOMONAS REINHARDI*. REGULATION OF UPTAKE AND BREAKDOWN

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1. Introduction

In the course of our investigation on the regulation of arginine metabolism in the green alga *Chlamydomonas reinhardi*, it was found that in the wild-type strain the pool of arginine was very low on minimal medium [1]. The addition of arginine to this medium did not result in a parallel increase of the arginine pool, although arginine rapidly disappeared from the medium.

It was then observed that the amount of arginine in the pool could be strikingly increased in wild-type cells by growing them on media supplemented with arginine but poor in NH_4^+ ions. As we had indications that the activity of the enzymes of the arginine catabolic pathway was very high in these conditions [1, 2], the increased arginine pool could be explained by assuming that the permeability of the cells to arginine was also regulated by ammonium and (or) arginine. This led us to investigate the effect of NH_4^+ ions and arginine on the regulation of uptake and breakdown of arginine in *C. reinhardi*. The results show that high concentrations of NH_4^+ in the culture medium greatly lower the penetration of arginine into the cells and lead to the repression of the first enzyme arginine deiminase of the catabolic pathway. Neither the uptake system nor arginine deiminase seem to be inducible by arginine.

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2. Experimental procedures

The wild-type (WT) strain 137 C, mating type (–) of *Chlamydomonas reinhardi*, kindly supplied by Dr. R.P.Levine, has been used throughout this study.

Media, conditions of growth and preparation of cell-free extracts have been described previously [1]. Various 'minimal' media have been used, which only differ in the concentration of NH_4Cl (400 N medium is the usual minimal medium containing 400 mg/l NH_4Cl). The permeability of the cells to arginine was determined by addition of ^{14}C-labelled arginine to liquid cultures in the logarithmic phase. Cells were collected and washed on Millipore filters. The radioactivity was measured in a Packard Tricarb Spectrometer.

Arginine was measured by the method of van Pilsum [4] after precipitation of proteins with 5%. trichloroacetic acid. Arginine deiminase was assayed as described earlier [2]. Protein was determined according to Lowry [5].

3. Results

3.1. *Effect of ammonium on arginine uptake in the wild-type strain*

Ammonium could interfere with the permeability of the cells to arginine in two different ways, by acting either as a repressor of the synthesis of the enzyme(s) involved in the active transport of arginine or as an inhibitor of the permeation system.

To check these possibilities, WT cells were grown for 3 days in media containing high (400 N) or low (20 N) concentrations of NH_4Cl. Cells from each cul-

ture were centrifuged, washed and resuspended, respectively, in 400 N and 20 N media. The uptake of arginine was measured for 4 hr after addition of 0.6 μCi/ml ^{14}C-arginine and 50 μg/ml arginine. The results of a typical experiment are shown in fig. 1. The penetration of arginine into the cells is greatly dependent upon the concentration of NH_4^+ in the culture medium during growth. The initial velocity of arginine uptake in cells grown on 20 N is about 20 times higher than in cells grown on 400 N (result of 4 independent experiments). The permeability of the cells to arginine is only slightly modified by the concentration of NH_4^+ during the uptake experiment itself. These results suggest that an active transport system for arginine (permease) is operative in *C. reinhardi*. This system is strongly repressible by NH_4^+ ions.

From now on, and without knowing anything about the nature and specificity of this uptake system, we propose to represent it by *Parg*.

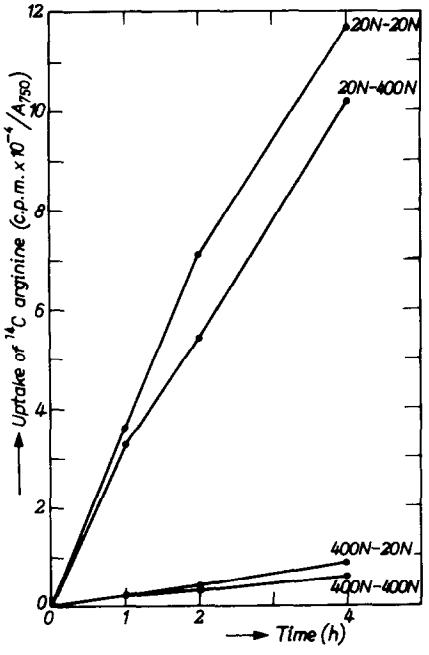

Fig. 1. Uptake of ^{14}C-arginine by WT cells in various conditions (20 N–20 N: growth on 20 mg/l NH_4Cl, arginine uptake on 20 mg/l NH_4Cl, arginine uptake on 400 mg/l NH_4Cl, et.). The values of the uptake of ^{14}C-arginine are related to the absorbance (A) of the culture at 750 nm.

3.2. *Effect of arginine during growth on arginine uptake*

To discover whether arginine is able to induce *Parg*, we did the following experiment: WT cells were grown on 20 N and 400 N media supplemented with various concentrations of arginine. The uptake of ^{14}C-arginine (0.6 μCi/ml) was measured in the presence of 50 μg/ml arginine, in 20 N medium for cells grown on 20 N, and in 400 N medium for cells grown on 400 N. It can be seen (fig. 2) that *Parg* is not modified by 10 μg/ml arginine. For higher concentrations of arginine (25 to 500 μg/ml), the uptake is reduced to a fairly constant extent. However, it remains higher than in cells grown on 400 N medium. Exogenous arginine has no significant inducing effect on the permeability of the cells grown on high ammonium concentration (400 N). This is not surprising in view of the finding that the arginine pool is very low whether or not medium is supplemented with arginine (see table 1). On the contrary, it appears that arginine at relatively low concentrations (from 25 μg/ml) acts as a repressor of *Parg* when the cells are grown 20 N media. It was interesting to note that increasing the concentration of arginine from 25 to 500 μg/ml did not significantly reduce the permeability. This could be explained by our finding that even in fully derepressed cells *Parg* is saturated by concentrations of arginine as low as 10 μg/ml. The conditions

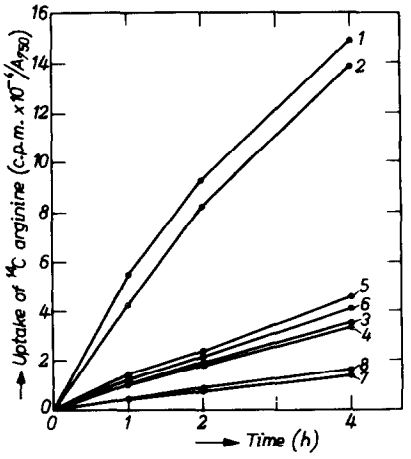

Fig. 2. Uptake of ^{14}C-arginine in cells grown on 20 N medium (1), on 20 N medium supplemented respectively with 10 (2), 25 (3), 50 (4), 100 (5) and 500 (6) μg/ml arginine, on 400 N medium (7) and on 400 N + 100 μg/ml arginine (8)

of saturation remain during the whole growth period on 20 N + at least 25 μg/ml arginine. As expected, after growth on 20 N + 10 μg/ml arginine, arginine had almost completely disappeared from the medium.

3.3. *Regulation of arginine deiminase (enzyme K)*

It was previously observed that the specific activity of enzyme K was very low in cells grown on minimal medium, but higher in cells grown on 1/10 of the normal concentration of NH_4Cl (40 N) or on media containing arginine as the sole nitrogen source [3]. These data suggest that NH_4^+ and perhaps, also arginine, are involved in the regulation of this enzyme. To check these hypotheses, experiments have been performed in which cells were grown on varying concentrations of NH_4Cl and arginine. The activities of enzyme K and the amounts of arginine in the pools were measured and are reported in table 1. No direct correlation between the arginine pool and the activity of K (compare for example 20 N and 400 N, or 20 N and 20 N + 100 arg) was found. However, whether or not arginine is present, there is a clear correlation between the concentration of NH_4^+ in the medium and the activity of K. These data strongly suggest that enzyme K is regulated by NH_4^+ but is not inducible by arginine.

3.4. *Derepression of uptake and breakdown of arginine by NH_4^+ starvation*

From the experiments above described, it has been found that *Parg* and K are both repressed in cells grown on high concentrations of ammonium. The derepression of *Parg* and K has been followed after transfer of the cells from 400 N medium ammonium-free media. The results show (fig. 3) that derepression begins very soon after transfer and, in agreement with the data of fig. 1 and table 1, is much stronger for *Parg* than for K.

Table 1
Specific activities of arginine deiminase (K) and arginine pools in various growth conditions.

Medium (μg/ml)	Specific activity of K (μmole citrulline/mg protein/hr)	Arginine pool (μmole/mg protein)
20 N	0.36	0.05
400 N	0.09	0.04
0 N + 100 arg	0.47	0.47
20 N + 100 arg	0.29	0.35
400 N + 100 arg	0.10	0.07

4. Discussion

Only few data have been published on the effect of arginine and ammonium of uptake and breakdown of arginine in bacteria. In *E. coli*, arginine [6] and NH_4^+ [7] did not affect the uptake of arginine, while in *B. subtilis* both the arginine permease and the arginase were inducible by arginine [8]. In the yeast *S. cerevisiae*, the rate of arginine uptake decreased in cells grown in the presence of NH_4^+ [9], as the result of inhibition by NH_4^+ of an unspecific permease, while the specific arginine permease was insensitive to this ion [10]. On the other hand, evidence has been presented that in yeast two enzymes of the catabolic pathway (arginase and ornithine amino transferase) were repressible by NH_4^+ and fully inducible by arginine even in the presence of NH_4^+ concentrations giving strong repression in the absence of arginine [11].

In this paper we show that, in *C. reinhardi*, NH_4^+ gives parallel but not coordinate repression (fig. 3) of

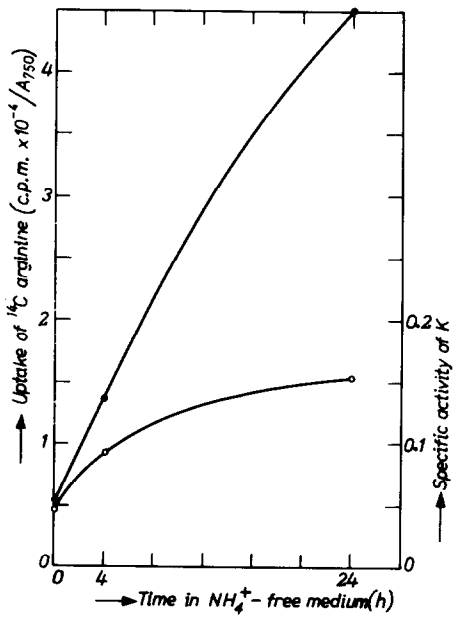

Fig. 3. Derepression of *Parg* (●—●) and K (○—○) during starvation in NH_4^+-free medaium.

uptake and breakdown of arginine. No inducing effect of arginine has been detected even in conditions where the pool of arginine was very high (table 1, fig. 2) Instead of induction, there was a significant repressing effect of arginine on its uptake system when cells were grown on low concentrations of NH_4^+. This repression could result from the direct action of arginine, but more likely from NH_4^+ produced by the breakdown of arginine and acting as a repressor. These hypotheses might be verified using mutants unable to produce NH_4^+ from arginine in the cell (arginine deiminase-less mutants) but so far this kind of mutant has not been isolated in *Chlamydomonas*. We know from previous experiments that the additions of arginine to a high ammonium medium does not change the activity of the arginine biosynthetic enzymes nor does it modify significantly the arginine pool [1, 12]. This may be easily explained by assuming that all arginine entering the cell in these circumstances is immediately broken down. On the contrary, cells grown in a low ammonium medium supplemented with arginine have a much higher pool, which means that in these conditions arginine from outside is not immediately broken down. These facts might explain why most of the arginine-requiring mutants of *C. reinhardi* are inhibited in growth by ammonium [13]. Biochemical aspects of this problem will be published in another paper.

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