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The urate uptake system in *Chlamydomonas reinhardtii*

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Urate was accumulated against a concentration gradient in cells of *Chlamydomonas reinhardtii* by an inducible mediated transport system lacking passive diffusion and distinguishable from the urate oxidase activity. The system has a K_t of 0.9 μM and an optimal pH of 6.2. Addition of ammonia to cells actively consuming urate rapidly inhibited the transport, whereas urate oxidation was not affected. Dark conditions halved urate uptake, which was not followed by a parallel decrease in urate oxidase activity. Nitrogen starvation diminished urate uptake by cells which, in contrast, exhibited high levels of urate oxidase after different periods of nitrogen deprivation. Metabolic inhibitors such as NaCN, NaN_3, 2,4-dinitrophenol and *N*-ethylmaleimide severely inhibited urate transport, whereas they did not appreciably affect urate oxidase in vivo. We conclude that urate uptake in *C. reinhardtii* is an inducible energy-dependent mediated transport process different from the enzyme-catalyzed intracellular urate oxidation.

Introduction

Purines and other nucleic acid breakdown products are utilized by a variety of organisms as sources of nitrogen and energy or as precursors in nucleic acids synthesis [1]. As a prerequisite, these compounds must enter the cells through different transport systems according to the type of cells.

A common pattern in the utilization of purines and purine derivatives is that their metabolic conversions take place immediately after their transport. Thus, it has been very difficult to distinguish between the transport process proper and the subsequent enzyme-catalyzed conversions of the transported substrate. For this reason, there are so relatively few reports in which these transport systems are distinguished from the following metabolism of these compounds [1], and the scarce existing systems utilize membrane vesicles as study models [2].

Studies on the purine uptake and metabolism in algae are rather scarce [1,3,4]. Uric acid is utilized by green algae as a nitrogen source for growth [4–7], although the mechanisms by which this compound is translocated into the cell are unknown. In the present work the uptake of urate by *Chlamydomonas reinhardtii* cells is established as an inducible energy-dependent transport system distinguishable from the metabolic drag caused by the subsequent enzyme-catalyzed urate oxidation.

Materials and Methods

Cells of *C. reinhardtii* 6145*c* (a gift from Dr. Ruth Sager, New York) were grown phototrophically as previously described [4] with 4 mM ammonia or 1 mM uric acid as sole nitrogen sources.

Cells were centrifuged at 20000 × *g*, 10 min, and then broken by freezing at −40°C and thawing with gentle stirring in 0.1 M Tris-HCl buffer (pH 9.0). The homogenate was centrifuged at 27000 × *g*, 10 min, and the resulting supernatant was used as source of enzyme.

Urate and ammonia uptake was measured by

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determining their disappearance from the media after removal of the cells by centrifugation. For short-term experiments, 1 ml aliquots of culture media were centrifuged at 11 000 rpm, 1 min, in a Beckman Microfuge 11. Intracellular urate content was determined by centrifuging 10–15 ml of cell culture at $20000 \times g$, 10 min, and storing the washed sediment at −40°C. Frozen pellets were then thawed with 1 ml water, vigorously shaken and centrifuged as above. Urate was estimated in the supernatant [8]. All the steps were performed at 0–4°C.

Growth was measured turbidimetrically by following the absorbance of the cultures at 660 nm. Cell volume was calculated by measuring the packed cell volume as described by Gfeller and Gibbs [9].

Urate oxidase (urate: O_2 oxidoreductase, EC 1.7.3.3) was assayed spectrophotometrically at 292 nm [4]. One unit of urate oxidase activity is defined as the amount of enzyme which oxidizes 1 μmol of urate per min under optimal conditions of assay. Specific activity is expressed in mU/mg protein. Protein was determined according to Bradford [10], using bovine serum albumin as standard. Ammonia was estimated colorimetrically by the Conway microdiffusion method [11] or by the phenol-hypochlorite procedure [12]. Urate was measured directly at 292 nm (molar extinction coefficient $12.2 \cdot 10^3$) or enzymatically by a colorimetric assay [8]. Chlorophyll was determined as described by Arnon [13].

Kinetic experiments were performed by following the disappearance of urate in diluted culture media at 30–60-s periods and plotting the corresponding progress curve ($|S|$ versus t). Parameters were calculated by means of the integrated Michaelis-Menten equation as described by Cornish-Bowden [14].

Results

Chlamydomonas reinhardtii cells used urate for growth as sole nitrogen source at a constant rate after a lag phase required to induce the urate-uptake system (Fig. 1). After the lag phase, urate was accumulated in cells against a concentration gradient, reaching a maximum of 5.4 mM at an extracellular concentration of 0.12 mM (Fig. 1, arrow). When urate in the medium was exhausted the intracellular urate concentration dropped sharply, which indicates a rapid urate assimilation. Urate oxidase specific activity increased steadily until the intracellular urate was depleted. The urate-uptake system was dependent on de novo protein synthesis, since it was not induced in the

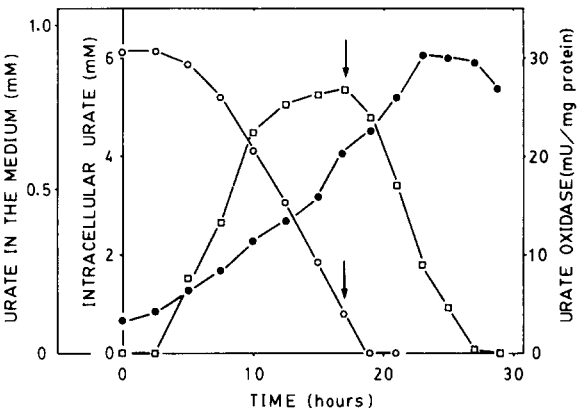

Fig. 1. Time course of urate uptake and accumulation by cells of *C. reinhardtii*. Cells grown on ammonia were washed and transferred to a medium containing urate as sole nitrogen source, ○, urate concentration in the medium; □, intracellular urate concentration; ●, urate oxidase activity.

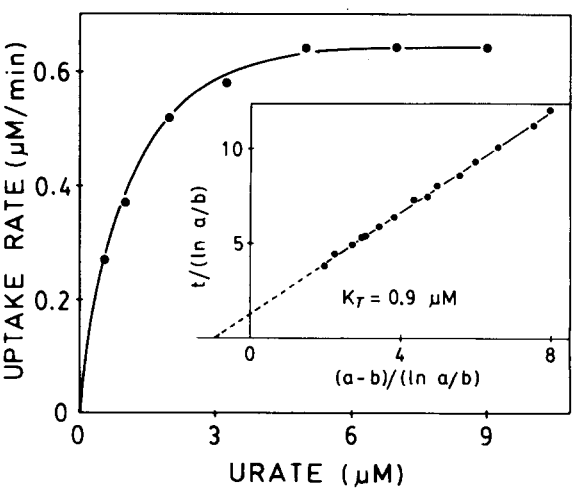

Fig. 2. Direct plot of urate uptake rate vs. urate concentration in *C. reinhardtii* cells. Slopes from the progress curve of urate disappearance in cell cultures containing 1 μg Chl/ml are represented. Inset: integrated plot of Michaelis-Menten equation for the uptake process. t, time in min; a, initial urate concentration (9 μM); b, urate concentration at the time t.

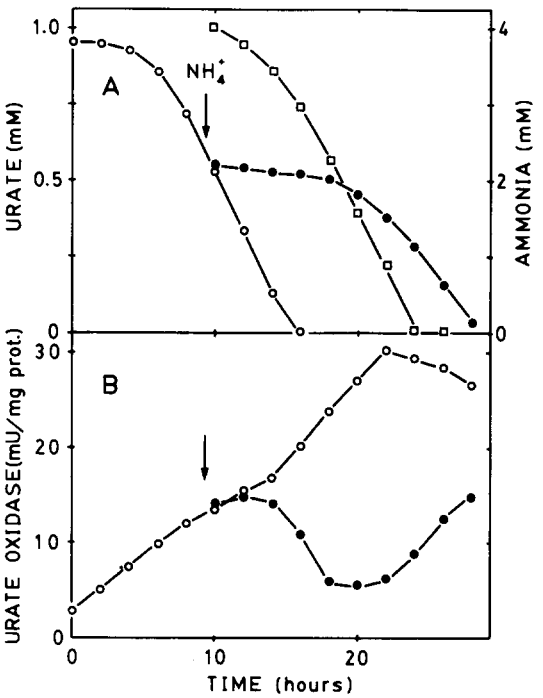

Fig. 3. Time course of ammonia effect on urate uptake (A) and oxidation (B) by *C. reinhardtii* cells. Ammonia 4 mM (↓) was added to cells linearly consuming urate (○); ●, urate uptake and oxidation in the presence of ammonia; □, ammonia uptake.

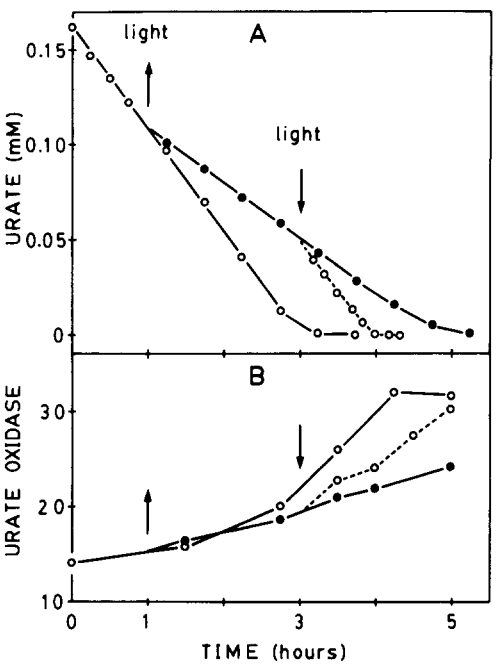

Fig. 4. Effect of light-dark transition on urate uptake (A) and oxidation (B) by *C. reinhardtii* cells. Cells linearly consuming urate in the light were transferred to dark conditions (↑) and after 2 h light conditions were restored (↓); ○, urate uptake and oxidation in the light; ●, urate uptake and oxidation in the dark. Urate oxidase activity is expressed in mU/mg protein.

presence of cycloheximide (1 μg/ml) in cells grown on ammonia and transferred to media containing urate (results not shown).

When the urate uptake by cells previously induced with urate was followed at 30–60-s periods, a mediated transport system lacking a diffusion component was found (Fig. 2). A Michaelis constant for transport (K_t) of 0.9 μM, markedly different from the apparent K_m of urate oxidase for urate (12 μM), and a V_{max} of 0.74 μmol/min per mg Chl were calculated.

The optimum pH of urate uptake was 6.2, whereas that of urate oxidase activity was about 9, which clearly distinguishes urate uptake and oxidation processes.

Ammonia prevented urate from entering the cells (Fig. 3). Addition of ammonia to *C. reinhardtii* cells actively consuming urate inhibited urate uptake, which was resumed after 10 h when ammonia concentration in the medium was still 2 mM and urate oxidase levels were minimal. Similar proportional inhibitions were found when different concentrations of added ammonia, ranging between 0.1 and 4.0 mM, were used (results not shown).

The entrance of urate was light-dependent. Urate uptake was halved when cells were transferred from light to dark conditions, whereas urate oxidase activity remained practically unchanged (Fig. 4). In the dark, urate uptake exhibited saturation kinetics with a K_t of 2.1 μM, twice the corresponding K_t value in the light.

Nitrogen starvation also influenced urate uptake without appreciably changing urate oxidation specific activity. Cells induced with urate and subjected to different nitrogen starvation periods were affected in their capacity to consume urate (Fig. 5). Similar urate oxidase levels, ranging between 18 and 25 mU/mg protein, sufficient to support maximum uptake activity, were present in control and starved cells.

Similarly, differences in response to metabolic inhibitors were also found for urate uptake and enzymatic oxidation activities (Table I). NaCN,

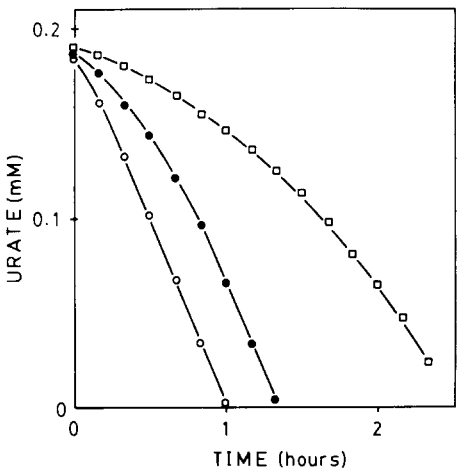

Fig. 5. Effect of nitrogen starvation on urate uptake by *C. reinhardtii* cells. Cells grown on urate 1 mM were allowed to exhaust urate and then kept 1 h (●) and 5 h (□) under conditions of nitrogen starvation. After these treatments, cells were diluted up to 9 μg Chl/ml and uptake of urate by treated and control non-starved (○) cells was measured as described in Materials and Methods. Urate oxidase activity at the beginning of the experiment was 18 (○), 23 (●) and 25 (□) mU/mg protein.

NaN_3 and *N*-ethylmaleimide turned out to be strong inhibitors of urate uptake without markedly affecting urate oxidase specific activity. 2,4-Dinitrophenol and *p*-hydroxymercuribenzoate inhibited uptake to a minor extent but still distinctly affected urate uptake and oxidation.

Discussion

The results presented in this paper demonstrate for the first time the presence in a green alga of an inducible energy-dependent urate transport system distinguishable from the urate oxidation process.

C. reinhardtii cells used urate as sole nitrogen source after a lag period required to induce the uptake system. Likewise, urate was accumulated in algal cells against a concentration gradient, reaching an inside-to-outside concentration ratio of 45 (Fig. 1). Under these conditions, urate transport was distinguishable from any metabolic drag caused by urate oxidation, since urate oxidase levels changed steadily irrespective of urate concentration until internal urate was depleted. Similar concentrating urate uptakes have been reported in several yeasts [15,16], bacteria [17] and *Chlorella pyrenoidosa* [6], and in *C. reinhardtii* a ratio of 3000 between urea concentration inside and outside the cells has been found [18].

Urate was translocated into the cells by means of a mediated transport system lacking a diffusion component with a K_t of 0.9 μM, much lower than the apparent K_m of urate oxidase for urate (12 μM) [4]. A similar K_t value has been reported for the urate-uptake system of *Alcaligenes eutrophus* [19]. The difference between K_t and K_m figures for urate uptake and oxidation indicates that both are separate processes.

TABLE I

EFFECT OF DIFFERENT METABOLIC INHIBITORS ON URATE UPTAKE AND OXIDATION IN *C. REINHARDTII* CELLS

Cells actively consuming urate were divided into glass flasks and the listed reagents were added at the indicated final concentrations. After 30 min, the urate uptake was determined during 1 h at 15 min intervals. Urate oxidase was measured at the end of the experiment. 100% of urate uptake and urate oxidase activities of untreated cells corresponded to 0.6 μmol/min per mg Chl and 14 mU/mg protein, respectively.

Inhibitor	Concentration (M)	Urate uptake activity (%)	Urate oxidase activity (%)
Sodium cyanide	$1 \cdot 10^{-5}$	24	82
	$1 \cdot 10^{-4}$	0	90
N-Ethylmaleimide	$1 \cdot 10^{-5}$	29	68
	$1 \cdot 10^{-4}$	0	65
Sodium azide	$5 \cdot 10^{-5}$	86	84
	$5 \cdot 10^{-4}$	0	73
2,4-Dinitrophenol	$1 \cdot 10^{-4}$	77	88
	$1 \cdot 10^{-3}$	16	92
p-Hyhroxymercuribenzoate	$1 \cdot 10^{-4}$	84	96

Another feature characteristic of a mediated active transport of the urate uptake system in *C. reinhardtii* is its dependence on pH. The optimum pH for uptake turned out to be 6.2, very different from the optimum pH (8.8–9.1) of urate oxidase activity [4]. The same optimum pH of 6.2 has been reported for the urate uptake in *A. eutrophus* [19].

The urate uptake system was very sensitive to energy poisons. Inhibition by azide and cyanide provides evidence that the transport process is energy dependent. The entry of urate was practically inhibited by 1 mM 2,4-dinitrophenol, suggesting that a proton gradient is necessary for the urate transport to proceed. The effect of these metabolic inhibitors and the stimulating effect of light on the uptake (Fig. 4) are consistent with the proposal that, as suggested for *Neurospora crassa* [20], the mechanism of urate translocation is via an active transport and not by metabolic drag. The fact that these treatments did not affect urate oxidase activity to a noticeable extent corroborated this distinction between urate active transport and its enzymatic oxidation. Besides, SH-group reagents strongly inhibited urate uptake in *C. reinhardtii*, thus indicating that SH-groups might be involved in the urate-uptake system. Similar results have been reported for the system responsible for xanthine and uric acid uptake in *A. eutrophus* [19] and for nucleoside transport in *Escherichia coli* [21]. The different sensitivity of uptake to *p*-hydroxymercuribenzoate and *N*-ethylmaleimide indicates that SH-groups are not easily accessible from the outside [22].

Experiments presented here demonstrate that urate uptake was rapidly inhibited by ammonia, whereas urate oxidase remained practically unaffected during short-term transport inhibition (Fig. 3), which separates the urate transport and metabolic oxidation processes. The fact that inhibition of urate uptake was relieved after several hours in the presence of ammonia (Fig. 3A) indicates that the synthesis of the urate transport system is not repressed by ammonia, thus establishing another difference with the urate oxidase system [4].

Nitrogen starvation also influenced urate uptake. When *C. reinhardtii* cells actively growing on urate were subjected to different starvation times, a lag in reaching the same uptake rate as that of the control cells was observed. For its part, urate oxidase activity was not noticeably changed by the treatment, which corroborates the distinction between urate transport and assimilation.

Acknowledgements

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References

1 Munch-Petersen, A. and Mygind, B. (1983) in Metabolism of Nucleotides, Nucleosides and Nucleobases in Microorganisms (Munch-Petersen, A., ed.), pp. 259–305, Academic Press, London
2 Bongaerts, G.P.A., Van der Drift, C. and Vogels, G.D. (1984) Anton. Leeuwenhoek J. Microbiol. 50, 177–182
3 Vogels, G.D. and Van der Drift, C. (1976) Bacteriol. Rev. 40, 403–468
4 Pineda, M., Fernández, E. and Cárdenas, J. (1984) Physiol. Plant. 62, 453–457
5 Birdsey, E.C. and Lynch, V.H. (1962) Science 137, 763–764
6 Ammann, E.C.B. and Lynch, V.H. (1964) Biochim. Biophys. Acta 87, 370–379
7 Cain, J. (1965) Can. J. Bot. 43, 1367–1378
8 Fossati, P., Prencipe, L. and Berti, G. (1980) Clin. Chem. 26, 227–231
9 Gfeller, R.P. and Gibbs, M. (1984) Plant Physiol. 75, 212–218
10 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
11 Conway, D.J. (1957) Microdiffusion Analysis and Volumetric Error, pp. 90–132, Crosby Lockwood, London
12 Solorzano, L. (1969) Limnol. Oceanogr. 14, 799–801
13 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
14 Cornish-Bowden, A. (1981) Fundamentals of Enzyme Kinetics, pp. 34–37, Butterworths, London
15 Roush, A.H. and Domnas, A.J. (1956) Science 124, 125–126
16 Roush, A.H., Questiaux, L.M. and Domnas, A.J. (1959) J. Cell. Comp. Physiol. 54, 275–286
17 Rouf, M.A. and Lomprey, R.F., Jr. (1968) J. Bacteriol. 96, 617–622
18 Dagestad, D., Lien, T. and Knutsen, G. (1981) Arch. Microbiol. 129, 261–264
19 Eschmann, K. and Kaltwasser, H. (1982) Arch. Microbiol. 131, 191–196
20 Tsao, T-F. and Marzluf, G.A. (1976) Mol. Gen. Genet. 149, 347–355
21 Doskocil, J. (1976) Mol. Cell. Biochem. 10, 137–143
22 Kepes, A. (1979) in Membrane Biochemistry (Carafoli, E. and Semenza, G., eds.), pp. 25–35, Springer Verlag, New York