ENERGY-DEPENDENT TRANSPORT OF URATE AND XANTHINE IN THE UNICELLULAR GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

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INTRODUCTION

Purines and purine derivatives are utilized by many organisms as sources of nitrogen and energy or as precursors in nucleic acids synthesis. Prior to their utilization these compounds must enter the cells through different transport systems depending on the type of cells. Up to now it has been very hard to distinguish between the transport process proper and the subsequent enzymatic oxidation of the transported substrate, since purines and other nucleic acids breakdown products have been observed to be utilized immediately after their transport into the cells. Thus, it is small wonder that there are so few reports in which these transport systems are distinguished from the subsequent metabolism of these compounds (Syrett, 1981; Munch-Petersen and Mygind, 1983).

Apart from our previous characterization of urate uptake and urate and xanthine metabolism in *Chlamydomonas reinhardtii* (Pineda et al., 1984, 1987; Pineda and Cárdenas, 1985; Pérez-Vicente et al., 1987, 1988), studies on purine uptake and metabolism in algae are rather scarce and centered mainly upon the ability of these compounds to be used as nitrogen sources for growth (Syrett, 1981; Devi Prasad, 1983), whereas the mechanisms by which these compounds are translocated into the cells have been but poorly studied.

In this paper we review our present knowledge about the uptake of purines by *Chlamydomonas reinhardtii* cells. Data on transport of urate and xanthine in this unicellular green alga strongly support the conclusion that both purines are translocated through inducible mediated transport systems, exhibiting Michaelian kinetics, lacking a diffusion component, energy-dependent, and distinguishable from the enzyme-catalyzed intracellular substrate oxidation.

MATERIALS AND METHODS

Growth Conditions and Preparation of Enzyme Extracts

Cells of *Chlamydomonas reinhardtii* 6145c (from the collection of Dr R. Sager, Sydney Farber Center, New York) were cultured phototrophically as previously described (Pineda et al., 1984). Cells were collected by
centrifugation at 20000 g, 10 min, and broken by freezing at -40°C and thawing with gentle stirring in 0.1 M Tris-HCl buffer, pH 8.5 (xanthine dehydrogenase) or 9.0 (urate oxidase). The homogenates were centrifuged at 27000 g, 10-20 min, and the resulting supernatants used as source of enzymes.

**Enzyme Assays**

Xanthine dehydrogenase (EC 1.2.1.37) activity was measured spectrophotometrically by following NAD⁺ reduction at 340 nm in a reaction mixture containing in a final volume of 1 ml 100 μmol Tris-HCl, pH 8.5, 0.6 μmol hypoxanthine, 1 μmol NAD⁺ and the adequate amount of enzyme extract (Perez-Vicente et al., 1988). Urate oxidase (EC 1.7.3.3) activity was assayed spectrophotometrically by following the decrease in absorbance at 292 nm due to urate oxidation in a reaction mixture containing in a final volume of 1 ml 100 μmol Tris-HCl, pH 9.0, 50 nmol urate and an adequate amount of enzyme extract (Pineda et al., 1984). One unit of enzyme is defined as the amount of enzyme which catalyzes the reduction of 1 μmol NAD⁺ (xanthine dehydrogenase) or the oxidation of 1 μmol urate (urate oxidase) per min under optimal conditions of assay.

**Xanthine and Urate Uptake**

Xanthine or urate uptake was determined by following their disappearance from the media after removal of the cells by centrifugation. For spectrophotometric measurements, 1 ml aliquots of the culture media were centrifuged at 13500 rpm, 1-2 min, in a Beckman Microfuge 11. In radioactivity experiments, samples of 0.2 ml were centrifuged (13500 rpm, 30 s) in polypropylene microcentrifuge tubes (0.4 ml) containing 0.1 ml of a 3:2 mixture of silicone DC-550 and bis(3,5,5-trimethyl hexyl)phthalate. Absorbance or radioactivity was measured in the supernatants.

Kinetic experiments were performed by following the disappearance of purines in culture media at 30-60 s periods and plotting the corresponding progress curves ([S] versus t). Parameters were calculated by means of the integrated Michaelis-Menten equation as described by Cornish-Bowden (1981).

**Analytical Procedures**

Cell growth was measured turbidimetrically at 660 nm. Xanthine was determined enzymatically with milk xanthine oxidase at 292 nm (Krenitsky et al., 1986) or directly at 268 nm (millimolar extinction coefficient 9.15). Urate was measured enzymatically by the colorimetric assay of Possati et al., (1980) or directly at 292 nm (millimolar extinction coefficient 12.2). When necessary (6-14C)xanthine or (2-14C)urate was used and radioactivity was measured in a Beckman LS 3801 liquid scintillation counter. Protein was determined according to Bradford (1976), using bovine serum albumin as standard. All spectrophotometric assays and determinations were performed in a Bausch & Lomb Spectronic 2000.

**RESULTS AND DISCUSSION**

Chlamydomonas cells grown on ammonium and transferred to either xanthine or urate media took up these purines after a lag phase needed to induce the corresponding transport systems (Fig. 1). Neither xanthine nor urate were consumed significantly in the presence of 10 mM chloral hydrate, a reported inhibitor of protein synthesis in Chlamydomonas (McMahon and Blaschko, 1971), which indicates that both systems are repressed in algal cells cultured in ammonium. Very recently we have also