| Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism" | **Vol. 32** | Fasc. 3 | (91)-(94) | 18. 12. 1992 |
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# Sulfate Uptake in *Chlamydomonas reinhardtii*

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K e y w o r d s : Eukaryotic microalgae, S-deprived cells, sulfur metabolism.


## S u m m a r y

PEREZ-CASTIÑEIRA J.R, PRIETO J.L. & VEGA J.M. 1992. Sulfate uptake in *Chlamydomonas reinhardtii*. - Phyton (Austria) 32(3): (91)-(94).

Sulfate uptake by the green alga *Chlamydomonas reinhardtii* is a light-dependent saturable process with maximal rate at 0.3 mM sulfate. Kinetically, the transport appears to be multifasic with at least two Km values about 10 and 40 μM. It can be efficiently inhibited by sulfite and chromate. Sulfate uptake was significantly enhanced in S-starved cells, the initial uptake rate being doubled after 30 min under S-deprived conditions.


## I n t r o d u c t i o n

Many organisms including eukaryotic algae can grow with sulfate as the only sulfur source, showing a rapid transformation to S-amino acids, sulfolipids and other S-containing metabolites (SCHMIDT 1986). The first step in sulfate utilization is the uptake of the divalent anion into the cells. Sulfate uptake has been characterized in cells from different autotrophic organisms, including higher plants (LASS & ULLRICH-EBERIUS 1984), green algae (BIEDLINGMAIER & SCHMIDT 1989) and cyanobacteria (GREEN & GROSSMANN 1988). However, the available information for sulfate transport systems in green algae is still scanty. In this paper we report studies on the sulfate uptake in *C. reinhardtii*.


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## Materials and Methods

*Chlamydomonas reinhardtii* wild type (21 gr) cells were grown autotrophically in the liquid medium as previously described with 10 mM $NH_4Cl$ as nitrogen source and 0.3 mM sulfate as sulfur source (LEON & VEGA 1991). For sulfate uptake studies, cells were collected, washed twice and resuspended in S-free medium up to 20 µg chlorophyll $ml^{-1}$. The algae suspension was placed at 25 °C in a Warburg vessel under continuous illumination with white light (30 W $m^{-2}$) and the experiment was started by addition of $^{35}S$-labelled sulfate (supplied by Amersham International, Bucks, U.K.). For kinetic studies the total amount of radioactivity could increase up to 0.5 µCi $ml^{-1}$, whereas for other experiments (performed with 0.3 mM sulfate) 0.05 µCi $ml^{-1}$ was routinely used. 500 µl samples were taken every 2 to 3 min, dispensed into microfuge tubes, which already contained 500 µl of S-free medium, and centrifuged at 14,000 rpm for 30 sec. Supernatants were discarded and the corresponding cell pellets washed with 500 µl of S-free medium and then dissolved in 500 µl of Beckman "Ready Protein" scintillation liquid. Samples were counted in a Beckman LS-6000IC apparatus.

## Results and Discussion

Sulfate uptake was absolutely dependent on light, being negligible in the darkness (results not shown). This uptake was linear with respect to time for up to 30 min of incubation for all concentrations tested. The slopes of the straight lines thus obtained were taken as a measure of the uptake rate, which was maximal at 0.3 mM sulfate concentration (Fig. 1). Inside, an Eadie-Hofstee plot of the data is shown. Two slopes corresponding to Km values about 10 and 40 µM were fitted, with Vmax values corresponding to 0.1 and 0.2 µmol sulfate mg chlorophyll$^{-1}$ $h^{-1}$, respectively (average of two experiments). A third Km value of around 0.5 µM was eventually obtained in one experiment, but we still need to check this point. A Hill coefficient of 0.9 was obtained from these results suggesting a certain degree of negative cooperativity supposing *C. reinhardtii* has a single permease for sulfate; however, we cannot exclude the possible presence of two transport systems with different affinities towards sulfate. Two or even three different Km values for sulfate uptake have been also previously reported in higher plants (NISSEN & NISSEN 1983) and *Chlorella fusca*, where the Km found were 13, 39 and 280 µM (BIEDLINGMAIER & SCHMIDT 1989). By contrast, in the cyanobacterium *Anacystis nidulans* the sulfate uptake only shows a single Km value of around 0.75 µM (UTKILEN & al. 1976).

Table 1. Effect of various concentrations of sulfite and of sulfate analogues on sulfate uptake by *Chlamydomonas reinhardtii*. Uptake was measured at 0.3 mM sulfate.

| | Sulfate uptake (% of control) | | |
|---|---|---|---|
| | 0.3 mM | 1 mM | 3 mM |
| Sulfite | 81 | 52 | 41 |
| Selenate | 42 | 19 | 19 |
| Chromate | 22 | 0 | 0 |
| Molybdate | 75 | 67 | 28 |
| Tungstate | 81 | 80 | 80 |

Different anions were tested as possible inhibitors of sulfate uptake by *C. reinhardtii* cells. Sulfite exerted around 50 % inhibition when present at very high concentrations (1 to 3 mM) with respect to sulfate (0.3 mM). As expected, selenate was a strong inhibitor of uptake, although not as strong as chromate was, the latter producing around 80 % inhibition at 300 µM, and total inhibition when present over 1 mM in the medium. Molybdate and tungstate had a smaller effect than chromate, tungstate being only slightly inhibitory (Table 1). These results suggest the existence of specific membrane-binding sites for sulfate which can discriminate among $XO_4^{2-}$ type anions according to their size. The latter fact agrees with data obtained for some photosynthetic organisms (for further discussion see the above references for plants and *C. fusca*).

A time-dependent stimulation of sulfate uptake was observed when *C. reinhardtii* cells were previously incubated in S-free culture medium, the initial rate being doubled after 30 min (not shown) and steadily increasing up to 24 h of treatment (Fig. 2). This effect has been observed in other organisms such as *C. fusca* (BIEDLINGMAIER & SCHMIDT 1989) and *A. nidulans* (GREEN & GROSSMAN 1988); however, whereas in *C. fusca*, sulfur starvation conditions seems to affect strongly the kinetics of sulfate uptake, in *A. nidulans* only Vmax values were modified (GREEN & GROSSMANN 1988). Kinetic studies with S-starved *C. reinhardtii* cells are currently in progress, in order to establish possible changes in the affinity of the specific permease towards its substrate under this circumstances.

Acknowledgements

This work was supported by the DGICYT, Spain (Res. Grant n° PB90-0880). J.R.P.-C. was a recipient of a Ph.D. studentship from Ministerio de Educación y Ciencia (Spain).

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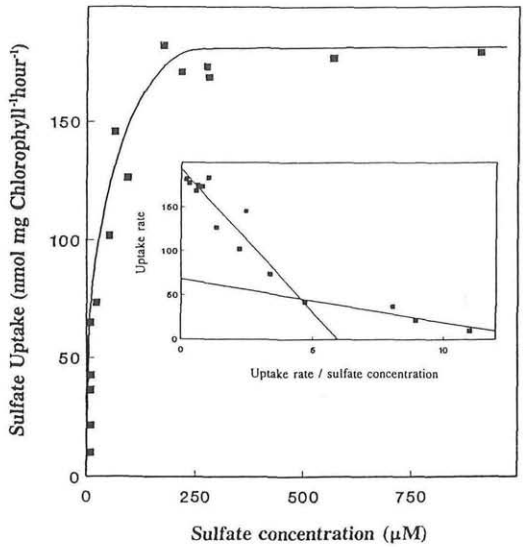


Fig. 1. Dependence of the rate of sulfate uptake on external sulfate concentration. Data from a typical experiment are shown. Inset shows an Eadie-Hofstee plot of the data.

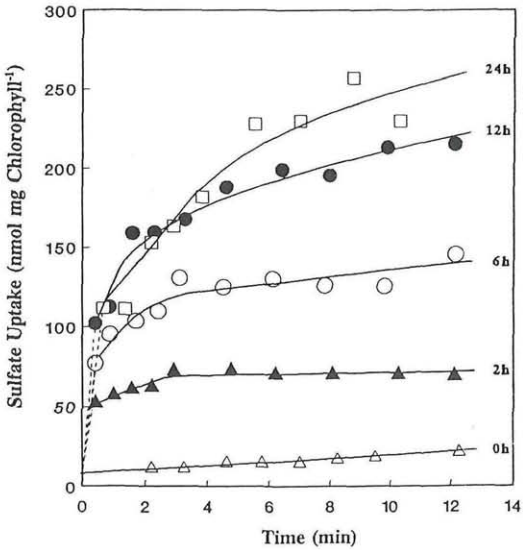


Fig. 2. Time-course of sulfate uptake by S-starved *C. reinhardtii*. Cells were harvested, washed and resuspended in S-free medium and pre-incubated at 25 °C in the light for 0, 2, 6, 12 and 24 h, then 0.3 mM sulfate was added and sulfate uptake measured.