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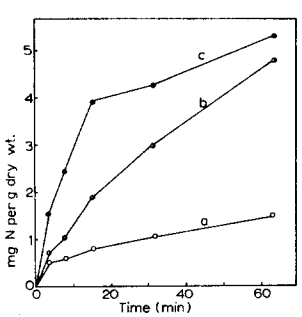
Oscillatory transport of ammonium ions by *Scenedesmus quadricauda*

Chlorococcal algae of the genera Chlorella and Scenedesmus have been used for studying physiological processes in algae both under growth and nongrowth conditions. In search for a suitable medium for *Scenedesmus quadricauda* we have examined in some detail the uptake and release of various inorganic cations and anions by this species and their mutual interactions during transport[1,2]. In the present communication we wish to report some of the properties of the transport system for NH_4^+ in this alga.

The experimental organism was *Scenedesmus quadricauda* (Turp) Bréb. strain Greifswald/15 of the collection of the Algological Laboratory at Třeboň. It was grown under static conditions[3] at 26°, with fluorescent tube illumination (about 0.05 cal·cm^{-2}·min^{-1}, Phar). The suspension was mixed and saturated with a mixture of air and CO_2 (95:5, v/v) at a flow rate of 50 l/h per l of suspension. The nutrient solution used contained 40 mM urea, 5 mM $MgSO_4 \cdot 7\ H_2O$, 5 mM KH_2PO_4, microelements and EDTA. After growth the cells were centrifuged, washed and, at a density of some 3 % (referring to dry weight), were used for incubation experiments. NH_4HCO_3 at the appropriate concentration was added to the suspension at zero time, and 10-ml samples of the suspension were removed at 30-sec intervals, filtered through a Whatman No. 1 filter attached to a water pump and the filtrate analyzed. Where efflux was studied, the suspension was preincubated for 2 h with NH_4HCO_3 (300 mg N per l), cells were separated by filtration and resuspended in an NH_4^+-free medium. The sampling procedure was the same as with influx. NH_4^+ was determined in the filtrate colorimetrically by Nessler's method. The standard deviation of the estimation was 1.09 %. It should be noted that the culture was not starved for nitrogen, the urea present in the medium being readily incorporated as such into the metabolic pathways of the cell[4].

It was found that NH_4^+ is taken up from the medium by a temperature-dependent process (Fig. 1) against its concentration gradient (Fig. 2). It may be seen from Fig. 3 that the NH_4^+ taken up over a period of 2 h are lost again when cells



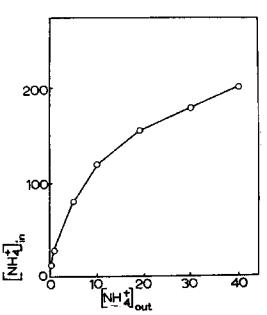


Fig. 1. Uptake of NH_4^+ by *S. quadricauda* at 5° (a), 15° (b) and 35° (c) under illumination from an initial concentration of 7 mM NH_4^+.

Fig. 2. Relationship between external NH_4^+ concentration and its intracellular steady-state level in *S. quadricauda*, determined from the decrease of NH_4^+ concentration in the medium after 90 min of incubation and expressed in mmoles/l of total cell water.

are resuspended in an NH_4^+-free medium and thus can be assumed to be present in a free form inside the cells. The maximum ratio of distribution attained at low concentrations of NH_4^+ was about 20:1, the half-saturation constant of uptake was 1.25 mM.

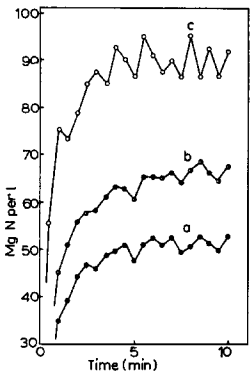


Fig. 3. Oscillatory efflux of NH_4^+ from *S. quadricauda*, determined in the medium after preincubation of cells with NH_4HCO_3 (300 mg N per ml) for 2 h and after transfer to an NH_4^+-free solution. a, at 5°, with 30 mg cells per ml; b, at 35°, with 30 mg cells per ml; c, at 35°, with 60 mg cells per ml.

The efflux of NH_4^+ was subjected to graphical analysis and the efflux curve decomposed into at least three components. The fastest component of the efflux was found to be associated with an activation energy of 5.1 kcal/mole, the value being rather low and suggesting only minimum interaction with membrane components during the transport process. A peculiar observation was made when studying the efflux curve, which was found to display periodic changes in the concentration of NH_4^+ present in the medium and, hence, in the cells. The oscillation amplitude was as much as 10 times greater than the standard deviation of the mean of NH_4^+ estimation and therefore considered to be significant. Moreover, the period of the oscillations was reproducible from one experiment to another, its mean value being 77 sec, with very little effect of temperatures between 5 and 35°. The amplitude, on the other hand, increased with temperature (Table I). It is noteworthy that application of 0.5 mM 2,4-dinitrophenol and of 5 μg/ml valinomycin had no effect on the oscillation frequency. Estimation of the pH of the external medium during NH_4^+ outflow showed it to decrease from any preset value (between 4 and 8) by a slow, nonoscillatory process. Hence there does not appear to be a correlation between the movement of NH_4^+ and H^+.

TABLE I

OSCILLATION PARAMETERS OF NH_4^+ EFFLUX AT DIFFERENT TEMPERATURES

Temp.	*Period (sec)*	*Amplitude (μg N per ml cells)*	*Number of experiments*
5°	76	8.2	10
15°	79	10 7	10
25°	77	16.5	10
35°	80	23.0	30

In a series of preliminary experiments a practically identical type of oscillation was found in the efflux of K^+ from *S. quadricauda*. In their insensibility to temperature and inhibitors, the oscillations resemble those in the uptake of sugars and amino acids by the yeast *Saccharomyces cerevisiae* (prepared for publication) and suggest a more widespread mechanism to be involved. While it is rather unlikely that the NH_4^+ is pumped back and forth actively, the hypothesis presented is that rhythmic changes of cell volume or at least of a part of it are somehow implicated in the phenomenon.

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