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SODIUM-DEPENDENT UPTAKE OF NITRATE AND UREA BY A MARINE DIATOM

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Summary

Uptake of nitrate and urea by *Phaeodactylum tricornutum* is shown to be a sodium dependent process inhibited by lithium or potassium. The half-saturation constant for sodium (K_{Na}) was 2.6 mM for nitrate uptake and 71 mM for urea uptake. It is suggested that sodium dependent uptake mechanisms may be characteristic of marine plants.

Sodium is the predominant cation in seawater, but the physiological basis for its requirement in the growth of marine phytoplankton is unclear [1]. Nitrate and urea uptake by the marine diatom *Phaeodactylum tricornutum* are active processes resulting in accumulation of these nitrogen sources against their concentration gradients [2, 3]. The major difference between the two systems is the effect of NH_4^+; NO_3^- uptake is inhibited in the presence of NH_4^+, whilst urea uptake is unaffected. We show here that active uptake of NO_3^- and urea by *Phaeodactylum* is Na^+-dependent. Uptake of NO_3^- or urea was followed by measuring the appearance of NO_3^- or [^{14}C]urea within the cells [2, 3]; in our view this is preferable to measuring disappearance from the medium because medium measurements do not distinguish clearly between uptake of nitrogen and its subsequent assimilation. However, as stated below, our observations were also confirmed by measurements made on the medium.

When cells of *Phaeodactylum* were resuspended in medium which contained mannitol, LiCl or KCl as substitutes for NaCl there was no uptake of either NO_3^- or urea. Addition of Na^+ to the medium containing mannitol resulted in uptake of NO_3^- and urea, but there was no uptake when Na^+ was added to medium containing either Li^+ or K^+ (Fig. 1). In the absence of Na^+ there was no disappearance of either NO_3^- or urea from the medium.

The results shown in Fig. 1 suggest that the presence of K^+ inhibited the uptake of NO_3^- and urea. To confirm this, cells of *Phaeodactylum* were incubated in medium containing a constant concentration of Na^+ and uptake of NO_3^- and urea was followed in the presence of differing concentrations of K^+ (Fig. 2). With increasing concentrations of K^+ there was, in general, a concomitant decrease in the rates of uptake of NO_3^- and urea. There was, however, a stimulation of the uptake of urea in the presence of 10 mM KCl.

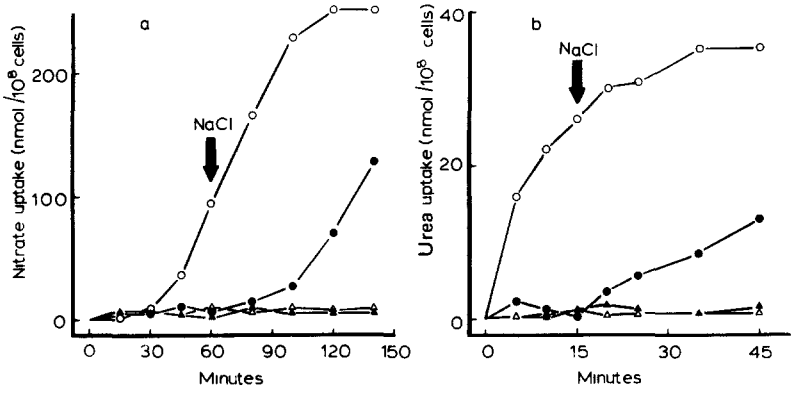

Fig. 1. Sodium dependence of uptake of (a) NO_3^- and (b) urea by *Phaeodactylum*. Cells were grown in an artificial sea-water medium (ASP-2 of Provasoli et al. [16]) with either NH_4^+ or urea as nitrogen source [2, 3]. After harvesting and washing [2, 3], NH_4^+-grown cells were pre-incubated for 3 h in N-free ASP-2 medium to allow development of the NO_3^- uptake system [3]; they were then washed and suspended in the experimental media and used for studies of NO_3^- uptake. Studies of urea uptake were made with urea-grown cells, washed and suspended in the experimental media. For the experiments cells were suspended in either (i) ASP-2 medium containing 0.2 NaCl (○—○); (ii) Na^+-free ASP-2 medium + 0.4 M mannitol (●—●); (iii) Na^+-free ASP-2 medium + 0.2 M LiCl (△—△); (iv) Na^+-free ASP-2 medium + 0.2 M KCl (▲—▲). Temperature was 20° C and the light intensity (from fluorescent tubes) 50 $W \cdot m^{-2}$. At zero time was added either (a) KNO_3 to give a concentration of 1 mM or (b) ^{14}C-urea to give 40 nCi and 10 μmoles/ml cell suspension [2, 3]. Uptake of NO_3^- or ^{14}C-urea was followed by collecting the cells from samples of cell suspensions on glass fibre filters and measuring appearance of NO_3^- or ^{14}C-urea in the cells [2, 3]. It has been shown [2] that 80% of the ^{14}C taken up by *Phaeodactylum* as [^{14}C] urea, remains in the cells in free [^{14}C] urea. NaCl was added to each sodium-free cell suspension (a) after 60 min to give a final concentration of 50 mM or (b) after 15 min to give a final concentration of 100 mM.

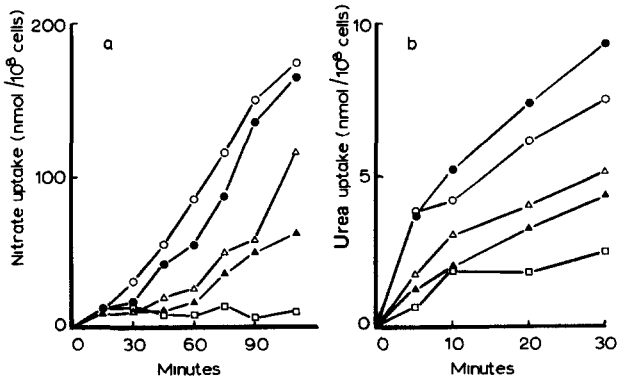

Fig. 2. Effect of KCl on NO_3^- and urea uptake by *Phaeodactylum*. Conditions were as described in Fig. 1. The cells were resuspended in ASP-2 medium containing a constant concentration of NaCl; 50 mM for (a); 100 mM for (b). Uptake was followed in the presence of KCl (○—○); 10 mM KCl (●—●); 30 mM KCl (△—△); 50 mM KCl (▲—▲); 100 mM KCl (■—■). Osmotic balance was maintained by the addition of mannitol.

The relationships between rates of NO_3^- and urea uptake and Na^+ concentration were hyperbolic and hyperbolae were fitted to the experimental points by computer [4]. Table I shows that the half-saturation constant or K_{Na} for NO_3^- (2.6 mM) was considerably lower than that for urea uptake (71 mM). This difference in the half-saturation constants and the differing response of uptake of NO_3^- and urea to the presence of 10 mM KCl, support the view that the uptake systems for NO_3^- and urea are separate and distinct [2, 3].

TABLE I

KINETIC CONSTANTS FOR SODIUM-DEPENDENT UPTAKE OF NITRATE AND UREA BY *PHAEODACTYLUM*

Cells of *Phaeodactylum* were resuspended in ASP-2 medium [16] containing a range of concentrations of Na^+. Osmotic balance was maintained by the addition of mannitol. For NO_3^- uptake the NaCl concentrations were 0, 2.5, 5.0, 7.5 and 10 mM, and for urea uptake 0, 25, 50, 100, and 200 and 300 mM. Uptake was followed as described in Fig. 1 and the initial rates of uptake calculated. Hyperbolae were fitted to the experimental points by the iterative procedure of Bliss and James [4] by computer. Standard errors are given in parentheses.

Uptake	Half-saturation constant for sodium (K_{Na}) (mM)	V (nmol/10^8 cells/h)
NO_3^-	2.58 (± 0.56)	140 (± 94)
Urea	70.8 (± 14.1)	359 (± 25)

Our results show a marked Na^+ dependence for the uptake of NO_3^- and urea by *Phaeodactylum*. It is noteworthy that a similar dependence of uptake upon Na^+ has been shown for some other microscopic marine plants, namely for the uptake of glucose and amino acids by the marine diatom *Cyclotella cryptica* [5], PO_4^{3-} uptake by the marine fungus *Thraustochytrium roseum* [6] and glutamate uptake by membrane vesicles of the halophilic bacterium *Halobacterium halobium* [7, 8]. Na^+-dependent uptake of organic solutes is now well studied in animals and with these systems Li^+ and K^+ do not substitute for Na^+ and, indeed, these cations inhibit the Na^+-dependent uptake of solutes as they do in our studies with *Phaeodactylum* [9, 10]. Sometimes a low concentration of K^+ stimulates uptake of an organic solute by animal cells e.g. uptake of glycine by ascite cells [11].

Schultz and Curran [12] have outlined two explanations to account for the Na^+-dependence of organic solute transport in animal cells. Either Na^+ is required as a cofactor for some reaction, possibly ATP hydrolysis, associated with the carrier for the substance transported or a Na^+ gradient exists across the membrane which drives the movement of the substance with associated Na^+ cotransport. In *Phaeodactylum* the ratio of external to internal Na^+ concentration can be of the order of 4500 so there is very considerable Na^+ gradient across the cell boundary [13]. If such a Na^+ cotransport system exists it may well be that K^+ is simultaneously transported out of the cells.

It is tempting to suggest, therefore, that active transport systems in marine plants may resemble those of animals in being based on Na^+ cotransport whereas those in freshwater plants and higher plants may be based on proton cotransport [14]. However, caution is necessary since a Na^+ cotransport system for the uptake of PO_4^{3-} has been demonstrated in a yeast which was not derived from a marine environment [15].

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