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The Uptake and Metabolism of Urea by *Chara australis*: IV. Symport with Sodium—A Slip Model for the High and Low Affinity Systems

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Abstract. We have previously investigated the electrogenic influx of urea in *Chara*, and the urea- and sodium-dependent membrane current. We have shown that there is a sodium-stimulated component of urea influx and a urea-stimulated component of sodium influx, and that these are of the same size. We conclude that the electrogenic inward transport of urea, and of its analogues acetamide and acrylamide, is by sodium symport, with a stoichiometric ratio of 1 : 1.

The kinetics of the fluxes and currents show two different K_M values for sodium in different cells and two different kinds of kinetics for the effect of urea on membrane current, one of which fits the Michaelis-Menten equation, while the other shows a maximum and fits the difference of two Michaelis-Menten terms, suggesting a phenomenon like *cis*-inhibition. Similarities in kinetic characteristics between the inhibitory site and the electrically silent uptake site (System II) lead us to suggest that the same protein may be responsible for both the low-K_M, electrogenic influx of urea (System I) and the high-K_M, electrically silent influx by System II. We suggest a "slip" model for urea uptake in *Chara*.

Key words: Sodium — Urea — Symport — Co-transport — Charophytes — Slip model

Introduction

Chara australis takes up urea by three pathways (Wilson & Walker, 1988*a*; Wilson, O'Donoghue & Walker, 1988) and rapidly metabolizes it, by means of a urease (Wilson & Walker, 1988*b*), to ammonia and carbon dioxide. The three uptake pathways described by these workers are: (i) passive diffusion, with linear kinetics; (ii) a high-affinity system (I), which is specific for urea and analogues, is active and electrogenic and saturates with a K_M of 0.35 μM and (iii) a lower affinity system (II), which is specific for urea, is not electrogenic and saturates with a K_M of about 7 μM.

System I has the same K_M for influx as for membrane current; transport by this mechanism is competitively inhibited by analogues such as acetamide and *N*-methyl urea, which share one amino group and the carbonyl group with urea. In the work quoted, the value found for V_{max} varied, but was of the order of 1 nmol m^{-2} sec^{-1}. System II has a similar V_{max} to that of system I and is not inhibited by analogues of urea. These values were obtained by trapping evolved CO_2, so that urea metabolism did not produce large errors.

Wilson et al. (1988) suggested that System I was a proton symport, an expectation based on the ruling paradigm for plant transport (*see* e.g., Sze, 1985). Following the finding that potassium is actively taken up into starved *Chara* cells by sodium symport (Smith & Walker, 1989), we investigated other electrogenic solute transport mechanisms in the *Charophyta*, including urea system I.

We found that external sodium is required for membrane current to be evoked by the sudden presentation of urea in *C. australis* (Walker, Smith & McCulloch, 1989) and a similar finding was made in *Nitella translucens* (Walker & Sanders, 1991). This has suggested strongly that the electrogenic mechanism is symport with sodium.

Sodium symport, in which inward transport is driven by the inward difference in the electrochemi-

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cal potential difference for sodium, is widespread in the animal kingdom. Cells in general have an inward driving force for sodium, and sodium symport is also found in prokaryotes. It occurs particularly in alkalophile organisms which cannot develop a large inward driving force for protons (Skulachev, 1985) but also, less explicably, in *E. coli*. The latter organism exhibits a mixture of proton and sodium symport for different substrates (Rosen, 1986).

The present paper reports further evidence for symport of urea with sodium in *Chara:* the cross-stimulated fluxes of urea and of sodium, and the kinetics of the electrogenic transport currents evoked by urea and its analogues in the presence of sodium.

Materials and Methods

C. australis R.Br. was grown in laboratory cultures in Adelaide and in Sydney. Internodal cells were cut from plants from selected cultures and kept in CPW (*see below*) for one to several days. Cultures varied in the size of the electric current evoked by the presentation of urea at micromolar concentrations; generally those with bigger responses were selected for electrical experiments.

Solutions were made from high-purity water freshly collected from a Milli-Q™ or an Abtech DMB-15 ion-exchange purifier. Solutions were used on the day they were made; fresh stock solutions of urea and analogues were made at least weekly. Solutions were held in borosilicate glass or polyethylene containers. The standard solution (CPW) contained 0.5 mM $CaCl_2$, 0.2 mM KCl, an appropriate concentration of Na_2SO_4, and, when buffered, 2 mM zwitterionic buffer (MES or MOPS) brought to a pH near its pK_a with $Ca(OH)_2$: variations are specified in the text. In some experiments with fast-flowing solution, it was not thought necessary to buffer the solution to prevent pH changes.

For measurement of membrane current, cells were voltage-clamped as described by Smith and Walker (1989) in a chamber in which 15 mm^2 of membrane was clamped and exposed to a fast flow of external solution. This chamber had a volume of about 0.35 ml, and solution flowed at 0.5 to 1 ml sec^{-1}. For measurement of kinetic parameters, concentrations were presented in various orders, for 10–20 sec, followed by a wash of 10–20 sec.

Measurement of sodium influx involved exposure of batches of cells to CPW labeled with ^{22}Na (or, in one experiment noted in the text, ^{24}Na) for periods of 5–20 min, followed by a rinse of 2–5 min in nonradioactive solution.

The measurement of urea influx is complicated by the rapid metabolism of urea to carbon dioxide, which escapes from the cell and from the solution if it is exposed to the atmosphere. The ^{14}C label is thus rapidly lost (Wilson & Walker, 1988*a*) and the influx can be underestimated by a factor of up to 5 unless the escaping CO_2 is trapped. After trials of the CO_2 trap method, we have preferred to estimate urea influx by measuring the loss of ^{14}C-urea from the external solution. This method yields the net influx, but we expect the efflux component to be small because the urea is so rapidly metabolized in the cytoplasm (Wilson & Walker, 1988*b*). In these experiments, 10 cells each of surface area about 10^{-4} m^2 were placed in 10 ml of solution; this gave an adequate depletion rate.

In general, kinetic curves have been fitted with the Briggs-Whittingham equation (Hill & Whittingham, 1955; Walker, Beilby & Smith, 1979). This equation is derived from a model in which a diffusion resistance lies between the bulk solution and the site of a process obeying Michaelis-Menten kinetics. Kinetic curves showing a maximum have been fitted to an equation ignoring unstirred layer effects. Wherever results were fitted to models, we used the least squares criterion (weighted where appropriate) and the Marquardt-Levenberg algorithm (Jandel Sigmaplot™).

Results

Flux Kinetics and Cross-Stimulation

Sodium Influx

Table 1*A* shows the results of a typical experiment in which the presence of low concentrations of urea stimulated the influx of sodium significantly, by 5–10 nmol m^{-2} sec^{-1}. The stimulations seen in Table 1 occur at both high and low sodium concentrations. The values at 30 and 400 μM indicate a K_M for sodium of about 30 μM for culture E and a much lower K_M for culture Sh. The effect of sodium concentration on the urea-stimulated component of sodium influx in an experiment on culture E, using 5 μM urea, is seen in Fig. 1, fitted to a Briggs-Whittingham equation. The parameters giving best fit to the data of Fig. 1 are: K_M, 4.2 ± 1.1 μM; V_{max}, 8.1 ± 0.2 nmol m^{-2} sec^{-1}; P_u, 0.8 ± 0.1 m sec^{-1}. (We use P_u for the permeability of the diffusion resistance, which consists of cell wall and unstirred layer of solution.) The K_M value in another experiment of this kind was 4.7 μM.

The stimulation by urea was independent of treatment time between 5 and 15 min (Table 1*B*), and independent of pretreatment time in urea from 0 to 120 min (Table 1*C*). In another experiment, the stimulation by urea was unaffected by overnight pretreatment in 5 μM urea (*data not shown*).

Urea and its analogue acetamide stimulated the influx of sodium at low concentrations (Fig. 2), and the shape of the urea graph in particular suggests the controlling influence of an unstirred layer of solution. The urea data fit the Briggs-Whittingham equation well, with a K_S of 0.06 ± 0.02 μM (we use K_S for the Michaelis constant found where the concentration being varied is not that of the solute whose transport velocity is being measured). The curve-fitting gives P_u as 8.0 ± 0.3 μm sec^{-1}, which is consistent with diffusion through a layer of water about 140 μm thick, a reasonable value in the ab-

Table 1. Effect of urea on sodium influx

A. Sodium influx measured over 5 min in CPW at pH 6.9, using ^{24}Na[a]

Treatment	Culture	
	E	Sh
30 μM Na^+ + 0 μM urea	3.5 ± 0.2	4.9 ± 0.3
30 μM Na^+ + 10 μM urea	9.0 ± 0.5	10.9 ± 0.6
Stimulation	5.5 ± 0.5	6.0 ± 0.7
400 μM Na^+ + 0 μM urea	13.2 ± 1.4	14.5 ± 1.8
400 μM Na^+ + 10 μM urea	23.2 ± 0.9	19.8 ± 1.2
Stimulation	10.0 ± 1.7	5.3 ± 2.2

B. Sodium influx from 400 μM Na^+ measured over various times in CPW at pH 6.8, in cells from culture Le[b]

Treatment	Influx period		
	5 min	10 min	15 min
0 μM urea	4.3 ± 0.4	4.4 ± 0.7	4.9 ± 1.0
10 μM urea	13.2 ± 2.8	13.5 ± 1.0	12.7 ± 1.0
Stimulation	8.9 ± 2.8	9.1 ± 1.2	7.8 ± 1.4

C. Sodium influx from 400 μM Na^+ measured over 15 min after various exposures to urea, in CPW at pH 6.9, in cells from culture Le[c]

Pretreatment and treatment	Pretreatment time		
	0 min	60 min	120 min
0 μM urea	3.9 ± 0.5	3.6 ± 0.3	4.2 ± 0.4
10 μM urea	11.0 ± 0.5	9.8 ± 0.4	11.5 ± 0.8
Stimulation	7.1 ± 0.7	6.2 ± 0.5	7.3 ± 0.9

[a] Urea present only during influx period. Values are mean and SEM of 8–10 determinations, in nmol m^{-2} sec^{-1}. Experiments of October, 1989.

[b] Urea present only during influx period. Values are mean and SEM of 8–10 determinations, in nmol m^{-2} sec^{-1}.

[c] Urea present during pretreatment and influx periods in cells given 10 μM urea treatment. Values are mean and SEM of 8–10 determinations, in nmol m^{-2} sec^{-1}.

sence of rapid stirring. Assuming the same unstirred layer permeability, the K_S for acetamide is found to be 0.86 ± 0.47 μM. The good fit of the Briggs-Whittingham equation to the stimulation of sodium influx by urea must imply that the urea is diffusing continually to the site of the stimulation, i.e., that it is being consumed or transported there. The stimulations of sodium influx produced by acetamide and urea are not additive (Table 2); this is to be expected if they stimulate by binding to the same site.

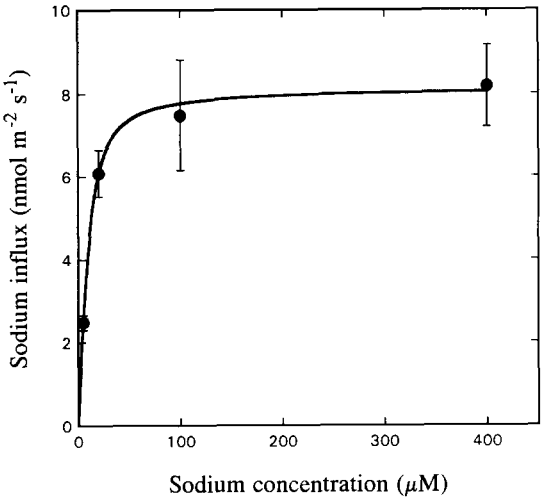

Fig. 1. The effect of concentration of sodium on the component of sodium influx stimulated by 5 μM urea. Cells from culture E, in CPW at pH 7.0. Points are mean and SEM of batches of 10 cells. Line is Briggs-Whittingham equation with parameter values (estimate ± SE): $K_M/\mu M$ = 4.2 ± 1.1; V_{max}/nmol m^{-2} sec^{-1} = 8.1 ± 0.2; $P_u/\mu m$ sec^{-1} = 0.8 ± 0.1.

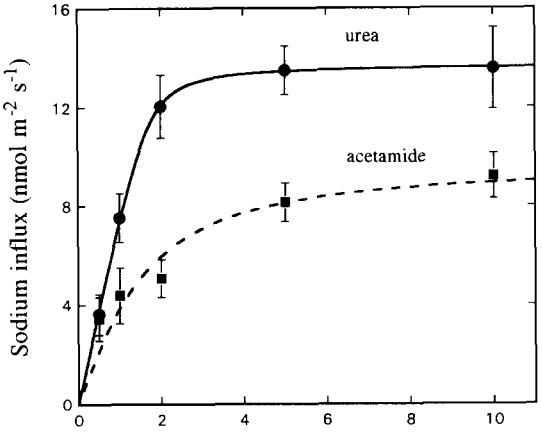

Fig. 2. The effect of concentration of urea (circles) and of acetamide (squares) on the component of sodium influx stimulated by urea at 100 μM sodium. Cells from culture J1, in CPW at pH 6.9. Points are mean and SEM of batches of 7–8 cells. Lines are Briggs-Whittingham equation with parameter values (estimate ± SE):

	Urea	Acetamide
$K_S/\mu M$	0.064 ± 0.024	0.86 ± 0.47
V_{max}/nmol m^{-2} sec^{-1}	13.7 ± 0.2	9.8 ± 1.3
$P_u/\mu m$ sec^{-1}	8.0 ± 0.3	[8.0]

The value in brackets was held constant during fitting—*see text.*

Urea Influx

Urea influx, from concentrations of 1–5 μM, measured by depletion of external concentration, was stimulated by 100 μM sodium, and also by 100 μM

Table 2. Effects of urea and urea analogues on sodium influx

Treatment	Influx
No substrate	4.73 ± 0.69
5 μM Urea	15.04 ± 1.15
5 μM Acetamide	13.44 ± 0.59
5 μM Urea + 5 μM acetamide	17.27 ± 0.86
10 μM Urea	13.15 ± 0.85
10 μM Acetamide	17.81 ± 1.05

Sodium influx from 200 μM Na^+ in CPW [1.0 mM $Ca(Cl)_2$] at pH 6.9, measured over 10 min in cells from culture J. Urea, acetamide or urea plus acetamide were present only during the influx period. Values are mean and SEM of 8–10 determinations, in nmol m^{-2} sec^{-1}.

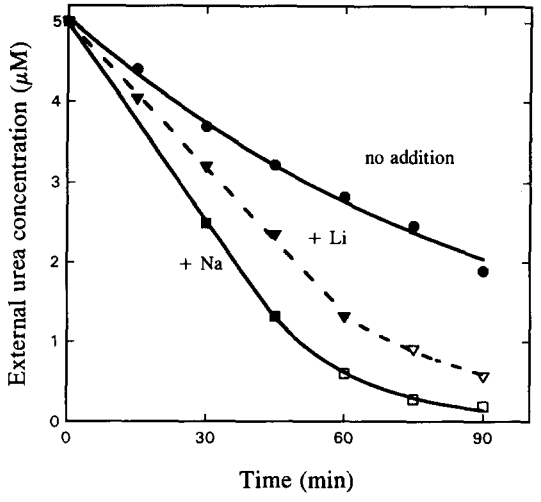

Fig. 3. The effects of 100 μM sodium (squares) and 100 μM lithium (triangles), compared with controls (circles), on the depletion of external urea by uptake into batches of 13 cells in CPW at pH 6.0. Points are single determinations of concentration. Lines are: for control, an exponential falling to zero; for added cations, linear regression on points down to 1.31 μM (filled symbols), and falling exponentials on points from 1.31 μM downwards (last filled symbol and open symbols).

lithium (Fig. 3). To smooth the data, we fitted models to the curves of concentration against time: a falling exponential when no cation was present, in the expectation of a linear dependence of flux on concentration, and a straight line followed by a falling exponential when cation was present, in the expectation of a Briggs-Whittingham kinetic. The initial slopes of the fitted lines yield flux increases of 3.3 and 1.2 nmol m^{-2} sec^{-1} at 5 μM urea due to sodium and lithium, respectively.

Stoichiometry

If the cross-stimulations seen in these experiments are measures of the same symport, the stoichiometry can be found from the values of V_{max} for influxes of

Table 3. Comparisons of saturated cross-stimulated fluxes

Date	Sodium influx	Urea influx
June 19, 1992	2.8 ± 0.5	2.7
June 25, 1992	3.1 ± 0.4	4.0
June 26, 1992	2.1 ± 0.4	2.0

Urea-stimulated component of sodium influx compared with sodium-stimulated component of urea influx, measured in cells of the same culture and on the same day. Concentrations used: 400 μM Na^+, 2 μM urea, pH 7.0. Values are mean (± SEM for Na^+) in nmol m^{-2} sec^{-1}.

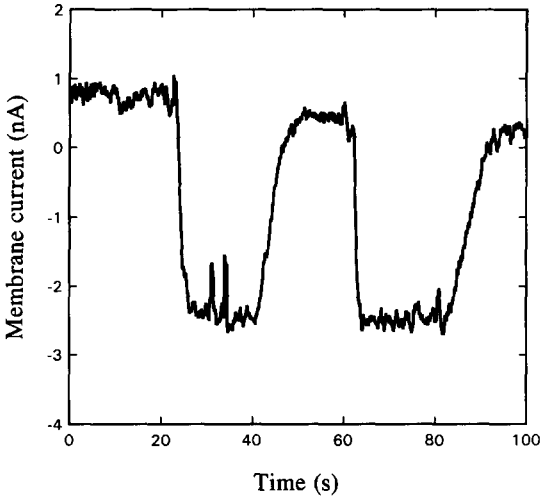

Fig. 4. The time course of membrane current in a cell exposed to 0.5 and then to 2.0 μM urea in the presence of 100 μM sodium in fast-flowing solution. Downward excursions of the trace are produced by 20 sec exposures to urea. Digitized at 8 Hz and filtered by running average of five data points.

sodium and of urea. The closest comparisons we can make are seen in Table 3, using the saturating concentrations of 2 μM for urea and 400 μM for sodium. A stoichiometric ratio of 1:1 is well supported by the data.

Current

Most cells showed a clear, rapid response to the presentation of external urea at micromolar concentrations in the presence of sodium; an example is seen in Fig. 4. Current responses at 3–5 μM urea were determined for a range of sodium concentrations to estimate the sodium K_M. In a survey experiment (October, 1989) the K_M was found for 12 cells from 5 different Adelaide cultures (La, M, Su, D and P) by measuring the response to 5 μM urea at 30 and 1,000 μM sodium. One cell gave a value of 44 μM, the 11 others gave a median of 3.3 μM (range

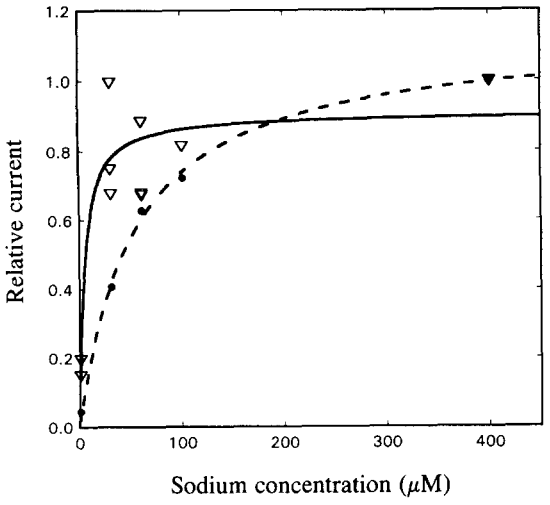

Fig. 5. The effect of sodium concentration on the change in membrane current evoked by 5 μM urea at pH about 6.0. In each experiment current is normalized to 1.00 at 400 μM sodium. One cell from culture E (filled circles and dashed line) and two cells from Sh (triangles and unbroken line). Points are means of 2–3 determinations. Lines are Michaelis-Menten equation with parameter values (estimate ± SE): Culture E: $K_M/\mu M$ = 53 ± 5; culture Sh: $K_M/\mu M$ = 5.2 ± 3.2

7–11 μM). Results from more concentrations of sodium for Adelaide cultures E and Sh (October, 1989) gave values of 53 ± 5 and 5.2 ± 3.2 μM, respectively (Fig. 5). As in the K_M values found from sodium fluxes, there are high and low values, around 5 and around 50 μM.

The dependence of membrane current on urea concentration in the range 0–20 μM has shown two types of behavior: (i) the current saturates, and is fitted by a Michaelis-Menten kinetic, but better by a Briggs-Whittingham (Fig. 6*A* and *B*); (ii) the current shows a maximum at about 2 μM and falls at higher concentrations (Fig. 7).

The first behavior was found by us with most Adelaide cultures except E (October, 1989): the latter behavior with Adelaide E (October, 1989) and Sydney cultures (1990). The first behavior was also found by Wilson et al. (1988) with Sydney cultures.

In the measurements reported here, the V_{max} for urea-evoked current was of the order of 10–40 nmol m^{-2} sec^{-1} (*cf.* Table 1), at least an order of magnitude greater than in most of Wilson's experiments.

Acrylamide and acetamide each evoked membrane current in *Chara* in the presence of added external sodium and a much smaller current in its absence. The values of K_M in the presence of sodium were: acrylamide, 0.15 μM (Fig. 8); acetamide, 1.5 μM (Fig. 9), each at pH 6.8. The values of V_{max} were equivalent to 6–12 nmol m^{-2} sec^{-1}, in the normal range for urea in these experiments. These experiments with Sydney cultures also show the second behavior with acrylamide and acetamide.

Discussion

Sodium Symport

The results of the present study further support the idea that system I (Wilson et al., 1988) catalyzes a symport of sodium with urea and its analogues such as acetamide and acrylamide:

(i) Both sodium and urea stimulate the flux of the other, and the magnitudes of the saturated flux stimulations show that the stoichiometric ratio sodium:urea is 1:1 (Table 3). We have not yet established the charge:matter ratio.

(ii) Sodium has K_M values of about 4 μM (Fig. 1) and about 30 μM (Table 1*A*) for its own urea-stimulated flux, and about 5 and 53 μM for urea-stimulated current (Fig. 5), values that agree reasonably well.

(iii) Urea has a low K_M (0.35 μM) for its own electrogenic influx (Wilson et al., 1988), a low K_S (0.06 ± 0.02 μM) for sodium influx (Fig. 2) and a low K_M (0.17 ± 0.10 μM) for current (Fig. 6 and *see below*), in good agreement given the difficulty of getting accurate values at these low concentrations.

Features of the Symport Kinetics

We have found two groups of values for the K_M for sodium in cells from different cultures: (i) low (4–5 μM), found in both electrical and flux studies, and (ii) high, (30–50 μM), found in electrical studies and suggested also by the sodium dependence of urea-stimulated sodium flux.

The kinetic data for current against urea concentration for many Adelaide cultures fit a simple Michaelis-Menten equation (Fig. 6), as did the data of Wilson et al. (1988). However, data from some Adelaide cultures (E in October, 1989) and from Sydney cultures (1990) show a current maximum at about 2 μM followed by a fall as urea concentration rises further (Fig. 7). These data suggest a model involving random binding of driver ion and substrate—either a slip model (Komor & Tanner, 1974, 1975) or a *cis*-inhibition model (Sanders, 1986). In a slip model, if the substrate molecule binds first it undergoes electrically silent transport, while if the driver ion binds first, the substrate binds second and there is electrogenic transport. *Cis*-inhibition by substrate occurs, e.g., if the transport protein exhibits a higher V_{max} when the driver ion binds first

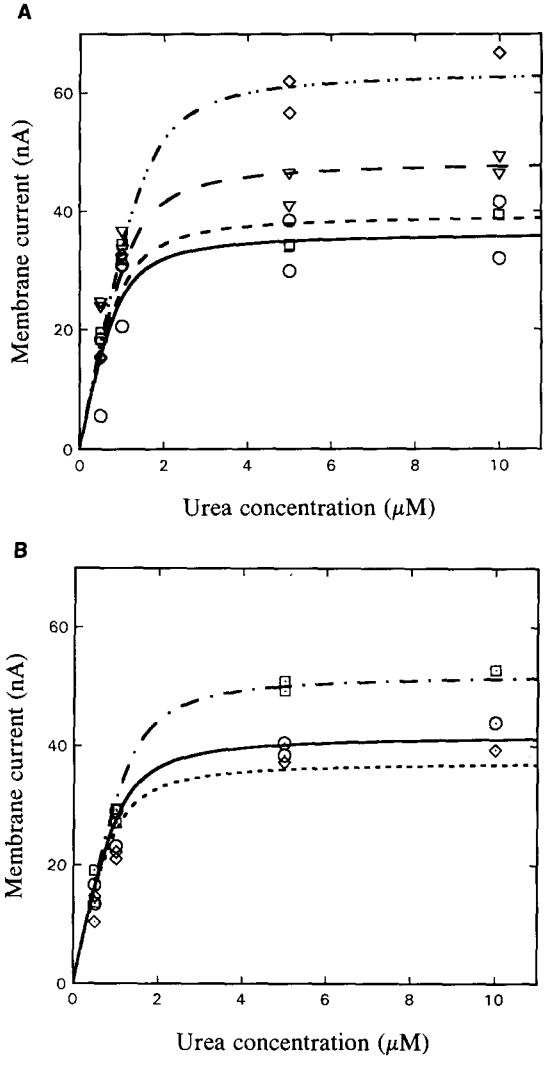

Fig. 6. (*A*, *B*) The effect of urea concentration on the current through $15 \cdot 10^{-6}$ m^2 of membrane, in the presence of 400 μM sodium at pH about 6.0. Concentrations presented in up-down order. Each symbol shows data of one cell from culture Ph. Each point represents a single determination. Lines are Briggs-Whittingham equation fitted to the data with a single K_M and P_u for the whole set of data, but with an individual V_{max} for each cell. This fit gave parameter values (estimate ± SE): $K_M/\mu M$ = 0.17 ± 0.10; V_{max}/nA = 36 ± 2 to 64 ± 3; $P_u/\mu m\ sec^{-1}$ = 27 ± 5. (*A*) Cell 1, circles and unbroken line; cell 2, triangles and long dashes; cell 3, squares and medium dashes; cell 4, diamonds and dot, dot, dash. (*B*) Cell 5, dotted diamonds and unbroken line; cell 6, dotted circles and unbroken line; cell 7, dotted squares and dot, dash.

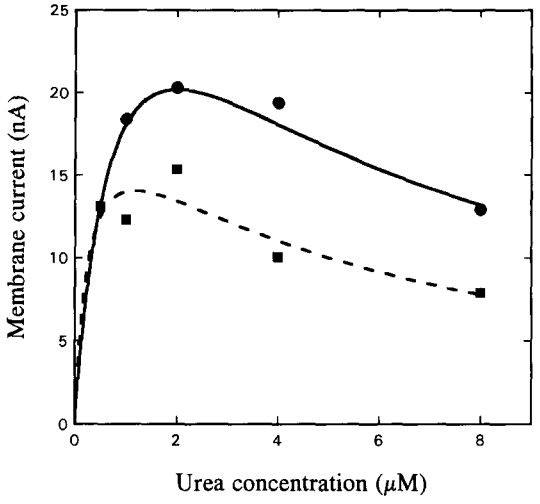

Fig. 7. The effect of urea concentration on the current through 15.10^{-6} m^2 of membrane, in the presence of 1.0 mM sodium at pH 7.2. Data of one cell of STh (circles) and one cell of SLi (squares). Points are single determinations. Lines are Eq. (1) with parameter values given in Table 4.

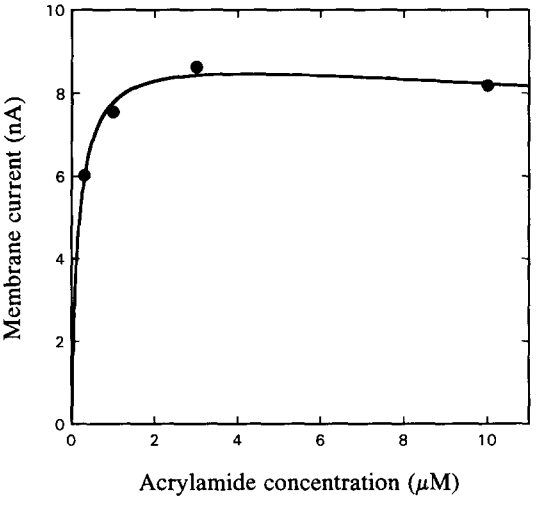

Fig. 8. The effect of acrylamide concentration on the current through $15 \cdot 10^{-6}$ m^2 of membrane, in the presence of 1.0 mM sodium at pH 7.2. Data of one cell from culture SLe. Points are single determinations. Line is Eq. (1) with parameter values given in Table 4.

than it does when the substrate binds first, each sequence producing electrogenic transport. We have, in view of our limited data, adopted a simplified model, by assuming that there is no current if the substrate binds first, which could be true of either model. This leads to a relationship between current (i) and substrate concentration (c) of the form:

$$i = i_{max}\, c\{(K_M + c)^{-1} - (K_I + c)^{-1}\} \ldots \quad (1)$$

where K_M corresponds to the binding of the substrate to the protein to which the driver ion is already bound and K_I corresponds to its binding to the bare protein. Table 4 shows the parameter values for the best fits of this three-parameter equation to the available data. We conclude that the data, for some cells at least, can be well represented by Eq. (1).

We suggest, therefore, that electrogenic sym-

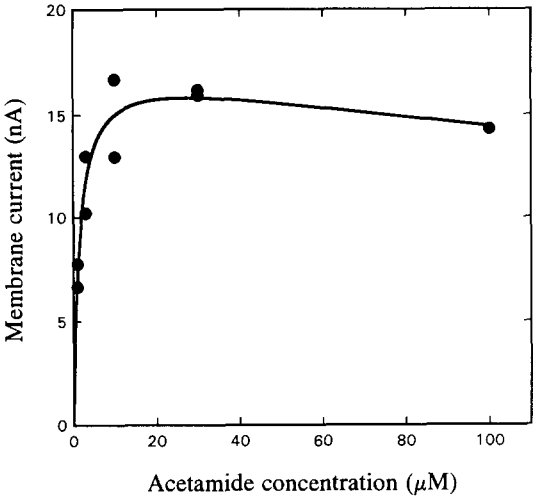

Fig. 9. The effect of acetamide concentration on the current through $15 \cdot 10^{-6}$ m^2 of membrane, in the presence of 1.0 mM sodium at pH 7.2. Data of one cell from culture SLe. Points are single determinations. Line is Eq. (1) with parameter values given in Table 4.

Table 4. Parameters for best fit of Eq. (1) to data for membrane current evoked by urea and analogues

Figure	Substrate	K_M/μM	K_I/μM	V_{max}/mA · m^{-2}
7	Urea	0.3	4.7	1.55
7	Urea	1.2	3.2	5.65
8	Acrylamide	0.16	110	0.61
9	Acetamide	1.5	500	1.17

K_M represents binding of substrate to protein after binding of Na^+, K_I binding of substrate to protein before binding of Na^+. Buffered media, pH 6.8. No allowance for unstirred layers was made, so that values of K_M may be too high.

port requires sodium to bind first, and that this binding reduces the K_M for urea from a value of about 5 μM (the K_I above) to a value of about 0.2 μM. The currents for the urea analogues (Figs. 8 and 9) fit the same model, though they fit a simple Michaelis-Menten equation almost equally well because their K_I/K_M is much higher than the K_I/K_M for urea—we interpret this as a higher discrimination against analogues by the protein without sodium.

Differences in Kinetics

Two different associations of kinetic properties have appeared in different cultures and at different times. Some cells show Michaelis-Menten kinetics for current against urea concentration and a low K_M for sodium, while others show Eq. (1) kinetics for current against urea concentration and a high K_M for sodium. These kinetic differences may result just from the difference in K_M for sodium: if the decrease in current at high urea concentrations is seen only when there is a sufficient concentration of free protein for urea statistically to bind first, it will be difficult to observe when the K_M for sodium is as low as 4 μM. We have, however, no physiological explanation for the appearance of two different values of sodium K_M; it does not appear to have a genetic cause, since culture E has shown different behavior at different times.

Electrically Silent Transport—Wilson's System II

We are now in a position to consider urea uptake by System II (Wilson et al., 1988), the specific, saturating, electrically silent process which is not inhibited by analogues such as methyl-urea. It was reported to have a K_M of 7 μM and a V_{max} similar to that of System I, in those experiments about 1 nmol m^{-2} sec^{-1}. These properties are notably similar to those of the urea-binding site on the System I protein when sodium is not bound: it has a K_M for urea (measured by K_I in Table 4) of about 5 μM and discriminates strongly against analogues such as acetamide and acrylamide, whose K_M values (measured by K_I), are 100–500 μM. We conjecture, therefore, that System II represents urea uptake by the same protein as System I, loaded with urea only. This implies adopting the slip model of transport, as used by Komor and Tanner (1974, 1975) for the proton-hexose porter of *Chlorella*. They found that at high pH the porter acquired a high K_M for hexose and became passive. Their interpretation of the high K_M transport as passive was later questioned by Sanders (1986), who argued that Komor and Tanner may have been wrong in concluding that sugar uptake by the high K_M mechanism was not accompanied by a driver ion. In the present case, it has been established by electrical methods (Wilson et al., 1988) that the high-K_M transport of urea (System II) is not electrogenic. Our suggestion involves some reinterpretation of the results of Wilson et al. (1988) but is not in conflict with their data. A possible kinetic scheme is shown in Fig. 10.

If our conjecture is correct, the current evoked by urea will show a maximum as urea concentration rises, but the influx of urea may well not. To predict the urea influx, Eq. (1) needs to be modified to take account of urea transport across the membrane by the cycle 2-1-7-6-2 (Fig. 10). This may help to explain why a maximum has not been seen in our urea influx experiments but has been obvious in at least some of our electrical studies.

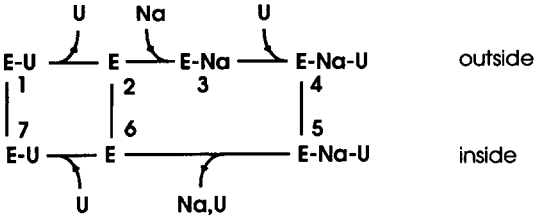

Fig. 10. Enzyme-kinetic model for urea transport in *Chara*. *E*, enzyme; *Na*, sodium; *U*, urea. States of the model are numbered *1* to *8*, and lines show possible transitions between states. Transitions 2-3-4-5-6-2 represent electrogenic transport of urea and sodium (System I); transitions 2-1-7-6-2 represent electrically silent transport of urea (System II).

Slip models are not favored by many writers because they provide a mechanism for a futile cycle (e.g., 2-3-4-5-6-7-1-2) in which driver ion energy is dissipated without substrate transport. In the present case, metabolism is thought to keep the internal substrate concentration relatively low (Wilson et al., 1988), and this may help to minimize energy loss.

Unstirred Layers and Kinetic Parameters

We have fitted our flux data to an equation that explicitly provides for the diffusion resistance of the cell wall and unstirred layer of solution, and found that for the nonelectrolyte urea the diffusion permeability was 8 μm sec^{-1}, while for sodium it was 0.8 μm sec^{-1}. The former value represents about 140 μm of water, but the latter value is unlikely to represent an unstirred layer 1.4 mm thick, and it is thought instead to result from the Donnan properties of the cell wall (Briggs, Hope & Robertson, 1961). Such diffusion resistances effectively mask the effects of the K_M on the curve, rendering our estimates of K_M from fluxes very approximate.

In the electrical experiments, rapid flow into the small chamber resulted in thinner unstirred layers, and it was possible to fit the urea data of Fig. 6 with an unstirred layer permeability of 27 ± 5 μm sec^{-1}. The same fit yielded a K_M of 0.2 ± 0.1 μM, which is less than the value for K_M, 0.35 μM, obtained by Wilson et al. (1988)—but their value was not corrected for unstirred layer effects. The value of P_u is reasonable for cells with fast flow (*cf.* 50 μm sec^{-1} of Walker et al., 1979). We have some confidence, therefore, in these parameter values.

Function of the Symport

It is of interest to ask whether urea symport can supply reduced nitrogen at a rate sufficient for cell growth. While *Chara* can reach 500 nmol m^{-2} sec^{-1} when using bicarbonate in the laboratory with light and substrate saturation (Lucas, 1975), it will frequently photosynthesize at a rate lower than this in nature. A photosynthetic rate of 200 nmol m^{-2} sec^{-1} over a 12-hr day would require uptake of reduced N at a rate of 5 nmol m^{-2} sec^{-1} over a 24-hr day, if the cell's N/C ratio is 0.05 (Walker et al., 1979) and so a urea uptake rate of only 2.5 nmol m^{-2} sec^{-1}, as there are two moles of reduced N per mole of urea. Most cells we have studied can easily match such a rate, which suggests that the symport is a potentially useful source of reduced N.

We have no explanation for differences in V_{max} values between different cultures and at different times. Our observations suggest no consistent differences between young and old material. The observed differences might reflect differences in the nitrogen status of the plants, but N-starvation of excised cells up to two weeks has not been found to increase V_{max} (*unpublished results*).

Conclusions—Two Chemiosmotic Circuits

We have confirmed a second sodium-driven symport in *Chara*, the first being that of potassium (Smith & Walker, 1989). The known proton-driven symport in *Chara* is that of chloride (Sanders, 1980), and protons also provide the driving force for carbon acquisition in bicarbonate solutions.

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