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Electrogenic Cl⁻ pump in *Acetabularia*

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Measurements of the transmembrane potential difference (V) under various conditions have demonstrated the operation of an electrogenic Cl⁻ pump in the outer plasma membrane (plasmalemma) of the unicellular marine alga *Acetabularia*. In preparations of partly purified membranes (containing plasmalemma), there is Cl⁻-stimulated, N,N'-dicyclohexylcarbodiimide-insensitive, vanadate-sensitive ATPase activity with a pH optimum around pH 6.5. These properties are consistent with the assumption that the electrogenic Cl⁻ pump is an ATPase. In order to investigate electrical details of this 'Mitchellian' type of charge-translocating enzyme, steady-state current–voltage curves of the electrogenic pump ($I_p(V)$) were measured *in vivo* under dark and light conditions and analysed by a two-state reaction kinetic model. This model with the resulting parameters predicts V-sensitive, unidirectional Cl⁻ effluxes through the pump. The predictions of this model agree with the experimental results. Green light causes a fast decrease of V, which is explained as a disturbance of the pump cycle. Relaxation studies on this effect and reaction kinetic analysis of $I_p(V)$ under different external Cl⁻ concentrations are used to develop a consistent three-state model of the pump that includes the order of and absolute rate constants of individual reactions, states of charge, stoichiometry, voltage-sensitivity and density of the pump molecules in the membrane.

Introduction

Electrical aspects are crucial in Mitchell's chemiosmotic theory. Nevertheless, rather little is known about the electrical properties of charge-transporting enzymes in membranes that can catalyse the conversion of metabolic energy to electrical energy and vice versa. The giant unicellular marine alga *Acetabularia* displays the operation of such a 'Mitchellian' type of enzyme (an electrogenic Cl⁻ pump) in its outer plasma membrane (plasmalemma). Since *Acetabularia* is a convenient object for quantitative, electrophysiological studies, it offers the opportunity for more detailed investigations of the electrical properties of such an enzyme.

The presence of an electrogenic Cl⁻ pump in *Acetabularia* has been demonstrated by various electrophysiological studies (see, for example, Saddler 1970; Gradmann 1970, 1975). The principal evidence came from the following observations.

1. The transmembrane potential difference (V) under normal resting conditions is far too large ($V_r = -170$ mV, inside negative) to be explained by conventional diffusion theory, which would yield a maximum of about -90 mV, the Nernst equilibrium potential for K⁺ (E_{K^+}) at physiological conditions: $[K^+]_i = 400$ mM, $[K^+]_o = 10$ mM.

2. Upon inhibition of the energy metabolism by either drugs or cold, V_r falls to V_d of about -80 mV, which can now be described by simple diffusion (Goldman equation).

3. V_r is rather insensitive to the external concentration of the major cations (H⁺, K⁺, Na⁺) but very sensitive to $[Cl^-]_o$. Replacement of Cl_o^- by other anions causes V to approach V_d and to become much more sensitive to $[K^+]_o$ and less sensitive to metabolic inhibition.

4. When V_d is obtained during metabolic inhibition, it is not sensitive to changes in $[Cl^-]_o$.

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RESULTS AND DISCUSSION

Identification of a Cl^- *ATPase*

The results presented in this section are from Goldfarb & Gradmann (1983).

Since, as a first guess, ATP is expected to drive the electrogenic Cl^- pump in *Acetabularia*, experiments have been designed to identify a Cl^- transport ATPase in preparations of partly purified membranes (Perlin & Spanswick 1980) from homogenates obtained by disruption with a nitrogen cavitation method (Wallach *et al.* 1960). For this purpose buffers were chosen to contain no physiological ions except Cl^- and (unavoidably) H^+ (OH^-): 25–50 mM Tris–SO_4, 0.55 M choline chloride (sorbitol for disruption and controls), 2 mM dithiothreitol (DTT), 0.5 mM $MgSO_4$, 10 µM phenylmethylsulphonylfluoride (PMSF) and 0.68 TIU aprotinin per 10 ml buffer, the last three components being used for disruption. [125I]Insulin was used as marker for plasmalemma (Legros *et al.* 1975).
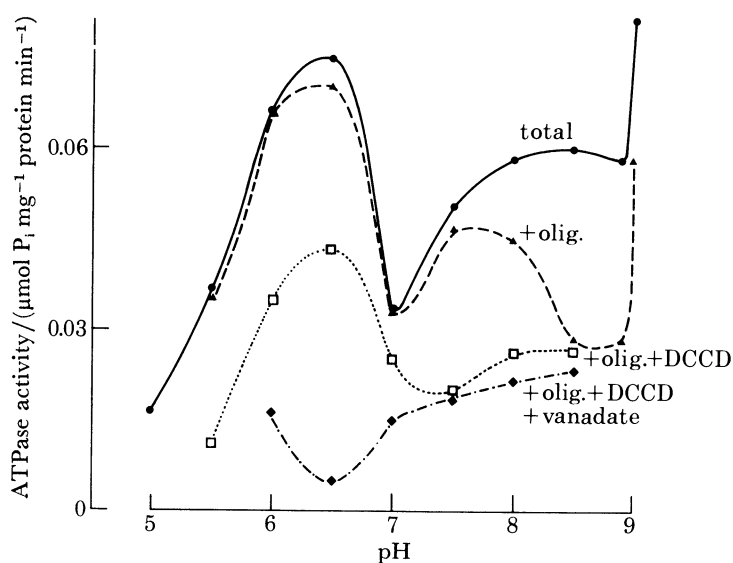

FIGURE 1. Effect of different inhibitors on Cl^--stimulated ATPase activity in partly purified (sucrose step gradients) membranes at different pHs. 10 min incubation in: 25 mM Tris-SO_4, 0.55 M choline-chloride, 6 mM Mg-ATP, 50 µM Na_3VO_4 or Na_2SO_4, inhibitors or 1 % methanol in blank; inhibitors, 200 µg oligomycin per milligram protein, 100 µM DCCD, 50 µM vanadate.

The top curve in figure 1 shows Cl^--stimulated ATPase activity of our preparations at various pHs and in the absence of inhibitors. Since Cl^- can stimulate H^+-translocating ATPases as a passively permeant co-ion (Perlin & Slayman 1982), it is necessary to discriminate which activities are due to primary active H^+ pumping and which are due to primary active Cl^- transport. This has been done with inhibitors (see figure 1).

Oligomycin, which specifically inhibits the mitochondrial H^+ ATPase with an alkaline pH optimum, decreases the activity at pH 8.5 in our preparations, to yield a maximum activity at a pH of 7.5, which is typical for vacuolar H^+ ATPases (Bowman 1981; Kakinuma *et al.* 1981). The further addition of N,N'-dicyclohexylcarbodiimide (DCCD), a potent inhibitor of virtually all known H^+ ATPases, causes this maximum to disappear, leaving a maximum ATPase activity at a pH of around 6.5, which is characteristic for ion transport ATPases in the outer plasma membrane of eukaryotic cells (see Glynn & Karlish (1975) for Na^+/K^+

ATPase, Bowman *et al.* (1980) for H⁺ ATPase in fungi, and Cambraia & Hodges (1980) for H⁺ ATPases in plants). In addition, these ATPases are sensitive to vanadate, both in our preparations and others so far investigated.

So we are left with a Cl⁻-stimulated ATPase with a pH optimum of around 6.5, which is vanadate-sensitive, DCCD-insensitive and does not require K⁺ or Na⁺ (or both) or Ca²⁺. These properties are completely consistent with the initial assumption, that the electrogenic Cl⁻ pump in the plasmalemma of *Acetabularia* is an ATPase.

For our immediate purpose, this result is sufficient. To compare our ATPase with others, further characterization is necessary. Briefly, it shares HCO_3^- stimulation (about sevenfold compared with Cl⁻ as substrate at a concentration of 67 mM) with animal anion ATPases (for review see DePont & Bonting 1981); however, this stimulation does not occur in the electrical properties (see below). Furthermore, there is about 40% GTPase activity compared with ATPase and no detectable phosphatase activity with ADP, CTP and UTP (at pH 6.5 in the presence of oligomycin and DCCD, vanadate-sensitive).

Current–voltage relations of active ion transport

For a discussion of electrical data in the next paragraphs, some theoretical considerations need to be introduced (for details see Hansen *et al.* 1981). The stationary current–voltage relation provides the complete electrical description of any system in its steady state. The current–voltage relation of an electrogenic pump ($I_p(V)$) is equivalent to the reaction kinetics of an active, charge-transporting enzyme. The driving force V can be compared with a substrate concentration and I_p with the velocity of the enzymic reaction, in moles of charges transferred per second. This needs to be discussed in more detail.

A cyclic reaction scheme of the electronic Cl⁻ pump is depicted in figure 1*a* (for reasoning and details see Mummert *et al.* 1981). This five-state model may be viewed as kind of a minimal model, having the smallest number of distinct reaction steps likely to be consistent with the enzymic properties of active transport systems. The pump 'X' with a total density of N mol m⁻² in a membrane exists in five distinct states (numbered 1 to 5) connected by five reversible reaction steps with the rate constants k_{ij} s⁻¹ (i, from; j, to). Approximate concentrations of the chemical ligands (Cl⁻, ATP and ADP) are given for normal conditions in *Acetabularia*. These concentrations enter the reaction system as products with their associated rate constants (for example $k_{32} = k_{32}' [Cl^-]_0^2$, with $k_{32}' = k_{32}$ at 1 M Cl_0^-, the exponent 2 stays for the (probably subsequent) binding of 2 Cl⁻). The voltage dependence of the charge transfer (between states 1 and 2) is obtained by considering the membrane to be a single energy barrier to the passage of charge (Läuger & Stark 1970). If this barrier is symmetric in the membrane (see below),

$$k_{12} = k_{12}^0 \exp\left(\tfrac{1}{2}zu\right) \quad \text{and} \quad k_{21} = k_{21}^0 \exp\left(-\tfrac{1}{2}zu\right), \tag{1}$$

where the superscript zero designates the transition rate at zero voltage, z is the charge number and $u = VF/RT$ is the normalized voltage. Current flow through such a transport system with one charge-carrying pathway (class I system) is just the difference between outward and inward flux of charge:

$$I = zF(N_1 k_{12} - N_2 k_{21}). \tag{2}$$

If all rate constants and N were known, $I_p(V)$ could be written explicitly. However, we have the inverse problem, i.e. to determine reaction kinetic parameters from measured $I_p(V)$'s. For this purpose we can formally reduce the explicit five-state model (figure 2*a*) to a two-state

model (figure 2 b), where the V-sensitive reaction step is picked out distinctly and the remainder, the V-insensitive part of the system, is summarized in one pair of V-insensitive gross rate constants $\mathcal{K}_{io}$ and $\mathcal{K}_{oi}$. The new subscripts (i for inside and o for outside) of the reduced model (b) denote that the indicated parameters (N_i, N_o, k_{io}, k_{oi}) are different from N_1, N_2, k_{12} and k_{21} in the explicit model (a). The conversion is accounted for by introducing V-insensitive 'reserve' factors r:

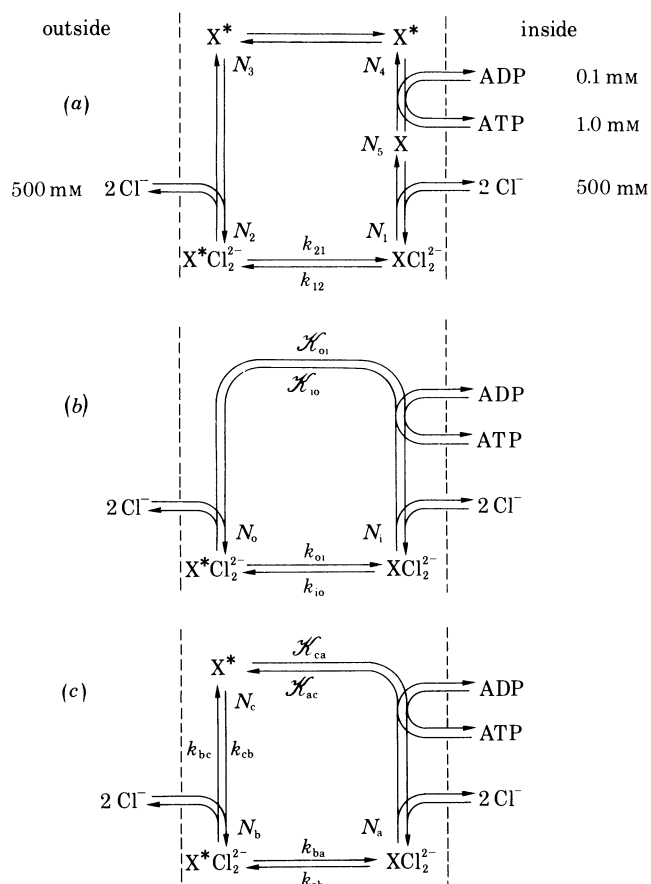$$k_{io} = k_{12}/r_i, \quad k_{oi} = k_{21}/r_o. \tag{3}$$

FIGURE 2. Reaction kinetic schemes for the electrogenic Cl^- pump in *Acetabularia*. (a) 'Real' model with a minimum of five distinct states and approximate substrate concentrations on the two sides of the membrane. (b) Two-state model, summarizing all V-insensitive reactions of (a) into $\mathcal{K}_{io}$ and $\mathcal{K}_{oi}$. (c) Three-state model (reduction of (a)), appropriate for description of $I_p(V)$ at different $[Cl^-]_o$.

The current–voltage relation of the two-state model is then

$$I = zFN(k_{io}\mathcal{K}_{oi} - k_{oi}\mathcal{K}_{io})/(k_{io} + k_{oi} + \mathcal{K}_{io} + \mathcal{K}_{oi}), \tag{4}$$

where V-sensitivity enters (4) via k_{io} and k_{oi} by (3) and (1). Equation (4) now describes all stationary current–voltage curves of any system with one charge-carrying pathway, including active ion transport systems.

Measurement of $I_p(V)$

To eliminate cable problems, we prefer to use spherical segments, tied off with two ligatures from the cylindrical stalk of the cells. With two inserted microelectrodes, one for measurement

of V, one for current application, it is possible to measure the current–voltage relation of the membrane under normal conditions ($I_m(V)$) and under conditions when the pump is inhibited ($I'_m(V)$). The difference (($I_m - I'_m)(V)$) is now $I_p(V)$. The exact procedure is described elsewhere (Gradmann *et al.* 1982).

Two examples of $I_p(V)$ for dark and (white) light conditions as obtained from the same cell, are given in figure 3. Dots represent measured values and lines are the result of fitting these data with (4). The numerical results for $N = 10^{-7}$ mol m^{-2} (see below) are given in the inset of figure 3. Several conclusions can be made from these curves.

1. If the ΔG of ATP hydrolysis is about 0.4–0.5 eV (1 eV $\cong$ 96 kJ mol^{-1}), the equilibrium potential of the pump (V at zero current) (E_p) of -190 mV means a stoichiometry of 2 Cl$^-$ per ATP (compare the explicit reaction scheme in figure 2a).
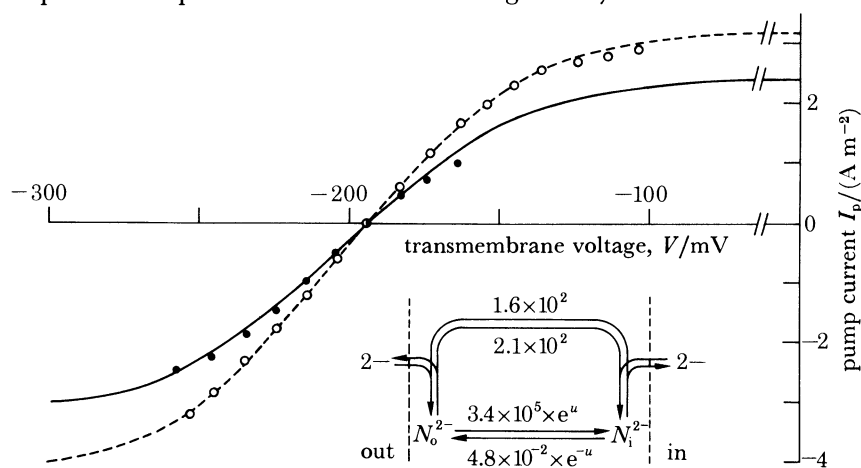

FIGURE 3. Example of $I_p(V)$ under dark ($\bullet$) and (white) light ($\circ$) conditions from one cell. Lines fitted by use of equation (4). Resulting parameters are given in the inset (compare figure 2b). All parameters are the same for both curves, except for N: light, 10^{-7} mol m^{-2}; dark, 0.76×10^{-7} mol m^{-2}. (Data recomputed from Gradmann (1975).)

2. The apparent symmetry around E_p means that (a) the assumption of a symmetric energy barrier is justified; (b) the thermodynamic equilibrium of the V-insensitive part of the reaction cycle ($\mathcal{K}_{io}$ and $\mathcal{K}_{oi}$ determine the saturation currents, as they become rate-limiting for large V) is close to zero potential. The next conclusion follows from this.

3. The energizing reaction must be localized in the V-sensitive reactions k_{io} and k_{oi}, which differ by about 7 orders of magnitude (7×58 mV $\cong$ 0.4 eV $\approx \Delta G$(ATP)) at short-circuit voltage zero. In terms of an explicit reaction scheme, charge transfer may be viewed as an individual reaction step, distinct from energization, which is primarily expected to be V-independent. In this case, there is only one possibility to account for the apparent V-sensitivity of the energizing step: it must be a very fast reaction, depleting that state immediately adjacent to the charge translocating step. This fast, energizing reaction could be a dephosphorylation of an intermediate state $X^*Cl_2^{2-}$ (inside), where the asterisk used in figure 2 would represent phosphorylated states.

4. The maximum slope of the curve is too large to be described with $z = -1$ (this cannot immediately be seen from the data); with $z = -2$ the fits improve. Also this finding clearly points to the mentioned 2:1 stoichiometry.

The good description of the difference between $I_p(V)$ in dark and light could only be obtained by introducing a change in N and not by a change of one of the kinetic parameters. This result means that (photosynthetically active) light only increases the number of active pump elements in the membrane and does not affect the kinetics of the individual (active) pump element. This interpretation is supported by the finding that none of the substrate concentrations, especially ATP, vary significantly in their steady-state value between continuous light and dark conditions (Gradmann 1975). There is a different situation in *Neurospora*, when CN^- causes a decrease in internal [ATP] (Slayman *et al.* 1970). In this case the change in $I_p(V)$ is described best by a decrease of that rate constant of the two-state model that implies the product with [ATP] (Gradmann *et al.* 1982).

Prediction of V-sensitive Cl^- *flux*

Mummert *et al.* (1981) have demonstrated the following identity: the two-state model predicts that unidirectional Cl^- efflux through the pump is given by

$$\phi(V) = nN\,k_{io}(k_{oi} + \mathcal{K}_{oi})/(k_{io} + k_{oi} + \mathcal{K}_{io} + \mathcal{K}_{oi}), \tag{5}$$

with n being a stoichiometry factor ($n = 2$ in our case). V enters (5) via k_{io} and k_{oi} again. Equation (5) is only correct for the reasonable assumption that Cl^- binding and dissociation are relatively fast reactions (see also below). If the kinetic parameters provided by the analysis of $I_p(V)$ (see figure 3 and inset) are inserted into (5), the absolute magnitude and V-sensitivity of unidirectional Cl^- efflux through the pump can be calculated explicitly.

Unidirectional Cl^- efflux including sensitivity to V can be measured in *Acetabularia* by tracer efflux experiments during the slow (minutes) changes in V in the course of action potentials, which are recorded simultaneously with the release of $^{36}Cl^-$. From such experiments one can plot unidirectional Cl^- efflux against V. If from this total Cl^- efflux, that portion is subtracted that is observed under conditions when the pump is inhibited, the remaining portion can be expected to the pump-specific. These final results can now be compared with the predicted results (figure 4). There is a good identity, which proves that the theory and experiments are valid.

Sensitivity of $I_p(V)$ *to* $[Cl^-]_o$

The results presented in this section are from Tittor (1982); some data are published in Hansen *et al.* (1982).

We can now look for more complicated cases in which substrate concentration $[Cl^-]_o$ as well as V are changed during an experiment. If $[Cl^-]_o$ is varied, which enters the explicit reaction scheme (figure 2a) by k_{32}, immediately adjacent to the charge transit, then a three-state model with six kinetic parameters (figure 2c) is appropriate (Hansen *et al.* 1981). This reduced model requires further different subscripts (a, b and c) to adjust the formalism to the elimination of states 4 and 5. Introducing the reserve factors r_a and r_c, it can be shown (U.-P. Hansen, personal communication) that

$$I = zFN(k_{ab}\,k_{bc}\,\mathcal{K}_{ca} - k_{ba}\,k_{cb}\,\mathcal{K}_{ac})/D \tag{6}$$

and

with

$$D = k_{ab}\,(k_{bc} + k_{cb} + \mathcal{K}_{ca}) + \mathcal{K}_{ac}\,(k_{ba} + k_{bc} + k_{cb}) + k_{ba}\,(\mathcal{K}_{ca} + k_{cb}) + \mathcal{K}_{ca}\,k_{bc},$$

with

$$k_{ab} = k_{12}/r_a, \quad k_{ba} = k_{21}, \quad k_{bc} = k_{23}, \quad k_{cb} = k_{32}/r_c, \quad \mathcal{K}_{ac} = \mathcal{K}_{13}/r_a, \quad \mathcal{K}_{ca} = \mathcal{K}_{31}/r_c, \tag{7}$$

where the $\mathcal{K}$'s describe the V-insensitive and $[Cl^-]_o$-insensitive part of the reaction scheme.

If one $I_p(V)$ provides the information for four kinetic parameters (figure 3), then paired $I_p(V)$ for different $[Cl^-]_o$ can in principle yield eight parameters. In this situation we fitted the data by (6), allowing in turn the following changes with different $[Cl^-]_o$: (*a*) each of the six rate constants and N (leaving five rate constants in common for different $[Cl^-]_o$) and (*b*) any pair of the six rate constants (leaving four rate constants and N in common for different $[Cl^-]_o$).

Out of these 21 possibilities the best fits were obtained when k_{cb} and N were allowed to vary. A change in k_{cb}, which describes the binding of external Cl^- to the pump, is exactly what is expected upon a change in $[Cl^-]_o$, whereas a change in N was unexpected and calls for a reasonable interpretation.
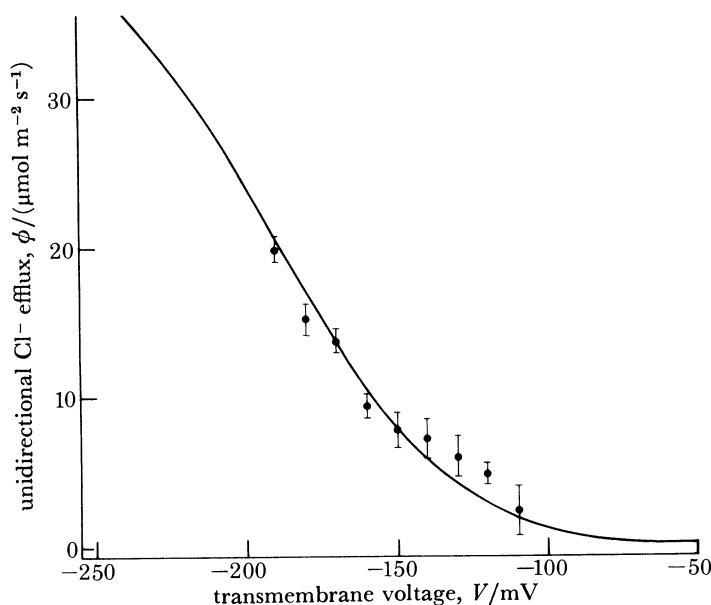

FIGURE 4. Voltage dependence of unidirectional Cl^- efflux through pump pathway. Dots and error bars (means $\pm$ s.e.m.) represent measurements; the curve is predicted by (5) and parameter values from the inset of figure 3 related on average $I_p(V)$ data (Mummert *et al.* 1981).

Changes in $[Cl^-]_o$ were accomplished in three different ways by using artificial seawater (Gradmann 1975) as a control medium: I, partial substitution of external Cl^- by I^-; B, partial substitution of external Cl^- by benzenesulphonate; and S, dilution of the entire ionic strength by isotonic sorbitol solution.

Average parameters of the three-state model for control conditions are given in table 1: k_{ab}^0, k_{ba}^0, $\mathscr{K}_{ac}$ and $\mathscr{K}_{ca}$ are similar to the comparable two-state parameters k_{io}^0, k_{oi}^0, $\mathscr{K}_{io}$ and $\mathscr{K}_{oi}$ (inset to figure 3). This similarity is in fact even closer, considering the different reserve factors of the two models. The parameters k_{bc} and k_{cb} turn out to be large. These numbers should be read as minima: increasing k_{cb} and k_{bc} by a common factor leads to fits of comparable quality. Therefore the above assumption in (5) of rapid dissociation and association of external Cl^- is confirmed. All results presented here are obtained with $z = -2$. When the fitting routines are carried out with $z = -3$ or $z = -1$, poorer fits are obtained throughout.

The change of k_{cb} with different $[Cl^-]_o$ is given in figure 5*a* for the three methods. We cannot explain the low k_{cb} for 423 mM Cl^- under sorbitol conditions. However, all the other

TABLE 1. AVERAGE ($\pm$ s.e.m., $n = 38$) KINETIC PARAMETERS (SECOND^{-1}) FOR THE THREE-STATE MODEL OF THE ELECTROGENIC Cl^- PUMP (FIGURE $2c$) FOR APPROXIMATE SUBSTRATE CONCENTRATIONS AS GIVEN IN FIGURE $2a$

(Data from $I_p(V)$ under different $[Cl^-]_o$, calculated with a total density of 10^{-7} mol m^{-2}; k_{bc}/k_{cb} = constant, $u = z(-\frac{1}{2}u)$ for $z = -2$ (compare equation (1)).)

$$
\begin{array}{ll}
k_{ab} & (1.78 \pm 0.30) \times 10^{-1} \times e^{-u} \\
k_{ba} & (8.70 \pm 1.43) \times 10^{5} \times e^{u} \\
k_{bc} & (1.00 \pm 0.12) \times 10^{6} \\
k_{cb} & (1.50 \pm 0.09) \times 10^{6} \\
k_{ac} & (3.35 \pm 0.47) \times 10^{2} \\
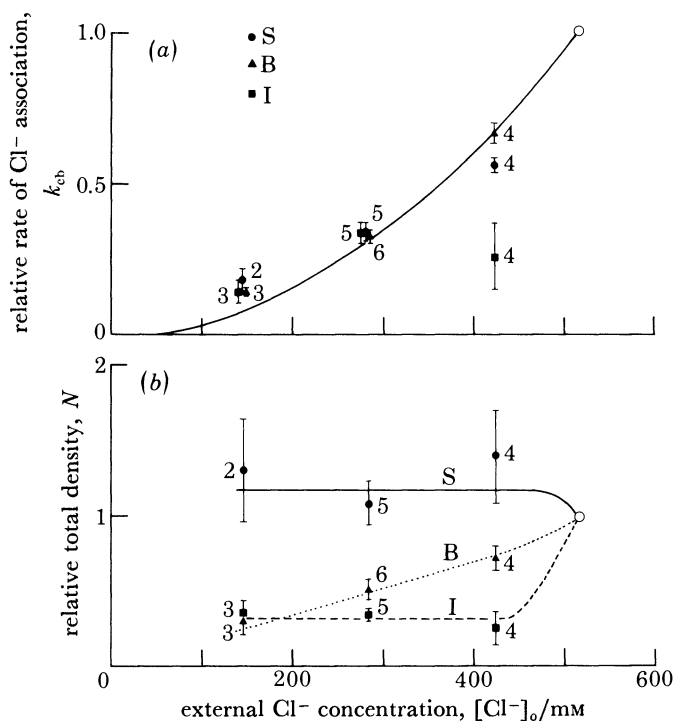k_{ca} & (5.22 \pm 0.65) \times 10^{2}
\end{array}
$$

FIGURE 5. Mean ($\pm$ s.e.m., n marked on points) results of reaction kinetic analysis of $I_p(V)$ at different $[Cl^-]_o$; relative parameter changes compared with control conditions (set at unity) using the three-state model (figure $2c$; equation (6)); conditions for (partial) replacement of external Cl^-; I, replacement by I$^-$; B, replacement by benzenesulphonate; S, dilution of (artificial) seawater by isotonic (1.1 M) sorbitol solution. For absolute (control) parameters see table 1. (a) Changes in k_{cb} (rate of association of external Cl^- to the pump): line, theoretical expectation for association of 2 Cl^- per pump cycle. (b) Changes in total density, N, of active pump molecules in membrane; lines are drawn to guide the eye.

results fit well around the theoretical parabola expected if k_{cb} describes the association of 2 Cl^-. The slightly too large values for 145 mM Cl^- may be due to considerable Cl^- efflux (compare data in figure 4), which may cause an increased $[Cl^-]$ right outside the membrane compared with the small $[Cl^-]_o$ in the free solution. These results strongly confirm the proposed stoichiometry of 2 Cl^- per electrogenic cycle, as formulated in the explicit reaction scheme (figure $2a$).

The pattern of the unexpected $[Cl^-]_o$-sensitive changes in N is not as uniform for the three different assays (figure $5b$). Under sorbitol conditions, there appears a slight increase in N, which saturates in the presence of about 200 mM sorbitol. Maybe the cell tends to maintain its

Cl^- fluxes under lower $[Cl^-]_0$ by an increase of the density of active pump elements in the membrane. When Cl^- is replaced by a different anion like I^- or benzenesulphonate there is a significant decrease in N (see figure 5*b*). The results of a few recent experiments with HCO_3^- (data not shown) fit exactly into this pattern. This finding allows the following interpretation: the pump has a non-specific binding site for external anions whereby only Cl^- can be transported, and all other anions form an inactive complex with the pump which will result in a decrease of the total density of active pumps. According to this interpretation, inert anions in the external medium can be used as inhibitors of the pump without affecting the energy metabolism of the cell.
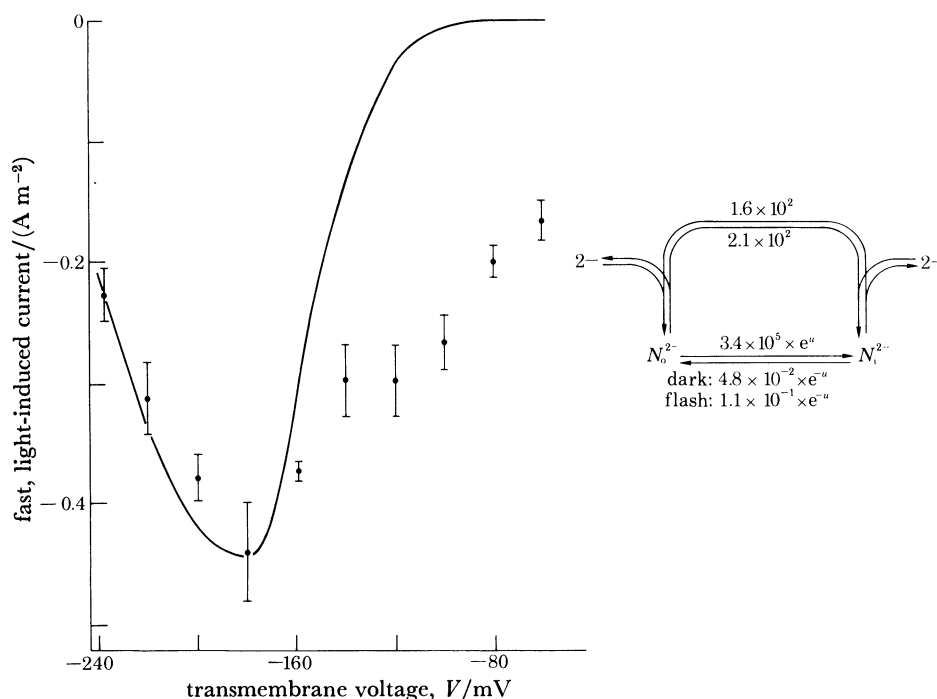

FIGURE 6. Current–voltage curve of fast photoelectric effect (voltage clamp data from Gradmann (1978)), means ± s.e.m. Line, fit with two-state model of the pump (figure 2*b*, inset); control (dark) parameters are the same as in figure 3; light-induced change in $I_p(V)$, as shown in the graph, accomplished by an increase in k_{io}.

Perturbation and relaxation

The reaction kinetic analysis presented so far is related on an assumption of N as a scaling factor. To obtain absolute parameters of reaction kinetics it is necessary to know N or the temporal behaviour of the pump. The temporal behaviour cannot be obtained from the steady-state $I_p(V)$. However, the time course of relaxation after a short disturbance can provide the required information.

It has been shown that green light directly disturbs the pump (Gradmann 1978). The current–voltage relation of this fast photoelectric effect (depolarization) does not intersect the V-axis within the range between -250 and $+200$ mV. Furthermore, it displays a characteristic peak around -190 mV which seems to be identical with E_p (compare figure 3). This current–voltage curve has been described by a somewhat complicated seven-state model of the pump (Gradmann 1978). Recent attempts to describe this effect by the simple two-state model (figure 2*b*) resulted in best fits when k_{io} was assumed to be increased by green light (figure 6),

with the same reaction kinetic control parameters as in figure 3. Although the measured currents exceed the theoretical currents for V more positive than -150 mV, the coincidence in the basic curve shape and the proper location of the peak renders this description valid as a first approach. It should be noted especially that changes in $N-$ as may be expected if the effect of green light were an inactivation of the pump – do not result in an acceptable description of the effect. Therefore, this perturbation and the following relaxation are considered to take place within the electrogenic reaction cycle itself.
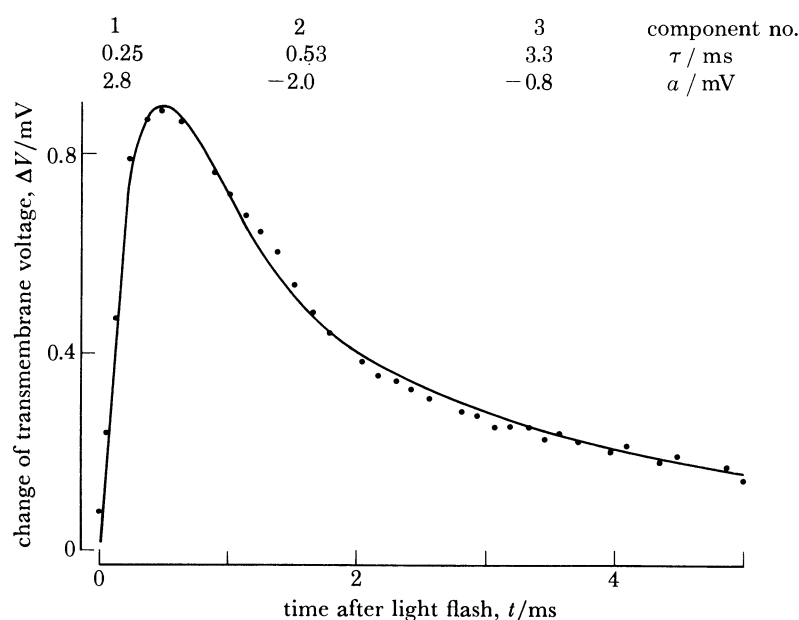

1	2	3	component no.
0.25	0.53	3.3	τ / ms
2.8	-2.0	-0.8	a / mV

FIGURE 7. Example of time course of change in V upon a short (3 μs) light flash at $t = 0$. V_r was -176 mV. Dots, measurements; the line is fitted to the dots with sum of three exponentials, amplitudes (a/mV) and time constants (τ/ms) of the three components are marked in the appropriate time range of their visible appearance.

An example of the time course of V with a short light flash is given in figure 7, which also includes the parameters of the description of this transient by the sum of three exponentials. A detailed discussion of the resulting parameters (including their sensitivity to voltage) with respect to the pump cycle and passive electrical membrane properties will be the subject of a future study (Gradmann & Hansen 1983). However, the results can already be used for a crude estimate of the absolute amounts of the rate constants k_{ab}, k_{ba}, $\mathcal{K}_{ca}$ and $\mathcal{K}_{ac}$ (in the order of $100\,\mathrm{s}^{-1}$) for V_r close to E_p (figure 7). With this reference, the absolute reaction kinetic parameters of the three-state model can be estimated. The resulting values are listed in table 1 for the actual substrate concentrations under control conditions (figure 2a).

Although the total density, N, turns out to be rather large, the large apparent capacity of the membrane of around 50 mF m^{-2} (Gradmann 1975) under conditions when the pump is operating, can be interpreted by a corresponding density of charges within the membrane that are redistributed by changes in V (Hansen $et\ al.$, in prep.). Furthermore, electron micrographs of freeze–fractured plasmalemma surfaces of $Acetabularia$ (H. Behrens, unpublished results) display a density of small particles also of the order of 10^{-7} mol m^{-2}. This finding is, of course, not convincing evidence by itself but it correlates well with the other results mentioned.

Conclusions

The electrogenic Cl^- pump in *Acetabularia* is likely to be an ATPase. Its electrical properties can be studied in detail. The results can be condensed in a consistent quantitative reaction kinetic scheme that includes the following features: order and absolute velocity of individual reaction steps, states of charge, stoichiometry and voltage-sensitivity of the entire system.

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