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Sodium Transport in Na^+-rich *Chlorella* Cells

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Summary. The rate of Na^+/Na^+ exchange as measured with $^{24}Na^+$ in Na^+-rich cells of *Chlorella pyrenoidosa* is governed by a single rate constant and saturates with increasing external Na^+ concentration. The K_m value for this process is 0.8 mM Na^+ and the maximum rate of exchange in illuminated cells is about 5 pmoles $cm^{-2}\,sec^{-1}$. These values contrast with a K_m of 0.18 mM K^+ and maximum rate of about 17 pmoles $K^+ \cdot cm^{-2} \cdot sec^{-1}$ for net K^+ influx. Although the Na^+/Na^+ exchange was only slightly sensitive to light it was inhibited by the uncouplers CCCP and DNP and by the energy transfer inhibitor DCCD. This inhibition of the rate of Na^+/Na^+ exchange was not accompanied by a loss of internal Na^+. Both the effect of external K^+ on $^{24}Na^+$ influx into Na^+-rich cells and the inhibition of net K^+ uptake by the presence of external Na^+ indicates that Na^+/Na^+ and K^+/Na^+ exchanges share the same carrier and that the external site of this carrier has a three to four times higher affinity for K^+ over Na^+.

Introduction

Like most other cells the green unicellular alga *Chlorella pyrenoidosa* maintains, under normal growth conditions, a high intracellular potassium concentration (Barber, 1968a; Shieh and Barber, 1971). This potassium content seems to be regulated by transport systems which are also capable of transporting other monovalent cations across the cell membrane. Potassium influx can be accompanied either by the efflux of sodium and hydrogen (Shieh and Barber, 1971; Barber and Shieh, 1972) or by the efflux of potassium ions during steady-state exchange (Barber, 1968b, c). Although the K^+/Na^+, K^+/H^+ and K^+/K^+ exchanges are dependent on metabolism and show saturation with increasing external potassium concentration they do not have the same rates of exchange. Closely coupled exchanges of this type seem to be a common feature of micro-organisms (Schultz and Solomon, 1961; Slayman and Slayman, 1968; Harold, Baarda and Pavlosova, 1970) but as yet it is not clear how many mechanisms are involved.

In order to clearly detect K^+/Na^+ and K^+/H^+ exchanges with *Chlorella* it was necessary to grow this photosynthetic organism in such away as to produce cells which were abnormally high in Na^+ and depleted of K^+

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(Shieh and Barber, 1971). In this paper we wish to report experiments conducted with these Na^+-rich cells. In particular we have concentrated our attention on the rate, kinetics and mechanism of Na^+/Na^+ exchange with the view of gaining a better overall understanding of cation regulation by *Chlorella*. Such studies on normally grown *Chlorella* cells are hampered by the low intracellular Na^+ concentration (Barber, 1968a).

Materials and Methods

The details of culturing, harvesting, and measurement of fluxes both by isotopic and flame photometric methods have been given in earlier papers (Barber, 1968a, b, c; Shieh and Barber, 1971; Barber and Shieh, 1972). All experiments were, except where stated, conducted in Na^+-culture medium containing 4 mM Na^+ (Shieh and Barber, 1971).

^{24}Na was purchased from the Radiochemical Centre, Amersham as sterile isotonic chloride solutions with specific activities of about 250 μCi/mg Na^+. Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was purchased from Calibiochem and N,N′-di-cyclohexyl carbodiimide (DCCD) from Koch-Light Laboratories.

Results

Kinetics and Rate of Na^+ Exchange. ^{24}Na was added as a tracer to suspensions of Na^+-rich cells which had either been illuminated or dark treated (see Fig. 1). Assuming that the cells and the external medium act as a steady-state two compartment system then the rate of increase of internal activity A_i is given by:

$$\frac{dA_i}{dt} = a\varphi^*_{oi} - a\varphi^*_{oi} \tag{1}$$

where φ^*_{oi} and φ^*_{oi} are the tracer influx and efflux respectively, a is the area of the rate limiting barrier and t is time. Since $\varphi^*_{oi} = S_o\varphi$ and $\varphi^*_{oi} = S_i\varphi$ where S_o and S_i are outside and inside specific activities and φ is the steady-state Na^+ exchange rate, then integration of (1) gives:

$$A_i = A_\alpha\left[1 - \exp\frac{(-a\varphi t)}{v_i c_i}\right] \tag{2}$$

where A_α is the final internal activity when $S_i = S_o$ and $v_i c_i$ is the total amount of internal sodium.

Eq. (2) is more conveniently written

$$A_i = A_\alpha(1 - e^{-kt}) \tag{3}$$

where k is a rate constant of reciprocal time units. According to Eq. (3) a plot of log $(A_\alpha - A_i)$ against t should give a straight line. As shown in Fig. 1. such a plot does yield straight lines for ^{24}Na uptake measured both under light and dark conditions. Fig. 1 also shows that there was only a very slight difference between the light and dark influxes.

Mean values for light and dark exchange rates are given in Table 1. The data has been quoted with ± SEM and compared with the rate of steady-state Na^+ exchange in normal K^+-containing cells.

Table 1. Mean value of the steady-state ^{24}Na initial influx

Type of cell	Light		Dark	
	μmoles/ (ml·packed cells·min)	pmoles ·cm^{-2} sec^{-1}	μmoles/ (ml·packed cells·min)	pmoles ·cm^{-2} sec^{-1}
Na^+-rich cells	1.24 ±0.09 (9)	5.53 ±0.40	0.82 ±0.08 (5)	3.66 ±0.37
K^+-rich cells	0.021 ±0.003 (6)	0.095 ±0.013	0.013 ±0.002 (5)	0.058 ±0.009

The figures in the parentheses represent the number of separate experiments and values are quoted as the mean ± SEM.

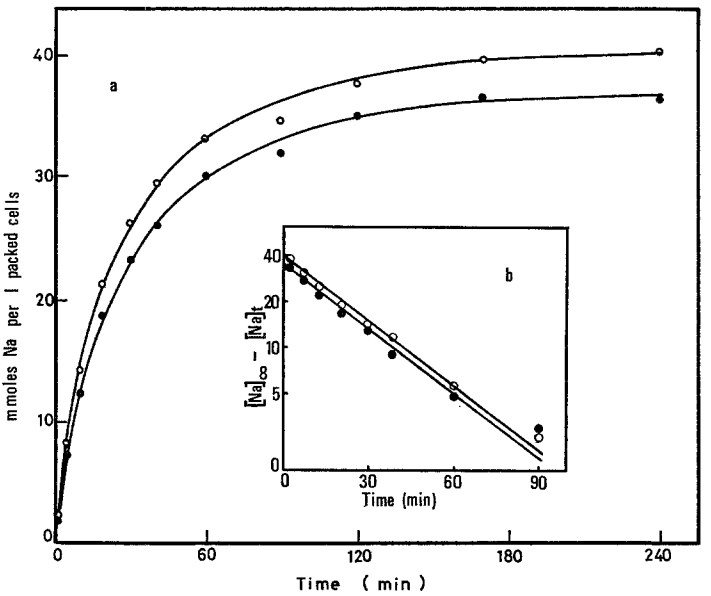

Fig. 1. (a) Steady-state influx of ^{24}Na into Na^+-rich cells measured in the light (open circles) and in the dark (closed circles). (b) Semilog plots of the same data. The cells were suspended in culture medium containing 4.0 mM Na^+ but no K^+: see Barber and Shieh (1972). The light was supplied by two 40 W white fluorescent tubes at a light intensity of 800 ft-candles. The temperature was 25 ± 0.5° C

Relationship between the ^{24}Na Influx and External Na^+ Concentration

Rates $^{24}Na/Na^+$ exchange with Na^+-rich cells were measured in the presence of different concentration of Na^+ in the external medium ranging from 0.2 mM to 7.0 mM and the results are presented in Fig. 2. The curve shows clearly that the rate of ^{24}Na uptake saturates as the

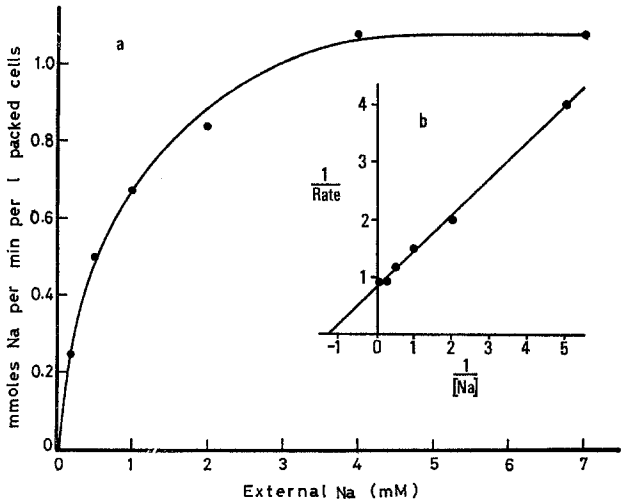

Fig. 2a and b. Effect of external Na^+ concentrations on ^{24}Na uptake into illuminated Na^+-rich cells. (a) ^{24}Na influx as a function of external Na^+ concentration. (b) A reciprocal plot of the Lineweaver-Burke type using same data. The cells were suspended in culture medium containing various levels of Na^+

external Na^+ level is raised and that a reciprocal plot of the Lineweaver-Burke type gives a straight line corresponding to a K_m of 0.8 mM and a maximum rate of 1.25 μmoles Na^+/min · ml packed cells.

Sensitivity of $^{24}Na/Na^+$ Exchange to Metabolic Inhibitors. As Table 2 shows the influx of ^{24}Na was inhibited by the uncouplers CCCP and DNP and by the energy transfer inhibitor DCCD. Flame photometric analyses of cells treated with these compounds showed that this inhibition of the rate of Na^+/Na^+ turnover is not accompanied by a loss of internal Na^+.

Net Na^+ Extrusion Induced by K^+. Fig. 3 shows the steady-state ^{24}Na uptake by Na^+-rich *Chlorella* cells. After the cells had reached isotopic equilibrium 3 mM KCl was added to the medium. It can be seen that a reduction of internal radioactivity occurred. In this case there was no change of internal or external specific activity since $^{24}Na^+$ was present in the external medium. Thus the reduction of internal activity corresponds to a fall in the intracellular Na^+ level. This K^+ induced net Na^+ extrusion from Na^+-rich cells has been detected by flame photometry and reported earlier (Shieh and Barber, 1971; Barber and Shieh, 1972).

Effect of External K^+ on ^{24}Na Uptake into Na^+-Rich Cells. Since the Na^+/Na^+ exchange shows saturation characteristics and is sensitive to metabolic inhibitors the question arises whether the Na^+ exchange occurs via the site which is responsible for the net K^+/Na^+ exchange; that is

Table 2. Effect of CCCP, DNP and DCCD on the steady-state ^{24}Na influx into illuminated Na^+-rich *Chlorella* cells

Experiment		Initial influx μmoles/(ml · packed cells · min)	% control
Exp. 1	Control	1.18	100
	CCCP 1×10^{-5} M	0.59	50
	5×10^{-5} M	0.07	6
Exp. 2	Control	0.84	100
	DNP 2×10^{-4} M	0.22	26
	5×10^{-4} M	0.05	6
Exp. 3	Control	1.4	100
	DCCD 1×10^{-4} M	0.63	45
	5×10^{-4} M	0.09	7

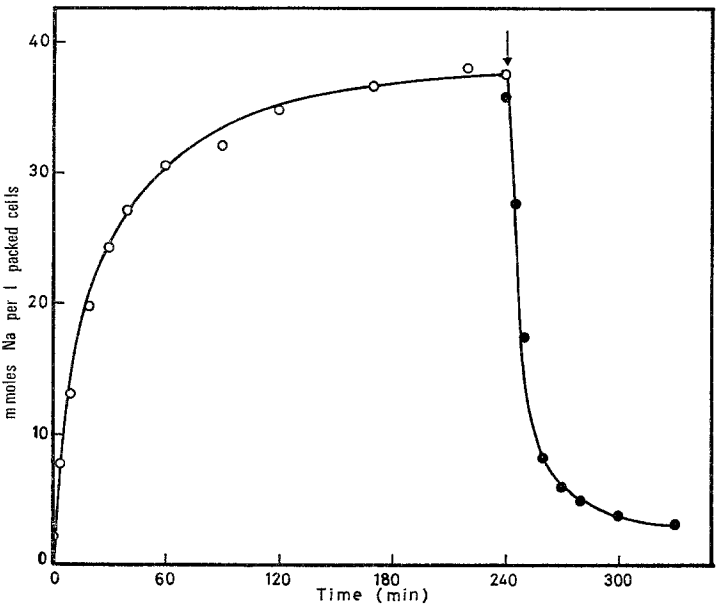

Fig. 3. Steady-state influx of ^{24}Na (open circles) into illuminated Na^+-rich cells suspended in K^+-free culture medium (see Fig. 1 legend). After the cells had reached isotopic equilibrium 3 mM KCl was injected into the suspension as indicated by the arrow and the resulting net Na^+ efflux followed by a fall of internal radioactivity (closed circles)

whether Na^+ and K^+ compete for the same exchange site on the outer side of the membrane. However studies into this question are complicated by the fact that when K^+ is present in the external medium net fluxes occur. As shown in Fig. 4. the net influx of K^+ and efflux of Na^+ show

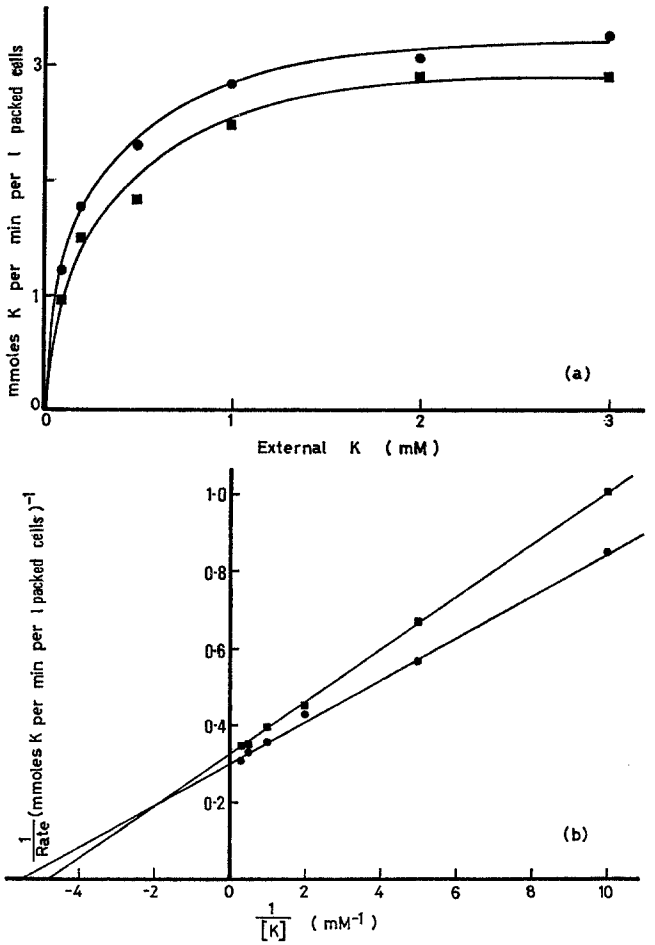

Fig. 4a and b. Effect of external K^+ concentrations on the initial rates of net Na^+ (closed squares) and K^+ (closed circles) movement in illuminated Na^+ rich cells (a) shows the initial rates of K^+/Na^+ exchange as a function of the external K^+ concentration (b) Reciprocal plots of the Lineweaver-Burke type using the data shown in (a). Other conditions were same as given for Fig. 1

saturation kinetics with increasing external K^+ concentration corresponding to Michaelis constants of 0.18 mM K^+ for net K^+ influx and 0.22 mM K^+ for net Na^+ efflux. The maximum rates for the net exchange were **3.35** μmoles K^+/ml packed cells · min and 3.0 μmoles Na^+/ml packed cells · min. Assuming that Na^+ competes with K^+ for this transport site then from comparison of the K_m values the affinity of the site for K^+ is at least four times greater than for Na^+.

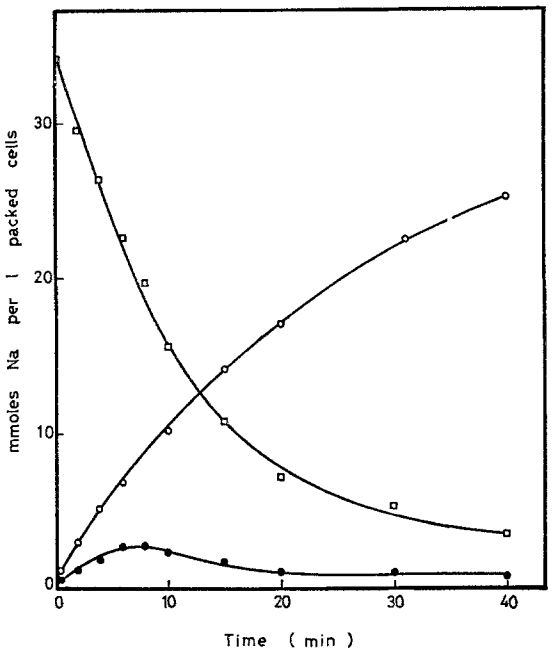

Fig. 5. Effect of external K^+ on the steady-state ^{24}Na influx into illuminated Na^+-rich cells. The cells were initially suspended in the 4 mM Na^+ culture medium and either ^{24}Na (open circles) or $^{24}Na + 3$ mM KCl (closed circles) was injected at zero time. The figure also shows the K^+-induced net ^{24}Na extrusions (open squares) from Na-rich cells which had been obtained from the same cells suspension but preloaded with ^{24}Na. Other conditions are given in legend of Fig. 1

Support for competition of K^+ for the Na^+ site associated with steady-state Na^+/Na^+ exchange in Na^+-rich cells comes from the data presented in Fig. 5. In this experiment 3 mM KCl was added simultaneously with ^{24}Na to a suspension of Na^+-rich cells. Also shown in Fig. 5 is the ^{24}Na uptake under conditions of no net Na^+ movement and the net Na^+ extrusion induced by adding 3 mM KCl to a suspension of Na^+-rich cells which has been preloaded with $^{24}Na^+$. It can be seen that a low level of $^{24}Na^+$ entered the cells when K^+ was added simultaneously with the isotope. Of relevant interest was the finding that only a slight rise of internal radioactivity above the final steady-state level was observed during the first few minutes of the uptake. For example, at six minutes the amount or $^{24}Na^+$ in the cells corresponded to 2.56 μmoles Na^+/ml packed cells while the actual level at this stage of the exchange was in the region of 23 μmoles Na^+/ml packed cells. If the ^{24}Na had been able to enter the cells at the same rate as in the absence of external K^+ (by using sites other than K^+ specific ones) then there should have been

radio-isotope in the cells corresponding to at least 6 μmoles Na^+/ml packed cells. Alternatively if the rate of ^{24}Na entry had been decreased by the presence of external K^+ then it is possible to account for the low level of radioactivity in the cells at this stage of the time course.

Discussion

In many respects Na^+/Na^+ exchange in Na^+-rich *Chlorella* cells is comparable with steady-state K^+/K^+ exchange in normal high K^+ containing cells (Barber, 1968b, c). Both processes show first-order tracer kinetics, saturate with increasing external Na^+ or K^+ concentrations and are sensitive to metabolic inhibitors. There are however some striking differences, the maximum rate of Na^+/Na^+ exchange in illuminated Na^+-rich cells is about 5 pmole Na^+ $cm^{-2} \cdot sec^{-1}$ with half maximum rate at 0.8 mM Na^+ while for K^+/K^+ exchange the rate is 1 pmole K^+ $cm^{-2} \cdot sec^{-1}$ and the K_m for this process is approximately 0.07 mM K^+. Both processes show the same characteristic of being inhibited by metabolic inhibitors without loss of internal cations. This indicates, as previously suggested for *Chlorella* (Barber, 1968c) and other microorganisms (Zarlengo and Schultz, 1966; Rothstein, 1964; Slayman and Slayman, 1968) that the cell membrane has a relatively low passive permeability to ions and that diffusion of Na^+ or K^+ in this alga represents only a small fraction of the carrier mediated fluxes. In fact little or no loss of ^{24}Na occurs when Na^+-rich cells loaded with radioisotope are suspended in distilled water. Efflux of this isotope only normally occurs, in absence of net K^+ influx, when Na^+ is present in the external medium.

The reason why the Na^+/Na^+ exchange is sensitive to metabolic inhibitors is obscure. Similar linkages between closely coupled homologous exchanges and metabolism have been found for other microorganisms (Epstein and Schultz, 1966; Slayman and Slayman, 1968; Harold *et al.*, 1967) and for redblood cells (Garrahan and Glynn, 1965). There is likely to be little or no energy utilized during exchange diffusion processes of this type and it has been suggested that the apparent sensitivity to metabolic activity reflects a requirement for, but not the hydrolysis of, ATP for formation or activation of the carrier (Garrahan and Glynn, 1965; Barber, 1968c). If in the case of *Chlorella* the Na^+/Na^+ carrier is converted to a K^+/Na^+ form then ATP hydrolysis would be expected to occur. The low sensitivity of the Na^+/Na^+ exchange rate to light most probably reflects the high rate of respiration relative to the photosynthetic rate found with Na^+-rich cells (Shieh and Barber, 1971).

In the case of *Chlorella* there does seem to be some evidence that the Na^+/Na^+ and K^+/Na^+ exchanges share the same carrier. Not only was

there an inhibition of ^{24}Na entry when K^+ was simultaneously added with the isotope but also the rate of K^+/Na^+ exchange was inhibited when Na^+ was present in the external medium. In the absence of external Na^+ the initial rate of net K^+ influx was 24.8 ± 2.7 pmoles $K^+ \cdot cm^{-2} \cdot sec^{-1}$ ($\pm$ SEM of 4 experiments), but when the external Na^+ level was 4.0 mM the rate was decreased to 17.8 ± 1.2 pmoles $K^+ \cdot cm^{-2} \cdot sec^{-1}$ ($\pm$ SEM of 11 experiments). Both effects can be accounted for by assuming a three to four times preference of the external site for K^+ over Na^+. Post *et al.* (1960) have demonstrated that external Na^+ inhibits active K^+ influx in human red blood cells and that the inhibition appears to be competitive. Sjodin (1971) also reported that inhibition of the K^+-activated Na^+ efflux from Na^+-enriched muscle cells by external Na^+ ions is of the competitive type. Sachs (1971) has further demonstrated that Na^+/Na^+ exchange measured with human red blood cells in K^+-free medium is accomplished by means of the carrier normally associated with K^+/Na^+ pumping. However in animal cells the situation is more complicated in that there is a passive leak of Na^+ and also there seems to be two components of the Na^+/Na^+ exchange (Sjodin, 1971). Apparent K_m values of 0.8 mM Na^+ for Na^+/Na^+ exchange and about 0.2 mM K^+ for K^+/Na^+ (exchange measured in the presence of 4 mM Na^+) also supports the concept that the outer membrane site has a higher affinity for K^+ than for Na^+. The lower affinity of the outer site for Na^+ may also account for the finding that the initial rate of K^+/Na^+ exchange is three or more times greater than Na^+/Na^+ exchange. Similar arguments have been made from observations with phosphate-deficient *Scenedemus* (Kylin, 1966) and with *Streptococcus faecalis* (Harold *et al.*, 1967).

Overall the results at present seem to indicate that Na^+/Na^+ exchange occurs via the carrier responsible for K^+/Na^+ exchange during net cation movement. A closer study of the effect of external Na^+ on net K^+ and Na^+ movement is required. With striated muscle such studies have indicated that there is a deviation from a Michaelis-Menten relationship for K^+/Na^+ exchange as a function of external K^+ when Na^+ is present in the bathing medium (Sjodin, 1971). Pronounced sigmoidal nature of activation curves of K^+/Na^+ exchange in presence of Na^+ seem to indicate that the effect of Na^+ is due to either an ion-induced allosteric transformation or to a multivalent binding site on the carrier. Slayman and Slayman (1970) have also come to a similar conclusion from studying net cation fluxes with *Neurospora* at various external pH. As yet we have not detected sigmoidal kinetics for K^+/Na^+ exchange in *Chlorella* either by introducing Na^+ to the external medium or by varying the external pH.

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