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THE EFFLUX OF POTASSIUM FROM *CHLORELLA PYRENOIDOSA*

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SUMMARY

1. Potassium efflux from Chlorella cells was studied by means of radiotracer.

2. The kinetics of the efflux suggested that the exchange of the majority of the internal K^+ is controlled by a first-order process.

3. The efflux rate under conditions of no net K^+ movement is approx. 1 pmole K^+ per sec per cm^2 in the light but falls to 0.3 pmole K^+ per sec per cm^2 for dark treated cells.

4. The efflux was sensitive to temperature and metabolic inhibitors in a way not expected for a simple passive leak through a relatively permeable membrane.

5. In the absence of external K^+ the efflux was very low and not until this cation or Rb^+ was replaced in the suspension medium did the cells readily lose tracer.

6. The results are tentatively interpreted in terms of a metabolically controlled K^+–K^+ exchange system possibly involving a membrane carrier.

INTRODUCTION

Recent investigations on *Chlorella pyrenoidosa* have shown that this alga is able to maintain a high internal level of K^+ (ref. 1). Both electrical measurements[1] and tracer influx studies[2] suggested that this accumulation was a result of an active transport mechanism possibly sited at the plasma membrane. Such a situation had been indicated by earlier work on this organism[3,4] although no detailed information about the electrochemical potential gradient had been previously reported.

The presence of a K^+ influx pump seems to be a characteristic of other microorganisms which have been studied to-date[5]. ROTHSTEIN[5] has speculated about the function of this accumulation believing that its purpose is to produce a high internal hypertonicity necessary for the growth and expansion of these walled cells. Moreover, he suggests that for those microorganisms living in dilute solutions the back leakage of internal ions is minimized by a relatively impermeable membrane and in many cases controlled by specific exchange systems.

In this paper the steady-state efflux of K^+ from Chlorella cells has been studied. The kinetics and magnitude of the rate of loss of internal tracer were consistent with

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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the previously reported influx data[2]. In addition the results were in agreement with the above suggestions since the efflux did not seem to act simply as a passive leak but showed a sensitivity to light, temperature and metabolic inhibitors. It was also found that the efflux rate dropped to a low level when there was no external K^+.

MATERIALS AND METHODS

The alga, *C. pyrenoidosa*, was cultured in the same manner as previously given[1]. Effluxes were measured by resuspending cells, which had been loaded with ^{42}K, in fresh inactive culture medium. Aliquots of cells were taken, filtered and assayed for internal radioactivity in essentially the same way as outlined earlier[2,6]. The duration of the experiments and the density of the cell suspensions were such that little or no back flow of tracer occurred. For some experiments a modified culture medium was used, details of which are given at the appropriate position in RESULTS.

The cells were usually loaded with ^{42}K by growing them in culture medium containing this isotope at an initial activity of 0.5 μC/ml. They were harvested about 24–48 h later by centrifugation and then quickly washed with distilled water before being resuspended in the inactive medium. Although this isotope has a short half-life the high intracellular K^+ content of this organism resulted in satisfactory levels of internal radioactivity even after 48 h of growth.

The experiments were conducted over 2–3 h and it was found that those suspensions which were illuminated over this period of time tended to increase their external pH. This rise was compensated by the use of thin suspensions, often less than 0.5 %, and by the occasional addition of small quantities of dilute HNO_3. This acid was used because the external medium contained a high concentration of nitrate[1].

The isotope ^{42}K came in two forms, as sterile isotonic KCl solution from the Radiochemical Centre, Amersham, and as spectroscopically pure K_2CO_3 from the Atomic Energy Establishment, Harwell. The internal radioactivity of the cells was assayed by means of a Nuclear Chicago gas-flow counter and carried out to an accuracy of approx. 1–3 %.

RESULTS

Kinetics and magnitude of K^+ efflux

In Fig. 1 the efflux of K^+ from Chlorella cells fully loaded with isotope is shown. It can be seen that the plot of the log internal radioactivity as a function of time gives a straight line for both light and dark conditions. This suggests that this cellular efflux is governed by a single rate constant and that the cells are essentially behaving as a one-compartment system for the majority of internal K^+. Thus, assuming no back flow of isotope, the internal activity A_1 at any time t is given by $A_1 = A_1' \exp(-kt)$ where A_1' is the initial internal activity and k is the efflux rate constant.

From these semilog plots a value of the internal K^+ concentration could be estimated by taking the intercepts log A' and knowing the external specific activity of the loading solutions. Indeed, as long as the harvesting and washing procedures were rapid the intercepts gave concentrations very close to the values measured by flame photometry[1]. From the slope of the lines a value of the rate constants can be found and the effluxes calculated in a similar manner as previously described[6]. This

TABLE I

LIGHT AND DARK K^+ EFFLUX RATES

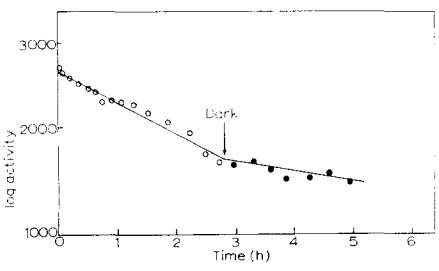
The efflux rates were measured for cells suspended in culture medium at pH 6.8. The suspensions were maintained at 25 ± 0.5° and the illumination was furnished by two 150-W incandescent spotlights giving an intensity of 500 ft candles at the reaction vessels. Results are quoted ± S.E. (number of experiments on which mean is based).

Conditions	*Efflux rates*	
	pmoles/sec per 5 mm³ packed cells	*pmoles/sec per cm²*
Light	17.0 ± 1.2 (11)	0.92 ± 0.10
Dark	6.2 ± 1.5 (6)	0.33 ± 0.10

has been done for both light- and dark-treated cells suspended in culture medium and the values are presented in Table I.

K^+ efflux from cells loaded over a short time

Although the majority of the intracellular K^+ exchanged according to first-order kinetics it was found that when cells were loaded over short periods of time, such that the cytoplasmic specific activity was low, a faster exchange compartment could be detected. In Fig. 2 an experiment using cells which had been loaded for about 1 h is presented. This plot shows the normal light and dark effluxes together with a rapid initial loss. Subtraction of the slower efflux curves from their faster components yielded straight lines of half-times of 10 min. In other experiments the half-times ranged from 4–10 min and as such represented values much longer than expected for cell-wall exchange or unstirred layer effects. The magnitude of this first compartment in terms of cell K^+ was small being in the region of 1–3 %.



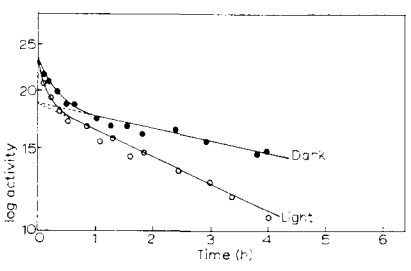


Fig. 1. The efflux of ^{42}K from cells which were initially fully loaded with isotope showing the effect of placing the illuminated suspension in the dark. The plot is log internal radioactivity in counts per min against time.

Fig. 2. The light (open circles) and dark (closed circles) efflux of ^{42}K from cells which were initially loaded with tracer over a short period of time, approx. 1 h.

Requirement of external K^+

During some preliminary experiments conducted in various solutions it was found that the efflux of this cation only occurred when external K^+ was present. For example, it was observed that when cells were suspended in water or NaCl solutions

there was very little efflux of radioactivity. This property was investigated further using culture-medium conditions or a slight modification of this.

The curves in Fig. 3 were obtained from illuminated cells which had been grown in radioactive culture. Fully loaded cells were harvested and quickly washed in the normal way by centrifugation. Half of these cells were suspended in Na^+ culture medium,

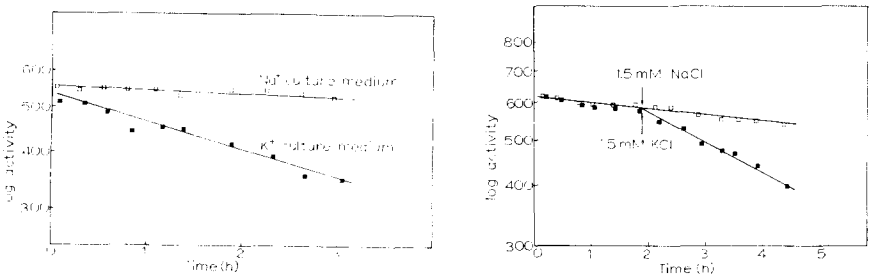


Fig. 3. The efflux of ^{42}K from illuminated cells which were initially fully loaded with tracer. The cells were suspended either in normal culture medium (closed squares) or in a modified medium having all its K^+ salts replaced by the corresponding Na^+ salts (open squares).

Fig. 4. A ^{42}K efflux experiment demonstrating the effect of injecting 1.5 mM NaCl (open squares) and 1.5 mM KCl (closed squares) into suspensions of illuminated cells in Na^+ culture medium (see text).

that is, a solution containing all the normal constituents except every K^+ compound was replaced by the corresponding Na^+ salt. The other half was resuspended in normal K^+ containing medium. The experiment shows quite clearly that an efflux of ^{42}K occurred into the normal medium but was reduced to a very low value for those cells bathed in the K^+-deficient solution.

This observation was further demonstrated by an injection experiment. Cells were initially suspended in Na^+ culture medium and placed in two seperate vessels which were both illuminated. Efflux into this solution was allowed to occur for 2 h (see Fig. 4), and as before the efflux was very low even after injecting 1.5 mM NaCl into one of the vessels. However, the injection of 1.5 mM KCl into the other vessel resulted in an immediate loss of cellular ^{42}K at a rate comparable to that found for cells suspended in normal medium.

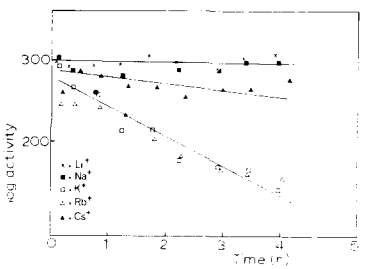
Effect of other alkali metal cations on ^{42}K efflux

The above results showed that the efflux of ^{42}K responds quite differently in the presence of external Na^+ or K^+. In Fig. 5 the effect of other alkali metal cations on the rate of loss of ^{42}K is shown. Fully loaded cells were suspended in Na^+ culture medium and placed in five different vessels each containing the chlorides of Li^+, Na^+, K^+, Rb^+ and Cs^+ at a concentration of 2 mM. It can be seen that in the presence of Li^+ or Na^+ the efflux was virtually undetectable while some loss of cytoplasmic ^{42}K did occur in the presence of Cs^+. In the case of Rb^+ and K^+ the slopes of the lines corresponded to the normal rate constants.

Temperature sensitivity

The effect of temperature on the efflux of ^{42}K from Chlorella cells, both under light and dark conditions, was measured at 5, 15 and 25°. The rate of loss of the isotope at 5° was very low over the period of these experiments. At 15 to 25° the loss

of ^{42}K was increased and Fig. 6 shows plots of log internal activity against time for cells suspended in the light and dark at these temperatures. In both cases the change from 15 to 25° on the same suspension resulted in a marked increase in the rate of tracer efflux. The rates gave temperature coefficients (Q_{10}) for this temperature change of 2.0 for dark treated cells and 2.3 for illuminated cells.



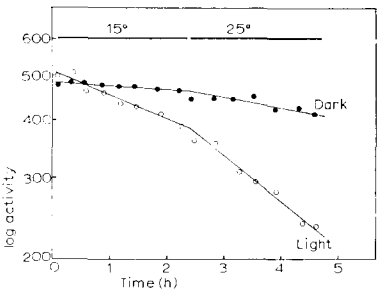


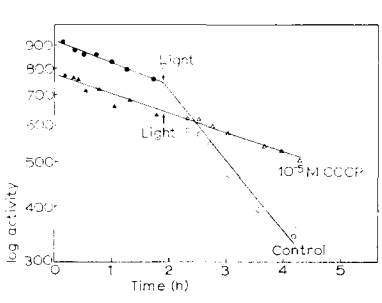
Fig. 5. Shows the efflux of ^{42}K from five sets of illuminated cells suspended in Na^+ culture medium (see text) each containing, in addition, 2 mM chlorides of Li^+, Na^+, K^+, Rb^+, and Cs^+.

Fig. 6. The temperature sensitivity of the light (open circles) and dark (closed circles) effluxes of ^{42}K into culture medium is shown by changing the temperature from 15 to 25° on the same suspensions.

The effect of high CO_2 concentration, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and carbonyl cyanide m-chlorophenylhydrazone (CCCP)

When the suspensions were bubbled with 5 % CO_2 in air both the dark and light effluxes were reduced by values of approx. 50 % of those normally found for air conditions. A similar inhibition has been reported for the influx of this cation[2] and perhaps suggests there may be some coupling between the two processes.

The possible linkage of the inward and outward fluxes of K^+ was further demonstrated in a qualitative manner by experiments involving 10^{-5} M DCMU and 10^{-5} M CCCP. Both these compounds reduced the light stimulated K^+ influx at these concentrations[2,7] and it can be seen in Figs. 7 and 8 that they have a similar effect on the effluxes.



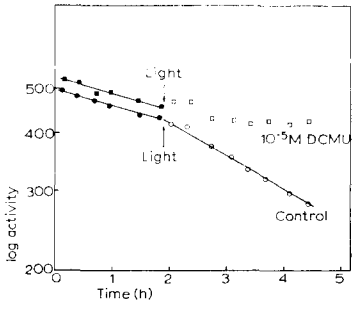


Fig. 7. A semilog plot of the dark and light efflux of ^{42}K from cells initially fully loaded with isotope showing the effect of 10^{-5} M CCCP (triangles) on this loss.

Fig. 8. A similar experiment to that shown in Fig. 7 but demonstrating the effect of 10^{-5} M DCMU (squares).

DISCUSSION

The kinetics of the tracer loss from Chlorella suggests that the majority of the cell K^+ exchanges according to a first order process and is consistent with previous studies[2]. The faster efflux fraction only detected with cells partly loaded with ^{42}K can be identified with the small initial uptake of this isotope found during the influx measurements[2]. As with the earlier work no conclusions can be drawn about the nature of this small compartment except that its half-times of exchange were rather longer than expected for cell-wall material or unstirred layer effects. The possibility still remains that this component arises because of the heterogeneous nature of the cell population within any particular suspension.

The light and dark efflux rates correspond closely with the influx rates measured under similar conditions[2] suggesting that the action of light is to stimulate the exchange of cell K^+ without causing a large change in the internal concentration. This is supported by flame-photometry measurements[1].

This photosensitivity of the efflux, which is believed to be passive[1], could mean that the action of light is to alter the driving forces on this process. However, there is no clear evidence that either the concentration or electrical potential gradients[8] change during light–dark transitions. Alternatively the action of light on the down-hill flux of K^+ could be to increase the permeability of the rate limiting membrane or possibly to speed up some metabolic reaction which is responsible for the regulation of the internal K^+ level. The latter possibility is supported from the sensitivity of the efflux to temperature which is not usually expected for a simple passive process unless a substantial energy barrier exists between the two phases. Moreover, it would seem unlikely that a passive leak through a permeable membrane would be reduced by metabolic inhibitors. In many respects the efflux seemed to respond to various experimental conditions in a comparable way to the influx[2], a situation not normally found for a 'pump and leak' system and more in accordance with an exchange mechanism associated with a relatively impermeable membrane.

Similar suggestions have been made for other microorganisms[5] and also indicated for some marine algae[9,10]. For the two most studied microorganisms, yeast and *Escherichia coli*, the uptake of K^+ by rapidly growing cells occurs *via* an exchange for metabolically derived H^+ (refs. 11, 12). In addition to this K^+–H^+ exchange there is also a K^+–K^+ component[13] and in the case of *E. coli* cells, which are prevented from rapid growth by chloramphenicol, the whole of the K^+ influx is balanced by a closely linked carrier mediated K^+ efflux[14]. In a similar way the K^+ efflux from Chlorella may be closely coupled to the majority of the influx in these present experiments but converted to a K^+–H^+ exchange process during net ion movement. Indeed, SCHAEDLE AND JACOBSON have detected H^+ extrusion during the net uptake of K^+ by K^+ starved Chlorella[15].

If the influx and efflux of K^+ is closely coupled together then Chlorella cells have a means of maintaining a constant level of this major intracellular cation under a variety of environmental conditions. Such a speculation is partly borne out from the observation that when the cells were suspended in distilled water little or no loss of this cation occurred. The absence of any external cations, for which the cellular K^+ could exchange, was not the reason for this for when the cells were suspended in Na^+ culture medium, containing a number of different cations, very little leakage occurred.

Not until K^+, or its closest biological analogue Rb^+, was present in the external medium did the exchange take place. Also there was no difference in the efflux rate if 1.5 or 6.5 mM K^+ was present in the external solution which represent levels at which the influx rate of this cation saturates[2].

These results further support the hypothesis that the efflux and influx of K^+ are coupled together as an exchange diffusion process. However, evidence has been presented elsewhere[2,7], that the influx of K^+ may be controlled by cellular levels of ATP. But, in considering an exchange diffusion mechanism there is no reason for metabolic energy to be utilized. The carrier is fully saturated and net movement does not occur. Such a process has been suggested many times for animal cells[16,17] but perhaps the most relevant arises from the work of GARRAHAN AND GLYNN[18] on red blood cells and their ghosts. They detected a Na^+–Na^+ exchange which required the presence of ATP for the formation of the carrier responsible for shuttling Na^+ backwards and forwards across the cell membrane. However, there was no evidence that the ATP was hydrolysed until the carrier was converted into a normal active Na^+–K^+ exchange system. Such an argument could be applied to K^+ turnover in Chlorella. In this case the carrier which is responsible for active transport and net accumulation is a K^+–H^+ exchange driven possibly by ATP. When no net ion movement occurs the carrier is saturated with K^+ in both directions and now requires the presence rather than the utilization of ATP. Thus the number of operational carriers would be controlled by the ATP available, which in turn would reflect the sensitivity of the transport mechanism to metabolism.

In discussing the possibility of exchange diffusion the relevance of the experiments involving K^+-free solutions has not been fully considered. It is most likely that in addition to there being a zero K^+ influx when no external K^+ is present there will also be a change in the membrane potential[1]. This potential would be expected to alter as the external K^+ concentration is reduced in such a way as to decrease the driving force on the passive efflux of this cation. To what extent the decrease of K^+ efflux is due to hyperpolarization of the surface membrane is not known, no attempt, for example, was made to measure this quantity for cells bathed in Na^+ culture medium. Nevertheless, if we assume the efflux to be in fact a passive leak through a membrane we can calculate the permeability coefficient and estimate the possible change in membrane potential required to reduce the efflux to the observed levels in K^+ deficient medium. This is possible by using the following equation derived from the theory[19,20] of GOLDMAN:

$$\Phi_0 = \frac{FEP_k}{RT} \frac{[K_i] \exp FE/RT}{(1 - \exp FE/RT)}$$

where Φ_0 is the passive efflux of K^+ in moles per sec per cm^2, P_k is the permeability coefficient in cm/sec, E is the membrane potential, $[K_i]$ is the internal K^+ concentration and the other symbols have their usual meanings. Taking the values of $[K_i]$ and E as previously reported for illuminated cells bathed in culture medium[1] and the efflux as 0.9 pmole K^+ per sec per cm^2, the calculation gives $P_k = 2.1 \cdot 10^{-8}$ cm/sec. This incidentally corresponds very closely to the earlier value estimated from tracer influx studies at low temperatures[2]. Using this value of the passive permeability coefficient for K^+ efflux and the above equation, it follows that a change from —40 to about —100 mV must occur, when the cells are taken from normal culture medium and

placed in a K^+ deficient solution, to result in the observed fall in the efflux rate. The development of such a membrane potential would not seem unreasonable when the cells are placed, for example, in Na^+ culture medium. However, if this argument was strictly correct it would be difficult to explain the similarity of the efflux rates when the external K^+ levels were somewhat different. It must be concluded that a change of electrical potential will almost certainly occur and as such will affect the diffusional component of the efflux, but it seems that the magnitude of this component is likely to be small.

In summary, it appears that the situation in Chlorella is comparable with that in other microorganisms. The efflux of K^+, under conditions of no net movement, seems to be linked to the majority of the K^+ influx possibly *via* a carrier mediated K^+–K^+ exchange. This may become adapted to a K^+–H^+ exchange during rapid growth and net uptake. However, it must be clearly recognised that this picture could be considerably altered if large changes of membrane potential occur or if the passive permeability properties of the rate limiting membrane are modified under the various experimental conditions.

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