Ion Absorption and Retention by Chlorella Pyrenoidosa. II. Permeability of the Cell to Sodium and Rubidium

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Summary. The Na and Rb permeability of Chlorella pyrenoidosa were estimated from the rates of radioisotope self-diffusion.

The isotopic exchange in absence of net ionic movements followed first order kinetics. This suggested that for sodium, which reached isotopic equilibrium in approximately 90 minutes, the cell behaved as 1 compartment with respect to isotopic exchange. Rubidium in 180 minutes approached isotopic equilibrium by 67%: thus, the existence of a single compartment for Rb has not been demonstrated. Net fluxes, calculated from the isotope exchange data, and expressed on a dry weight and surface area base showed that Na fluxes were approximately 7 times larger than Rb fluxes. Net Na fluxes of 90 milliequivalents per 100 g dry weight per hour were far in excess of the observed maximum net accumulation of Na. However, Rb fluxes of 13 milliequivalents per 100 g dry weight per hour were of similar magnitude as the rate of Rb accumulation. Thus, permeability could be a limiting factor for Rb but not for Na accumulation. Sodium and Rb fluxes in absence of net ionic movements were inhibited by low temperature, dark air and dark N₂ conditions. This change in flux rates was explained mainly on the basis of metabolically dependent changes in the cell surface layers.

Isotope fluxes of Rb were drastically reduced in dark air and dark N₂ in the absence or presence of net cation movements. Dark N₂ essentially eliminated net cation accumulation, whereas dark air had relatively little effect on the net K and Rb accumulation by Chlorella. Thus the 2 major factors involved in net cation accumulation in the Chlorella cell, permeability and processes leading to cation retention, respond differently to metabolic inhibition permitting a separation of these 2 important aspects of cation accumulation.

The use of radioactive isotopes for the study of transport phenomena has demonstrated that net accumulation and ion interchange are under most conditions not a valid estimate of ionic fluxes across the cell surface (5, 14). In most systems total fluxes exceed net movements and the cells remain permeable to ions after cessation of net ionic movements (14). The knowledge of ionic fluxes and cellular permeability is essential for the understanding and explanation of ion transport.

Attempts to define the meaning of ionic flux and permeability measurements presents considerable practical difficulties. It is not sufficient to estimate the rate of movement of a substance through the region of interest but it is also essential to estimate the energy gradients which induced the movement. For most biological systems including Chlorella the energy gradients involved in net movements are not known. However, self-diffusion of radioisotopes under ideal conditions circumvents this problem and represents the most elegant means for the estimate of the ionic permeability of cells (13).

In this publication measurements are reported of cell permeability to Rb and Na estimated from self-diffusion of their radioisotopes. The meaning of the reported data is somewhat limited since biological systems are not at equilibrium with their environment nor are the cell contents necessarily homogeneous and equilibrated throughout. The successful elimination of net ionic movement, however, should provide the best possible estimate of cellular permeability to Rb and Na.

Methods

The experimental conditions used in the series of experiments reported were the same as described earlier (28), except for the modifications mentioned below. To avoid any possible physiological shock Chlorella pyrenoidosa cells containing Na and Rb were grown for the isotope exchange experiments. For this purpose, the K content of the growing medium (28) was reduced to 0.085 meq/liter and

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0.125 meq/liter Rb or 1.25 meq/liter Na were added respectively. After harvesting and washing by centrifugation the cells were pretreated for 120 minutes in solutions of the same composition and pH as the experimental medium. This was done in order to saturate the cells with the ion and thereby avoid net changes in content during the influx experiments. For influx experiments the cell suspensions after pretreatment were diluted 1:1 with similar supporting media containing the desired amount of radioisotope. This eliminated the requirement for washing the cells after pretreatment. For the light-air system, except for a decrease in the cell number per unit volume, the conditions were the same for pre- and treatments. This eliminated any possible cell volume changes (26) and associated ionic fluxes (9). For the investigation of the interaction between metabolism and ion fluxes the cells were transferred from the 2-hour light-air pretreatment into dark N₂. For the efflux experiment reported in Table IV the cells were grown in radioactive solutions and then treated as described for K net uptake experiments (28). Except when mentioned otherwise, all cells were rinsed with Ca after treatment to remove surface and exchangeable cations. The radioactivity of the ashed samples was determined with a Tracerlab thin window proportional counter.

The isotope influx results were analyzed mathematically. A first order reversible model was found to be satisfactory (5,11). Assume the system to consist of 2 compartments, the cell and the suspension medium, in which the substance A is distributed.

\[ A^* + A_1 \rightleftharpoons A_2 \]

Let \( a \) be the initial amount of the radioactive isotope \( A^* \) in compartment 1 (the external phase) and \( X \) the amount of \( A^* \) transferred at time \( t \). Let \( k_1 \) and \( k_2 \) be the fractional removal coefficients for the forward and back reactions. The rate equation then takes the form (11)

\[ \frac{dX}{dt} = k_1 (a-X) - k_2 X. \]

If \( Xe \) is the value of X at equilibrium, then

\[ k_1 (a-Xe) = k_2 Xe. \]

Integration with the boundary conditions \( X = 0, t = 0 \) and \( X = Xe, t = t \) leads to

\[ k_{at} = 2.303 \log \left( \frac{Xe}{Xe-X} \right). \]

Then \( k_1 \), the fractional removal coefficient for influx, can be calculated from

\[ k_1 = \frac{\text{slope} \times 2.303 \times Xe}{a} \]

where the slope is obtained from a plot of \( t \) versus \( \log \left( \frac{Xe-X}{Xe} \right) \). The half time for equilibration \( t_{0.5} \) can be found from equation (III) by substitution of one-half \( Xe \) for \( X \)

\[ t_{0.5} = \frac{0.69 \cdot Xe}{k_1 a}. \]

Since \( k_1 \) is the fraction removed per unit time from compartment 1, the influx rates of the labeled element can be calculated from

\[ k_1 \times \text{labeled element in compartment 1} = \text{influx per unit time}. \]

Similarly the efflux rates would be

\[ k_2 \times \text{labeled element in compartment 2} = \text{efflux per unit time}. \]

The constant \( Xe \) represents the amount of isotope which must move from the outside solution into the cell to make the specific activities of the cell and outside solution equal. Since the cation content of the cells did not change measurably during the experimental period, \( Xe \) can be calculated for influx experiments from the amount of Na or Rb present in the cell and outside solution and the amount of radioactivity added to the outside solution. The fractional removal coefficients \( k_1, k_2 \) and \( t_{0.5} \) are functions of compartment size. If compartment size is expressed as the total amount of an ion in a compartment, then both concentration changes at constant volume and volume changes at constant concentration would lead to changes in the magnitude of \( k_1, k_2 \) and \( t_{0.5} \). This is not the case for the calculated flux rates. Here \( k \) is multiplied by the amount of labeled ion in the compartment, and since \( k \) is inversely proportional to compartment size (equation IV), the calculated flux rates become independent of compartment size. It should also be noted that the application of above equations is limited by the assumption that at \( t = 0, A^* \) is confined entirely to 1 compartment and that equilibrium with respect to the chemical concentration of the ion studied is present at all times within each compartment.

**Results**

The isotope influx of Na, in absence of net ionic movements is shown in figure 1. Sodium reached isotopic equilibrium between cells and solution in about 90 minutes. A comparable Rb system (fig 2) after 180 minutes progressed only 67% towards equilibrium. Figures 1 and 2 also show that the influx of Na and Rb are both inhibited by dark N₂ conditions. The early portion of the dark N₂ curve for Na has been omitted in figure 1. During the first 50 minutes in dark N₂, net losses of Na occurred from the cell, eliminating the possibility of satisfactory interpretation of the isotope movements during this period. The Na content in dark N₂ dropped from 39 to about 28 meq/100 g dry weight and remained constant thereafter. Such losses were only observed for Na saturated cells in dark N₂. Under the same conditions K or Rb saturated cells retained all their alkali metal cations. Figures 1 and 2 also contain the influx data replotted on the basis of reversible first order kinetics. The plots, \( \log \left( \frac{Xe}{Xe-X} \right) \) versus time, are linear over the time period reported. For sodium (fig 1), which eventually reached isotopic
equilibrium this demonstrates clearly that practically all sodium in the cell underwent isotope exchange at the same rate; that is, the cell behaved as one compartment with respect to the isotopic exchange of sodium. The light-air plot for sodium becomes uncertain when the system is close to equilibrium. This would be expected since as X approaches Xe, the fraction \( \frac{X}{Xe} \) becomes increasingly subject to large variations due to relatively small fluctuations of X.

The Rb exchange also satisfied first order kinetics (fig 2) for 180 minutes. However, since the cells did not reach isotopic equilibrium, no assurance is available that the curve would remain linear for a more extended period of time. The data, therefore, do not exclude the possibility that *Chlorella* may contain 2 or more Rb compartments.

Table I provides a summary of flux rates calculated from the radioisotope influx experiment in absence of net cationic movements. The flux rates were calculated from the appropriate fractional removal coefficients and the size of the respective compartments. The size of a compartment is expressed as the total amount of the ion present in the compartment. This is made possible by the assumption, implicit in the kinetic equations used, that equilibrium with respect to the chemical concentrations of the ion studied exists at all times within each compartment. Fluxes are reported on a dry weight and surface area basis. The surface area was estimated from cell counts and the assumption that *Chlorella*

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**Table 1. Sodium and Rubidium Fluxes Estimated from the Self-Diffusion of Isotopes In Absence of Net Ionic Movements**

The sodium suspension contained 5 meq/liter of Na phosphate pH 6.8. For the Rb experiment the suspension contained 5 meq/liter Rb and 7.5 meq/liter K as phosphate salts pH 6.8. Ionic composition of Na cells: Na 38.7 meq/100 g dry weight, K 22.6 meq/100 g dry weight. Ionic composition of Rb cells: Rb 38.0 meq/100 g dry weight, K 25.0 meq/100 g dry weight.

<table>
<thead>
<tr>
<th>Sodium fluxes</th>
<th>Rubidium fluxes</th>
</tr>
</thead>
<tbody>
<tr>
<td>meq/100 g dry wt per 60 min</td>
<td>meq cm² sec⁻¹</td>
</tr>
<tr>
<td>Light air</td>
<td>94.2</td>
</tr>
<tr>
<td>Dark N₂</td>
<td>9.35</td>
</tr>
</tbody>
</table>
Table II. Effect of Temperature, Darkness and Anaerobic Conditions on Rb\(^{86}\) Influx, in 40 Minutes, in Absence of Net Ionic Movements

Suspension contained 5 meq/liter Rb and 7.5 meq/liter K as phosphate salts at pH 6.8. Initials, K 24.9 and Rb 31.2 meq/100 g dry weight. Specific activity, 1 \(\mu\)eq. Rb = 25900 cpm.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Isotope influx Rb(^{86}) meq/100 g dry wt*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light air</td>
<td>22</td>
</tr>
<tr>
<td>Dark air</td>
<td>22</td>
</tr>
<tr>
<td>Dark N(_2)</td>
<td>22</td>
</tr>
<tr>
<td>Light air</td>
<td>4 - 5</td>
</tr>
<tr>
<td>Dark air</td>
<td>4 - 5</td>
</tr>
</tbody>
</table>

* Calculated from specific activities.

Table III. Demonstration of the Rapid Nonmetabolic Equilibration of Rb in the Ca Removable Fraction of Chlorella

Suspension, 5 meq/liter Rb and 5 meq/liter K as phosphate salts pH 6.8. Initial, K 19.6 and Rb 17.7 meq per 100 g dry weight. Specific activity, 1 \(\mu\)eq. Rb = 24179 cpm.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H(_2)O rinsed</th>
<th>Ca rinsed</th>
<th>Rb removed by Ca rinse</th>
<th>Rb(^{86}) uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light air</td>
<td>11.3</td>
<td>1.2</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Dark N(_2)</td>
<td>11.0</td>
<td>1.2</td>
<td>9.8</td>
<td>10.1</td>
</tr>
<tr>
<td>Light air</td>
<td>18.8</td>
<td>10.1</td>
<td>8.7</td>
<td>28.4</td>
</tr>
<tr>
<td>Dark N(_2)</td>
<td>10.1</td>
<td>0</td>
<td>10.1</td>
<td>12.3</td>
</tr>
</tbody>
</table>

* Calculated from specific activities.

Table IV. Na and Rb Exchange, in 60 Minutes, during Net Cation Accumulation

The Rb suspension contained 5 meq/liter Rb and 10 meq/liter K as phosphate salts pH 6.8. Composition of Na suspension, 5 meq/liter Na phosphate pH 6.8. Ionic composition of Rb cells, K 18.4 and Rb 18.5 meq/100 g dry weight. Initial specific activity, 1 \(\mu\)eq Rb = 59698 cpm. Composition of Na cells, K 21.8 and Na 19.2 meq/100 g dry weight. Initial specific activity, 1 \(\mu\)eq Na = 27628 cpm.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K</th>
<th>Rb</th>
<th>Rb(^{86}) efflux from roots meq/100 g dry wt*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light air</td>
<td>6.7</td>
<td>7.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Dark air</td>
<td>6.7</td>
<td>5.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Dark N(_2)</td>
<td>1.0</td>
<td>0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Calculated from specific activities.
reduced by 45%. It is evident, therefore, that dark air affects isotope exchange more than net accumulation and dark N₂ net accumulation more than isotope exchange.

Discussion

The exchangeable fraction of Chlorella (28) was found to reach isotopic equilibrium with the medium in less than 1 minute and was not dependent on metabolism. It is probable that this rapidly equilibrating nonmetabolic fraction, similarly to large algae (3, 6, 8, 15), is located in the cell wall of Chlorella. When the rapidly exchangeable fraction was eliminated by washing with Ca solution, the remaining isotopic exchange of both Rb and Na was linear when plotted logarithmically as a first order kinetic process. This shows that a constant fraction of isotope present in the cell or the external solution is transferred per unit time (5). Since Rb in the experimental period of 180 minutes moved only 67% towards isotopic equilibrium, no conclusion can be made as to the number of apparent Rb compartments present in the cell, even though the observed exchange followed a linear first order curve. However, essentially complete isotopic equilibration was attained with Na. This finding suggests that the Chlorella cell behaves as a single Na compartment with respect to Na exchange. This does not imply that the cell is uniform nor that it will necessarily behave as one compartment with respect to any other variable or component measured. For example, Na saturated cells will lose approximately 30% of their sodium in dark N₂ to solutions varying in concentration from 5 to 450 meq/liter Na. The loss stops when the Na content of Chlorella reaches about 24 meq per 100 g dry weight and will not continue even if the cells are then placed in distilled water. Thus, an unstable Na fraction exists in the cell saturated with Na which cannot be detected from isotope exchange measured in light air. It is probable that the net Na loss is not related to the state of Na in the cell but occurs as a result of decreases in cellular anions. Such losses do not occur with high Rb or K cells in dark N₂. The net Na losses may, therefore, be the result of specific interaction between Na and anion metabolism. As has been stated repeatedly, linear kinetics do not represent proof for the existence of only 1 cellular compartment. Kinetic evidence must be supported by independent physiological evidence. In Chlorella, for example, the rate-limiting step for cation entry could be located in the cell surface. Then the Chlorella cell would appear to represent a single compartment with respect to isotope exchange regardless of internal heterogeneity.

The Na and Rb fluxes observed in the Chlorella system fall into the range of values reported for other biological systems (3, 15, 21, 22, 32). When expressed on a surface area basis, they are very similar to values obtained for large algae. Since the large algae have a small surface to volume ratio when compared with Chlorella, a representation of the data on a volume basis would show that the exchange appears much slower in large algae than in Chlorella. This could lead to the erroneous conclusion that ions move into Chlorella more rapidly than into large algae. Isotope exchange experiments in large algae in absence of net ionic movements (3, 8, 15) have demonstrated that large algae contain 2 distinct internal compartments, the cytoplasm and vacuole. Chlorella under similar conditions shows only 1 internal compartment as would be expected from the effectively nonvacuolated structure of the cell.

The measured Na flux rates were approximately 7 times larger than those of Rb. Since Na has a larger hydrated radius than Rb the difference in flux rates cannot be explained by a screening process through a sieve type membrane (31). The lower flux rate of Rb could be explained on the basis of its comparatively greater interaction with cellular components. The process would be analogous to that occurring in ion chromatography (10, 20). The flux of Na into the cell was approximately equivalent to an entry of 90 meq Na per hour per 100 g dry weight of cells. This high rate of influx was far in excess of the maximum net accumulation of 18 to 24 meq per 100 g dry weight observed with Chlorella. For Rb, the observed influx was 12 to 14 meq per 100 g dry weight per hour. This was very close to the maximum observed net Rb uptake of 10 to 13 meq per 100 g. One can conclude the permeability could not have been a determining factor in Na accumulation, for Rb the flux rates were sufficiently low so that during rapid Rb accumulation, permeability could have limited the rate of net uptake.

For cations accumulated by Chlorella, the rate of isotope exchange and the measured ionic fluxes in and out of the cell were functions of cellular metabolism (fig. 1, 2, table II, III, IV). Compared with air-light, dark N₂, inhibited fluxes by about 90% and dark air by about 45%. An inhibition of ionic fluxes in presence or absence of net ionic movement has been observed in algae (1, 2, 3, 30), bacteria (27), higher plants (4) and animal systems (33). The molecular mechanism for the interaction between metabolism and permeability is not known. One of the earliest explanations for this phenomenon was the suggestion that the decrease in cytoplasmic motility as a result of metabolic inhibition could be the cause of the associated decrease in ionic movements (34). The interaction between cell metabolism and the magnitude of ion fluxes may also occur at or in the cell surface layers. Inhibition of metabolism could lead to changes in the shape and arrangement of molecules in the permeability barrier (2, 7, 12, 17) such that the permeability of the cell would be decreased (17), provided the treatment is not so severe as to induce a general breakdown of cellular integrity. It is generally believed that enzymes, when combining with a substrate molecule or when releasing a reaction product, will undergo a change in shape or vol-

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ume. Less specific intramolecular and extramolecular rearrangements may also occur as a result of charge density or charge distribution changes during enzymatic reaction cycles. Thus, during metabolism, the matrix of the membrane and associated components would be different from the inhibited state (17). Such a mechanism finds support in observed metabolically dependent shape or size changes of cells, cell organelles (16, 19, 24, 26) and their fragments (25). An additional possible explanation for the observed changes in flux rates is a compulsory, direct coupling of ionic movements to some aspects of phosphorylative or oxidative metabolism (23, 35). As a result, ionic fluxes would always reflect changes in metabolism.

Chlorella has been found to be highly permeable to sodium and rubidium in presence or absence of net ion accumulation. The composition difference between the cell and external solution is maintained by the cell surface layers which are apparently highly impermeable to anions (28). Thus, the complex colloidal cell interior and the external solution are reminiscent of 2 phases of a Donnan type system separated by a rigid selective membrane (20). In such a system net cation accumulation is dependent either upon the creation of new anions or upon a redistribution of anions between the 2 phases. Cation interchange can occur continuously between the 2 phases provided the membrane is permeable to positively charged ions (13). Net cation accumulation may, therefore, differ from isotopic exchange or ion interchange, not in the mode of entry into the cell, but in the compulsory coupling to anion creation in the cell. The interaction between net ion accumulation and metabolism can occur either as a result of metabolically dependent permeability changes or through a coupling between metabolism and the processes leading to anion creation in the cell. Evidence for such a dual control of cation accumulation is provided in tables II and IV. Since cell permeability and cation retention respond differentially to metabolic inhibition (table IV), it has become possible to separate in Chlorella these 2 aspects of cation accumulation. If permeability is not limiting, the rate of net cation accumulation would be determined by the rate of anion creation. The best demonstrated example of such anion metabolism is the synthesis of organic acid associated with excess cation accumulation (32). However, since any slowly or nondiffusible internal anion generated by the cell would be equally satisfactory, net cation accumulation could be associated with any other cellular process leading to the production of negative charges (18, 32). The rates of synthetic reactions may vary greatly and would be affected differently by specific metabolic inhibitors. This would always be reflected in the rate of net cation accumulation. Alternatively, the coupling of cation accumulation and anion creation may mean that cation accumulation under conditions of low permeability may determine the rate of synthesis of anionic molecules in a cell.

**Literature Cited**