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Ion Absorption and Retention by Chlorella pyrenoidosa. III. Selective Accumulation of Rubidium, Potassium and Sodium[1]

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Summary. The selective preference of *Chlorella pyrenoidosa* for alkali metal cations was found to have the order Rb>K>>>Na. It was demonstrated that a cation of higher preference can replace in the cell cations of lower preference by an ion interchange process.

The replacement of Na from the cell by K or Rb occurred against high external Na concentrations up to 450 meq/l Na.

It is suggested that the structural selectivity of the *Chlorella* cell may be amplified by a chromatographic type repetitive selection process, driven by metabolically dependent unsymmetric shape changes of cellular membranes.

The terms selectivity, ion discrimination, and ion preference have been used to describe the observation that close to a steady state, the ratio of 2 ions in the cell is significantly different from their ratio in the surrounding solution and environment. Equality of the 2 ratios would be expected to exist under the above condition in any simple unrestrained electrostatic system (10, 16).

One can express the preference of a cell for an ion by the separation factor (10).

$$\alpha_n^m = \frac{\frac{\bar{C}_m}{\bar{C}_n}}{\frac{C_n}{C_m}}$$

Where α_n^m is the separation factor for the ion pair m, n. $\bar{C}_m$, $\bar{C}_n$ are the concentration of m and n in the cell. C_m, C_n are the concentrations of m and n in the solution.

For monovalent cations in an ideal system the separation factor is identical to the selectivity coefficient derived more rigorously using the mass action law (10). If m is preferred the factor α_n^m is larger than unity; if n is preferred the factor is smaller than unity. Conveniently, the dimensionless separation factor does not suggest any cause for the observed ion discrimination, but simply restates, in a short form, experimental observations.

Discrimination between K and Na has been observed in cross-linked organic ion exchange resins, gels, glasses and zeolites (6, 10, 15). The range of separation factors found usually varied between 1.5 and 3 with a few values as high as 6 to 10. Small degrees of selectivity in living organisms could be explained on the basis of mechanisms proposed for non-living systems. However, some living systems can maintain separation factors as high as 1000 or more. Such extreme values suggest from the consideration of energy requirements for recognition and separation (6, 10, 16), the existence of special cellular processes and structures devoted to and capable of ion discrimination. A model widely used suggests the existence of special molecular entities instrumental in ion transport and possessing ion specific properties. These transport entities may be mobile or stationary and may undergo chemical changes during the transport act (2, 8, 12, 19, 23, 28, 29). Alternative explanations include sieve effects at the membrane involving ion size and charge (11, 28, 29), retention in the electrical field created by high concentrations of negatively charged cell constituents (6, 13, 15, 16) as well as differential solubility of organometallic salts in the cytoplasm (21).

The phenomenon of selective ion accumulation is one of the major unresolved problems of ion transport and represents the key to our understanding of the observed extreme ion distributions between cells and environment.

Methods

The experimental conditions and procedures used in this series of experiments were the same as described earlier (25, 26). To obtain cells containing approximately equal amounts of K and Na

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or K and Rb, the K content of the growing medium was reduced to 0.085 meq/liter, and 1.25 meq/liter Na or 0.125 meq/liter Rb were added respectively.

Except when stated otherwise cells were rinsed on the membrane filter with a 5 meq/liter $CaCl_2$ solution to remove exchangeable surface cations (25). All samples were then rinsed with water, ashed, dissolved in 0.1 N HCl, and analyzed for cations using an emission flame spectrophotometer (25).

Results

Chlorella pyrenoidosa accumulates in 120 minutes, depending on solution concentration, 9 to 13 meq of Na per 100 g dry weight. Net uptake is only observed in light-air and not when energy metabolism is inhibited by dark-N_2 conditions. A similar experiment with ribidium shows that in air-light *Chlorella* accumulates large amounts of rubidium (3), however, at the same time the cells lose potassium to the outside solution. The uptake of Rb and loss of K increases with increasing Rb concentrations in the outside solution and is still not complete after 120 minutes. At that time the K content of the cells in a 25 meq/liter of Rb solution has decreased from an initial value of 34.8 to 6.3 meq K per 100 g dry weight and it appears that the process might continue to a nearly complete loss of K from the cells. In dark-N_2 the Rb and K movements are reduced to 1 to 5 % of those occurring in air-light.

If cells containing 45 to 50 meq of K per 100 g dry weight are placed in K or Na solutions they will not accumulate either of the 2 cations. However, when placed in Rb solutions, 26.6 meq per 100 g dry weight Rb can enter the cell in a nearly stochimetric exchange for 23.4 meq per 100 g dry weight of cellular K. This process does not represent a physical exchange with cell surface ions since it exhibits distinct dependence on metabolism (7, 23, 26), and the cations involved cannot be removed by rinsing the cells with salt solutions (25, 26).

These results demonstrate that the total alkali metal cation content of *Chlorella* when reasonably close to a steady state with the outside solution remains constant between 45 to 50 meq per 100 g dry weight in solutions containing a single monovalent cation. In solutions containing 2 alkali metal cations this value may go up to approximately 60 meq per 100 g dry weight. Even in solutions with concentrations as high as 450 meq/liter K, Rb, or Na it was found that the cell content of alkali metal cations stabilizes between 50 and 60 meq per 100 g dry weight in air-light. This suggests the existence in the cell of effective physical and chemical controls over its internal cation composition.

The replacement of one ion by another in *Chlorella* (tables I, II and fig 1) appears similar to

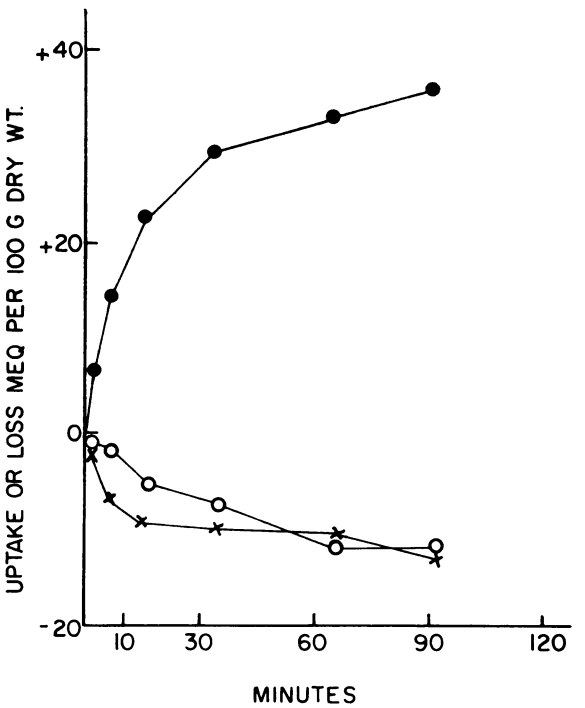

FIG. 1. Rubidium accumulation by *Chlorella* cells containing K and Na. The solution contained 5 meq/l of Rb phosphate (pH 6.8). Initial contents of cells K 22.0, Na 16.7 meq per 100 g dry wt. Rb, —●—; K, —X—; Na, —○—.

observations reported for a variety of biological systems including red blood cells, yeast, bacteria and red algae (2, 7, 27, 28, 29, 30). This type of ion movement has been utilized successfully for the study of relationships between ion transport and cell metabolism as well as for the evaluation of the exchangeability of ions in cell vacuoles. Since the degree of ion interchange depends on whether Na, K or Rb is involved it appears possible to utilize this exchange phenomenon for a study of the selective order of alkali metal cation in *Chlorella*. This possibility is underlined by the previously reported observation that ions move rapidly and continuously in both directions through the membrane as demonstrated by isotope self-diffusion studies (26). As a result the cell composition changes appear to reflect the different affinities of the ions for the cell.

The next series of experiments was conducted with cells containing approximately equal initial amounts of either K and Na or K and Rb. Assuming a uniform distribution of the 2 ions in the cell, either member of a pair should be provided an equal chance to leave the cell. Only if this condition is satisfied is it possible to assign some significance to the quantitative estimates of ion selectivity.

When cells containing approximately equal amounts of K and Na are placed in solutions

Table I. *Sodium and Potassium Accumulation by Cells Containing K and Na*

Phosphate solutions were buffered at pH 6.8. The initial cell composition for the K and Na experiments was 14.5 K, 14.3 Na and 21.8 K, 19.2 Na meq per 100 g dry weight respectively.

Time	Cells in 5 meq/l K phosphate		Cells in 5 meq/l Na phosphate	
	Change in content meq per 100 g dry wt			
Min	K	Na	K	Na
1	+ 3.0	0.0	—1.0	+2.0
15	+17 2	—7.6	0 0	+6.2
60	+21.9	—8.8	0.0	+9.1
90	+24.3	—9.1	0.0	+9.1

Table II. *Potassium and Rubidium Accumulation by Cells Containing K and Rb*

Phosphate solutions were buffered at 6.8. The initial cell composition for the K and Rb experiments was 21 5 K, 14.5 Rb and 18.2 K, 17.0 Rb meq per 100 g dry weight respectively.

Time	Cells in 5 meq/l K phosphate		Cells in 5 meq/l Na phosphate	
	Change in content meq per 100 g dry wt			
Minutes	K	Rb	K	Rb
1	+ 1.0	—1.3	— 1.0	+ 1.7
45	+ 9.3	—3.9	— 8.0	+15.6
60	+16.1	—6 6	—12.5	+25.1

containing K, Na leaves the cell and K enters (table I). However, placed in a solution containing Na, some Na accumulation occurs without concurrent K losses. If metabolism is inhibited by dark-N_2 conditions, net accumulation is eliminated, but exchange of Na for K continues at a reduced rate (fig 2). Similar experiments with K and Rb give a completely different picture (table II). Cells containing approximately equal amounts of K and Rb when placed in Rb solutions lose K and cells placed in a K solution lose Rb. Under dark-N_2 conditions, K, Rb movements are almost entirely eliminated (fig 3). If cells containing Na and K are placed in an Rb solution both K and Na are lost from the cell during Rb accumulation (fig 3).

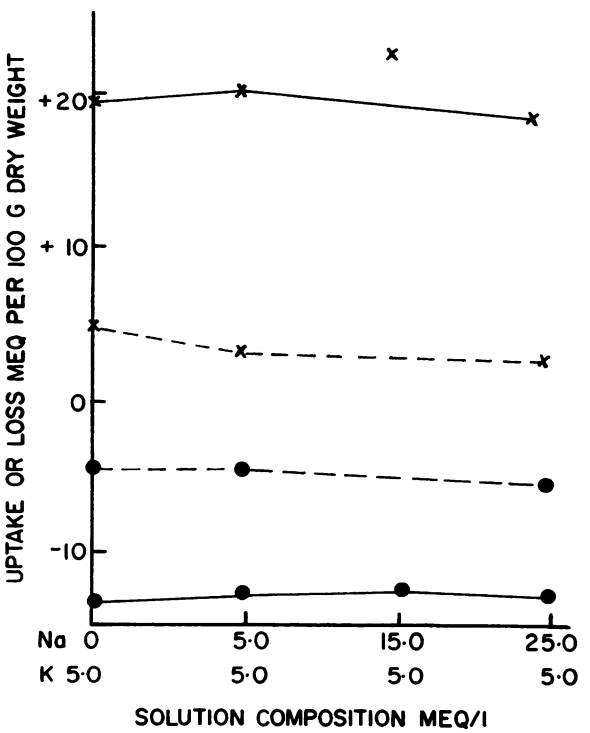

FIG. 2. Effect of Na concentration on K accumulation and Na loss from the cell. All solutions contained phosphate as the anion buffered at pH 6.8. Experimental period 90 minutes. Initial content K 22.0, Na 16 7 meq per 100 g dry wt. K, light-air —X—, dark-N_2 - - X - -; Na, light-air —●—, dark-N_2 - - ● - -.

To provide a more quantitative estimate of the discrimination between Na, K and Rb, *Chlorella* cells were placed in solutions containing different ratios of Na and K or K and Rb respectively (figs 2, 3). If the K/Rb ratio is 1 to 1, Rb enters the cell and K is lost from the cell. Only when the K to Rb ratios reaches a value between 1.5 and 2 does the loss of K from the cell stop; thus the cells prefer Rb to K. The relationship between K and Na is much more extreme. Over-all ratios tested, Na is lost from the cell and K enters (fig 2, table III).

Data in figure 3 show that the retention of Rb by the *Chlorella* system differs from its retention of K. Since *Chlorella* grows satisfactorily in Rb containing media, this difference is probably not the result of Rb toxicity, a problem repeatedly observed during culture experiments with plants and animals, but rather may reflect the differences in interaction of Rb and K with cell constituents. It therefore appears not possible to substitute Rb for K in transport experiments with *Chlorella*, as has been done for convenience in other systems.

Attempts were made to explore the limits of

Table III. *Effect of Extreme K-Na and Rb-Na Solution Ratios on Ion Movements in and out of* Chlorella *Containing K and Na*

In addition to chloride all solutions contained 5 mmoles of phosphate (pH 6.8). The initial cell composition for the K and Rb experiments was 20.2 K, 14.8 Na and 23.5 K, 25.9 Rb meq per 100 g dry weight respectively.

Solution composition		Composition change in 60 min		
meq/l		meq per 100 g dry wt		
		K	Na	Rb
450 Na	Light-air	— 1.2	+3.7	0
450 Na + 0.1 K	Light-air	+11.4	—5.3	0
450 Na + 1.0 K	Light-air	+16.4	—6.3	0
450 Na	Light-air	— 2.9	+6.2	0
450 Na + 0.1 Rb	Light-air	— 3.1	—2.7	+15.2
450 Na + 1.0 Rb	Light-air	— 7.3	—8.1	+23.6
450 Na + 0.1 Rb	Dark-N_2	— 3.1	—2.7	0

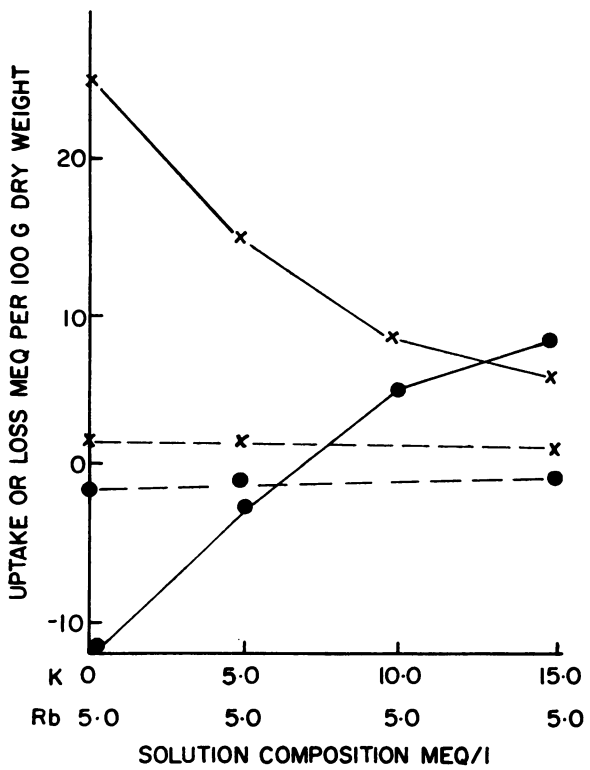

FIG. 3. Effect of K concentration on K and Rb movements. Experimental period 90 minutes. Initial cell content K 18.2, Rb 17.0 meq per 100 g dry weight. All solutions contained phosphate as the anion buffered at pH 6.8. Rb, light-air —X—, dark-N_2 - -X - -; K, light air —●—, dark-N_2 - - ● - -.

selectivity of the *Chlorella* cell with regard to the ion pairs K and Na as well as Rb and Na. For this purpose cells containing K and Na were placed in solutions containing 450 meq/liter Na and varying concentrations of K and Rb. The addition of 0.1 meq/liter K or Rb to a 450 meq/liter solution of Na completely prevented any sodium entry into the cell (table III), and in case of K induced an appreciable loss of Na from the cells. Increase in the K concentration to 1 meq/liter confirmed the 0.1 meq/liter results. If the Rb content of the medium is raised to 1 meq/liter, both Na and K are lost from the cells and the Rb entry is greatly increased. Since cells grown in low salt media lost small amounts of K and Na when first placed in the solution containing 450 meq/liter Na, the effect of 0.1 meq/liter Rb on K and Na movements may have been partially masked by this initial ion loss. The magnitude of ionic movements reported in table III is smaller than usually observed with *Chlorella*. This reduction of ion movements by high salt concentration has been observed consistently in all experiments.

Discussion

The metabolically dependent replacement of one ion by another of a different species will be referred to as ion interchange. Since Rb entry exceeds K loss (table II) and the Ca and Mg content of the cells remains constant, ion interchange and net ion accumulation can occur concurrently in the *Chlorella* system. Both processes are inhibited by dark-N_2 conditions. However, net accumulation appears more sensitive to the inhibition of aerobic metabolism than ion interchange. Similar differences in sensitivity to metabolic inhibition have been reported for net accumulation and isotope self-diffusion in *Chlorella* (26). A mechanistic aspect common to ion interchange and isotope self-diffusion is their independence from anion metabolism, and this may explain their lower sensitivity to metabolic inhibition when compared to net accumulation.

Transport studies with isolated cell parts (14, 18, 24) suggest that the over-all selectivity measured is the result of the summation of the different contributions of all cell parts and that the behavior of the cell components are not necessarily similar. Some evidence for cellular heterogeneity has been observed previously in *Chlorella* with respect to sodium (26). In the experiment described here exposure of *Chlorella* cells containing K and Na to a K or K + Na solution lead to the loss of approxi-

mately 50 % of Na in 15 minutes with little further loss after an additional 2 hours (table II). However, if K containing cells are placed in a K + Na solution, Na cannot enter the cell. Similarly Rb, which can almost completely replace K from the cell, replaces cellular Na only to a particular point. Thus Na, whose entry into the cell is prevented by K and Rb, can only be displaced partially from the cell by these ions and Na consequently appears to be trapped in the cell in a compartment possibly preferring Na to K and Rb.

Ion interchange for the ion pairs K − Rb is to a large extent reversible (table II) whereas for the ion pairs Na − K and Na − Rb the process occurs only in 1 direction, the replacement of Na from the cell by K or Rb (table III). The predominant direction of the interchange process can be considered to be the reflection of the selective preference of the cell (6, 7, 13, 15), since in systems permeable to the ions involved, such asymmetric movements are a prerequisite for the maintenance of a characteristic steady state cell composition (2, 7, 28, 29).

The selective order of the alkali metal cations found in the *Chlorella* system, Rb<K<<Na (fig 2, 3) is similar to the ranking observed in most animals, plants, and microorganisms (3, 7, 28). This ranking which follows the order of monovalent cations in Group I of the periodic table is not the only possible one and has been found to be partially or completely reversed in some organisms (2, 28, 29). A similar reversal of the selective order of monovalent cations has also been observed in non-living systems and has been shown to depend on the nature of the anionic charge intensity, the geometry of the matrix holding together the fixed negative charges, and the hydration of the system (6, 10, 16).

It is evident from figure 3 that the rates of K and Rb movements in *Chlorella* are dependent on the concentrations of the 2 ions in the suspending solutions. In kinetic experiments these 2 ions will show definite interactions. Sodium in the outside solution has over a wide range of concentrations no effect on the entry of K (fig 2). In kinetic experiments it would therefore appear that the 2 ions do not interact. But in fact, the presence of K or Rb in the outside solution completely prevents net Na entry into the cell and under most conditions actually induces the loss of Na from the cell. We can therefore conclude that in *Chlorella* the apparent lack of interaction between the external concentration of Na and the entry of K or Rb into the cell is the result of the overpowering preference of the cell for K and Rb, and not a reflection of the lack of interactions.

The magnitude of the discriminatory capacity of the cell as expressed by the separation factor is approximately 1.5 for the ion pair K − Rb, at least 4500 for the ion pair K − Na and between 450 to 4500 for the ion pair Rb − Na. Because of experimental uncertainties, the Rb − Na separation factor could not be approximated more closely. The separation factors for K − Na and Rb − Na exceed those found in better defined organic nonliving systems by a factor of 100 to 1000 and it is therefore not possible at the present to explain selectivity in many organisms without ad hoc assumptions (6, 16, 28, 29). Regardless of the molecular nature of the ion discrimination system, one could state that for a cell system which accumulates ion X in preference to Y, the cell is in a lower energy state when containing X. Since all systems proceed toward the lowest available energy state, ionic movements will occur between cell and environment to replace Y with X whenever possible. These movements, when considering the ions alone, could be against activity gradients and away from an identical ratio of the 2 ions in cell and environment. Selectivity may therefore be considered as a force (2, 6, 13, 15, 16) capable of inducing ion movements.

Such general energetic considerations provide a relatively simple framework for the organization of the experimental data reported. If, for example, Rb is added to a suspension of *Chlorella* containing Na and K or K alone, Rb will tend to replace both K and Na in the cell until the system reaches a minimum energy state. Similarly, K will replace Na in the cell even if Na has to leave the cell against an apparent concentration gradient (table III). Some indication for the existence of this type of phenomenon in *Chlorella* is provided by the observation that in dark-N_2 when the permeability of the cell is greatly reduced, Na leaves the cell in exchange for K and Rb at an appreciable rate (fig 2), but very little K for Rb exchange occurs under similar conditions (fig 3). This suggests that for the ion pairs, Na − K, and Na − Rb the energy of retention is sufficiently different to overcome the increased resistance of the outer cell membrane in dark-N_2. For K and Rb the energy of retention is similar, as a result the energy gradient is insufficient for penetration of the membrane.

Considering the magnitude of the separation factors, it appears necessary to assume that work has to be done by the cell to maintain such a large composition difference between cell and environment (2, 6, 10, 15, 16, 28, 29). This is emphasized by the results in table III, where Na extrusion from the cell occurs into a solution containing 450 meq/liter of Na. The energy input into the discrimination process may be partially the result of the coupling of ion movements to energy yielding chemical and physical reactions or it may have occurred to some extent during the synthesis of the cell and reside in its structural components.

From our present understanding of the chemical and physical properties of cell constituents, this structural selectivity would not be sufficient to explain the observed selectivity of living cells

(11, 16). A possible mechanism for the production of the observed large discrimination ratios would be a repitition of the selection process by low selectivity molecular entities, analogous to chromatography. This possibility has been incorporated into a wide variety of mechanical (9, 12), osmotic (19), and chemical (2, 8, 17, 19, 23, 28, 29) repetitive process transport mechanisms.

Recent demonstrations of sustained synchronized oscillations of proton and cation movements in mitochondrial suspensions (1) together with the reported metabolically dependent structural deformations of organelles (5, 20, 22) and membranes (4, 12, 22) make it attractive to suggest that amplification of ionic discrimination could be provided by reversible shape changes of macromolecular components of membranous structures (9). Such shape changes would result in volume changes (5, 19) or localized membrane displacements which in turn would induce solution flow permitting repeated screening or selection of ions during passage through highly structuralized components of the cytoplasm. It is essential that such shape changes be asymetrical either with respect to time or space, since under steady state conditions an isotropic system would not be able to maintain selectivity ratios in excess of its stationary discrimination capacity.

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