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PASSIVE POTASSIUM ION PERMEABILITY OF *HALOBACTERIUM HALOBIUM* CELL ENVELOPE MEMBRANES

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Summary

Cell envelope vesicles, prepared from *Halobacterium halobium*, were loaded with 3 M KCl, suspended in 3 M NaCl, and the loss of K^+ was followed at various temperatures. The Arrhenius plot of the K^+-efflux rates shows a break at 30°C, with higher energy of activation above the break. This temperature dependence is consistent with earlier studies of chain motions in liposomes prepared from isolated lipids. The efflux of K^+ is more rapid with increasing pH between pH 5 and 7. Since these vesicles do not respire under the experimental conditions it was expected that the K^+-efflux data would be related to the passive permeability of the membranes to K^+. The apparent K^+ permeability at 30°C is $1—2 \cdot 10^{-10}$ $cm \cdot s^{-1}$. This value corresponds to a 5-h half-life for retained K^+ in the envelope vesicles and to a probably much longer half-life in whole cells. The previously observed ability of *Halobacterium* to retain K^+ in the absence of metabolism can thus be explained solely by the permeability characteristics of the membranes.

Non-metabolizing *Halobacterium* cells are known to retain several molar K^+ against large concentration gradients for many hours [1—6]. Two different hypotheses have been proposed to account for this behavior. One of these [1—3,6], assumes that the *Halobacterium* membrane is not a barrier to K^+, and K^+ is retained in the cells because the cation is not free but bound to some cellular components. A number of difficulties arise from this hypothesis; one of these is that the cells do not contain enough anionic groups to bind all the intracellular potassium [6]. The other hypothesis [5] suggests that the *Halobacterium* membrane is passively very impermeable to K^+ and thus the cells can retain large concentrations of free intracellular K^+ without energy expenditure.

Cell envelope vesicles from *Halobacterium halobium* strain R_1, containing

bacteriorhodopsin, have been prepared and characterized [7—11], and they can carry out energetic membrane functions, such as active amino acid transport, when illuminated [7,9,10]. Since during their preparation these vesicles lose most of the cytoplasmic content of the cells, including respiratory substrates, they appear metabolically inert in the dark. Thus, any cation movements into or out of the vesicles should reflect only passive membrane permeabilities. We have attempted, therefore, to follow K^+ efflux from KCl-loaded envelope vesicles, and to relate the results to the properties of the whole cells.

Procedures for preparing *H. halobium* cell envelope vesicles, and for loading them with 3 M KCl by osmotic shock, have been described before [7,9]. Typically, such vesicles were stored overnight in 3 M KCl at 4°C in the dark (membrane protein 0.080 mg/ml) and were centrifuged and suspended in an equal volume of cold 3 M NaCl, containing $1 \cdot 10^{-2}$ M phosphate buffer. Very little extravesicle KCl was carried over with the vesicles and the initial K^+ gradient (K^+_{in}/K^+_{out}) was about 500. The suspensions were then warmed to the desired temperature and were incubated in the dark. Aliquots were withdrawn at equal time intervals, centrifuged, and the pellets were suspended in H_2O. *Halobacterium* membranes break apart in distilled water [12—14], and the K^+ that had been retained in the vesicles was determined with a Perkin-Elmer Model 303 Atomic Absorption Spectrometer.

K^+-release kinetics are shown in Fig. 1A at pH 5.0, at 22, 38 and 45°C. The efflux of K^+ is described by a single exponential of increasing rate with increasing temperature. The Arrhenius plot of first-order rate constants from such curves is broken at 30°C (Fig. 1B), the temperature dependence (activation energy) of the K^+ efflux being greatly increased above this temperature. Studies of the mobility and position of stearic acid-type spin labels in liposomes prepared from *Halobacterium* lipids also showed a general increase in

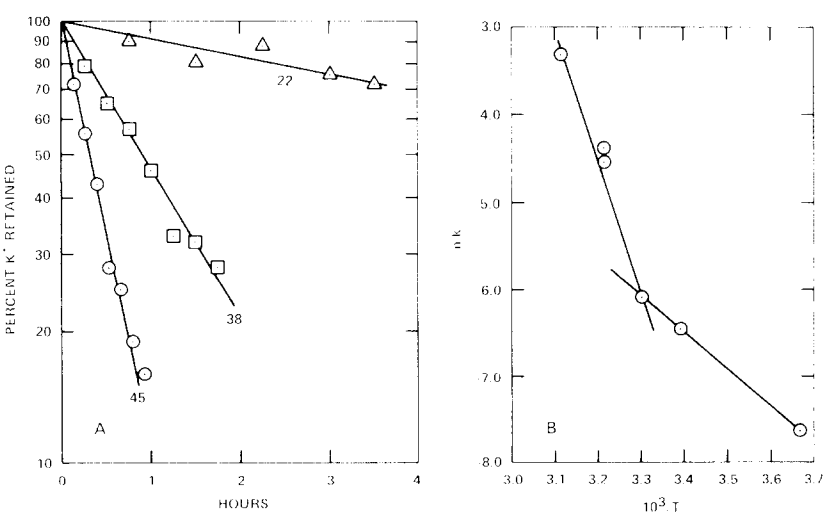

Fig. 1. K^+ efflux in *H. halobium* cell envelope vesicles. (A) Time course of K^+ loss at different temperatures. (B) Arrhenius plot of the first-order rate constant, k, (in min^{-1}), for K^+ loss in experiments similar to those in A.

TABLE I

DEPENDENCE OF K^+ EFFLUX ON pH

Experiments were carried out at 38°C, as described in text. With the half-life of K^+, above pH 6 the efflux kinetics progressively deviate from a single exponential, similarly to the behavior of whole cells [6].

pH	Half-life of K^+ (min)
5.0	55
5.5	44
6.0	25
6.5	23
7.0	18

lipid thermal motions above 30°C [15]. The increasingly rapid efflux of K^+ from the vesicles above 30°C may therefore reflect the behavior of the lipid phase in the membranes.

Table I shows that at pH > 5.5 the rate of K^+ efflux is increased. This is similar to another observation of ours with these vesicles [16], that at higher pH the decay of pH gradients, previously induced by illumination, is more rapid. The efflux of K^+ from the vesicles is not affected by metabolic processes under the conditions used, since the vesicles do not exhibit detectable respiration, and the efflux rates at 38°C are not changed when incubation is with 1 mM KCN present or in a nitrogen-saturated solution (not shown).

The rate of K^+ loss may depend on the flux of other ions, for example because of the need to maintain electrical neutrality across the vesicle membrane. The rate of K^+ fluxes therefore may not reflect the intrinsic permeability of the membranes to K^+ alone. An apparent permeability can be calculated from the efflux data, however. If the vesicles are assumed to be uniform spheres of 500 nm diameter, with an osmotic volume of 3 μl/mg protein [7], the permeability of *H. halobium* envelope membranes to K^+ at 30°C is $2 \cdot 10^{-10}$ cm·s^{-1}. A more accurate method for estimating permeability is from the membrane area of the lipids. Lipid phosphorus was determined in chloroform/methanol extracts of vesicle suspensions [17], and was found to be $4.2 \pm 0.7 \cdot 10^{-7}$ mol/mg protein. The total amount of lipids can be calculated from the known lipid composition of *Halobacterium* membranes [18], with the lipids contained in the purple membrane subtracted since these structures are crystalline [19] and are probably not permeable to K^+. With the assumption that the lipid bilayer thickness is 45 Å, an apparent permeability to K^+ of $1.4 \cdot 10^{-10}$ cm·s^{-1} is obtained. These values are not unusual for biological membranes: for comparison, the K^+ permeability of human erythrocytes is $2.4 \cdot 10^{-10}$ cm·s^{-1} [20]. Such K^+ permeability accounts for a 5-h half-life for K^+ in the vesicles at 30°C. In whole cells, where the surface-to-volume ratio is 3—4 times lower, and where the membranes are probably less leaky than in the vesicles, the half-life of K^+ would be much longer. It appears, therefore, that the permeability properties of the *H. halobium* membrane alone can account for the observed slow efflux of K^+ from whole cells [1—6], and intracellular binding of K^+ need not be postulated. A similar conclusion was made for *Streptococcus faecalis* by Harold and coworkers [21].

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