SODIUM EXCLUSION AND POTASSIUM RETENTION BY THE RED MARINE ALGA, PORPHYRA PERFORATA

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ABSTRACT

Cells of the red marine alga, *Porphyra perforata*, accumulate potassium and exclude sodium, chloride, and calcium. Various metabolic inhibitors including dinitrophenol, anoxia, and *p*-chloromercuribenzoate partially abolish the cells' ability to retain potassium and exclude sodium. Iodoacetate induces potassium loss only in the dark; reduced sulfur compounds offer protection against the effects of *p*-chloromercuribenzoate; dinitrophenol stimulates respiration at concentrations which cause potassium loss and sodium gain.

Following exposure to anoxia potassium accumulation and sodium extrusion take place against concentration gradients. These movements are retarded by sodium cyanide, but are stimulated by light.

Sodium entry, following long exposure to 0.6 M sucrose, occurs rapidly with the concentration gradient, while potassium entry against the concentration gradient takes place slowly, and is prevented by cyanide.

Porphyra perforata J.G. Agardh is a leafy red alga with blades only one cell in thickness. The individual cells, about 40 × 100 micra in size, lack a central vacuole and are embedded in a rubbery matrix consisting largely of galactan sulfate (1). There are no cytoplasmic connections between the cells as there are in higher red algae (2). The alga grows abundantly on rocks in the intertidal zone along the Pacific Coast. The simplicity of the plant and the ease with which it may be handled in the laboratory make *Porphyra* suitable material for permeability studies.

Ulva lactuca, a green marine alga, has been recently studied with regard to its ability to regulate cellular sodium and potassium. A role of metabolism in this aspect of cellular homeostasis has been indicated (3). The present study attempts to determine (*a*) the ionic constitutents of *Porphyra*, (*b*) the ion concentration gradients which exist between the cells and sea water, and (*c*) whether *Porphyra* metabolically regulates its sodium and potassium contents as does *Ulva*.

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Methods

Discs were cut from the *Porphyra* thalli with cork borers of 2 to 3 cm. in diameter, avoiding the fruiting portions at the margins of the blades. The cut discs and collected plants were kept in running aerated sea water before use.

Solutions of the various metabolic inhibitors were prepared in sea water and the pH was adjusted to 7.5 with HCl or NaOH. Sodium and potassium were determined by flame photometry, calcium (4) and amino acid nitrogen (5) by titrimetric methods, and chloride by a modified colorimetric method (6).

The algal tissues were prepared for analysis by (*a*) washing samples of three to five discs each (0.1 to 0.2 gm.) in 0.6 M sucrose for 2 minutes to free them of adhering sea water, (*b*) blotting each disc twice with tissue paper, (*c*) weighing (this weight is called the fresh weight), (*d*) digesting with concentrated nitric acid using heat, extracting with 5 per cent trichloracetic acid, or with boiling water and (*e*) making the resulting extracts up to volume. Flame photometry standards were prepared in trichloracetic acid when it was used for cation extraction. Boiling water was used for chloride and amino acid extraction. The analytical results are expressed as milliequivalents per kilogram fresh weight (m.eq./kg. FW).

All experiments were performed in the dark at an ambient room temperature (15–20°C.) unless otherwise indicated.

RESULTS

Justification of a 2 Minute Sucrose Dip.—Scott and Hayward (3) have shown that washing briefly in 0.6 M sucrose, followed by a triple blot with tissue paper removed the ions of sea water from the extracellular space in *Ulva*. This procedure has been successfully used for *Porphyra* with the substitution of a 2 minute sucrose dip in place of a 30 to 60 second one, and a double blot rather than a triple blot. While 0.6 M sucrose is hypotonic to sea water its use as a rinsing medium may be justified. Estimates based on fresh weight changes indicate that cell volume increases only about 5 per cent due to water influx in passing from sea water to 0.6 M sucrose. It seems unlikely that this water influx would result in gross changes in permeability or transport.

On exposure to sucrose solution the sodium content of *Porphyra* drops abruptly to a nearly constant value in less than 2 minutes (Fig. 1). There is little change in potassium during this time (a 24 hour exposure to the sucrose solution is required to remove half of the potassium of the discs). The ratio of potassium to sodium reaches a maximum at 2 minutes of washing, and thereafter falls slowly. Movements of chloride in this period are similar to those of sodium.

The 2 minute dip seems satisfactory because it removes the salts of the adhering sea water without greatly altering the potassium content of the discs (the remaining potassium is probably cellular). Sodium and potassium contents, as described here, refer to amounts of ions present in the tissue follow-

ing the standard 2 minute sucrose dip (except in Fig. 1 in which various dip times are shown).

Cell Ionic Constituents.—The task of estimating cellular ion concentrations in *Porphyra* is complicated by the adsorption of sodium (40 m.eq./kg. FW) and calcium (10 mM/kg. FW) on extracellular materials with cation exchange properties (probably galactan sulfate) (1). These values are subtracted from the total sodium and calcium measured only in Table I, for comparison with other algae.

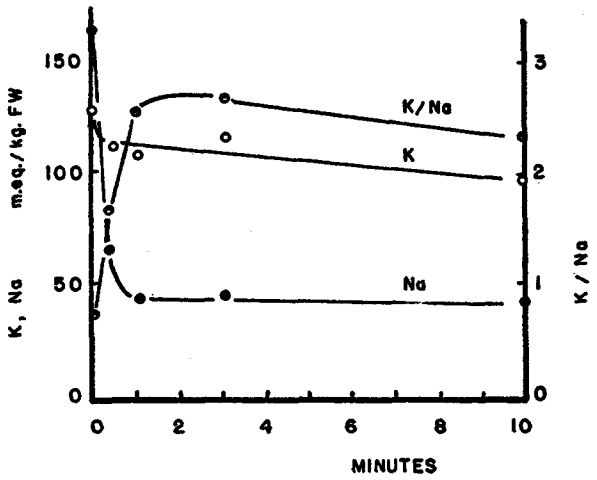

FIG. 1. Variation of the sodium and potassium contents and the K/Na ratio of *Porphyra perforata* with time in 0.6 M sucrose. Units milliequivalents per kilogram fresh weight (m.eq./kg. FW).

The osmotic volume of the *Porphyra* cells is estimated from the difference in sucrose apparent free space values for living (44 per cent) and killed discs (89 per cent), or 45 per cent of the thallus volume, on a fresh weight basis (7) (Table I).

Considerable variability in tissue potassium and sodium among different samples is evident from the various graphs and tables. Potassium contents measured in over 100 samples during a 2 year period ranged from 110 to 300 m.eq./kg. FW, while sodium contents varied between 38 and 110 m.eq./kg. FW. Variation in ion contents of samples taken from a given plant seldom exceeded 20 per cent, however. Much of the variability among different plants appears to be due to variation in the thickness of the blades. Thick discs (Table II), with greater fresh weight per unit area, show lower potassium, higher sodium, and greater sucrose apparent free space values than do thin discs of the same area. This observation supports the idea that most of the

sodium is bound in the extracellular mucilage while potassium predominates as the cellular cation in *Porphyra*. Actual cellular sodium contents are, however, uncertain because cell volumes and extracellular bound sodium were not separately determined for each sample.

TABLE I

Cellular Ion Constituents of Porphyra, Ulva, and Several Coenocytic Marine Algae

Alga	Units	K	Na	Ca	Cl	Source
Valonia ventricosa	M.ea./liter sap	576	35	Trace	608	(16)
Valonia macrophysa	M.eq./liter sap	500	90	1.7	597	(17)
Halicystis osterhoutii	M.eq./liter sap	6.4	557	8	603	(18)
Halicystis ovalis	M.eq./liter sap	349*	235*	—	600*	(19)
Ulva lactuca	M.eq./kg. tissue water	310	192	—	—	(3)
Porphyra perforata	M.eq./liter cell volume	482	51‡	0‡	81§	Present study

* The original data were expressed as per cent total halide. Above values are based on an assumed halide content of 600.

‡ Values corrected for amounts bound extracellularly.

§ Other anions present include PO_4, 2.5, SO_4, trace, NO_3, 69 to 148 (20), free α-amino acid nitrogen, 81 m.eq./liter cell volume. Amino acids found before hydrolysis include cysteic acid, aspartic acid, glutamic acid, threonine, proline, phenylalanine, leucine, and isoleucine (21).

TABLE II

Sodium and Potassium Contents and Sucrose Apparent Free Space Values (AFS) of Thick and Thin Discs of Porphyra perforata

K and Na in units m.eq./kg. FW.

	Plant No.	K	Na	AFS
				per cent
Thin discs	1	201	50.2	35.6
		205	58.5	
	2	187	55.3	43.6
		179	58.5	
Thick discs	3	116	81.6	46.0
		144	75.5	

The average sum of the cell cations determined is about 0.53 M. This value is roughly that of the concentration of cations in sea water, but sodium is excluded from the cells by a factor of 10 while potassium is accumulated some 40 times that of sea water.

Including the free α-amino acids determined, only 0.23 to 0.31 M anions have been accounted for. The apparent anion deficit may represent negatively charged cytoplasm, as has been suggested for certain root cells (8). If so, a Donnan equilibrium may be involved in maintaining a high cell potassium content, although the ratios Cl_o/Cl_i (6 to 7) and K_i/K_o (40) are not equal.

TABLE III

Effects of Sodium Cyanide on Sodium and Potassium Contents of Porphyra perforata

K and Na in units m.eq./kg. FW.
24 hour exposures (dark).

	NaCN concentration	K	Average	Na	Average
	m/liter				
Experiment 1	0	115		63.5	
		133		63.2	
		129	126	61.6	62.3
	0.0001	132		68.6	
		150		65.2	
		137	140	65.2	66.3
	0.0003	118		73.8	
		118		70.8	
		146	127	71.2	71.9
	0.001	139		68.8	
		125		75.7	
		157	140	70.3	71.6
Experiment 2	0	200		70.0	
		193	196	72.4	71.7
	0.005	166		92.2	
		177	171	87.8	90.0

Effects of Inhibitors on Potassium Retention and Sodium Exclusion

(*a*) *Cyanide.*—Sodium cyanide effects a loss of potassium and a gain of sodium only at the highest concentration used (Table III). That the lack of cyanide inhibition was not due to its failure to enter the cells is suggested by its inhibition of potassium uptake and sodium extrusion following anoxia (see Table VI).

(*b*) *Sulfhydryl Inhibitors.*—Iodoacetate causes a marked loss of potassium in the dark, but no significant gain of sodium (Table IV). Light largely prevents this loss. Respiration is only partially inhibited by iodoacetate in the dark, which may account for the selective loss of potassium (if it is assumed

that the magnitude of the concentration gradients of potassium and sodium determines the extent of inhibition). Sodium arsenate and reduced sulfur compounds did not relieve the iodaoacetate-induced potassium loss.

Sodium arsenite and *p*-chloromercuribenzoate (Table IV) cause potassium loss and sodium gain in both light and dark. Thioglycolate and reduced glutathione relieved the *p*-chloromercuribenzoate inhibition.

TABLE IV

Effects of Various Sulfhydryl Inhibitors on Sodium and Potassium Contents of Porphyra perforata

Units milliequivalents per kilogram fresh weight (m.eq./kg. FW).
± values represent standard deviations from the means.

Inhibitor	Concentration	Conditions	K	Na	No. of samples
Iodoacetate	0	Light and dark	183 ± 24	77.8 ± 11.7	12
	0.001 M	Light	176 ± 14.5	69.6 ± 13.7	6
	0.001 M	Dark	128 ± 10	76.9 ± 9.1	11
Arsenite ($NaAsO_2$)	0	Light and dark	211 ± 39	59.3 ± 2.9	8
	0.001 M	Light and dark	89.7 ± 23.4	74.0 ± 5.0	8
p-chloromercuribenzoate (PCMB)	0	Light and dark	224 ± 5	60.2 ± 3.0	4
	0.0001 M	Dark	170 ± 3	70.7 ± 3.0	2
	0.0005 M	Light and dark	100 ± 18	82.9 ± 2.3	6
PCMB plus thioglycollate	0.0001 M plus 0.005 M	Dark	203 ± 9	68.4 ± 1	2
PCMB plus glutathione	0.0005 M plus 0.005 M	Dark	206 ± 0	63.6 ± 1	2

(*c*) *Dinitrophenol.—Porphyra* responds in a typical manner to dinitrophenol (DNP). Respiration is stimulated some 70 per cent at a DNP concentration of 0.1 mM which causes considerable loss of potassium and gain of sodium (Fig. 2). Oxidative-phosphorylating respiration is apparently required for potassium retention and sodium exclusion in *Porphyra* as well as in other tissues (9, 10).

Effects of Light and Dark on Growth and Ion Retention.—Porphyra tissues kept in light or dark up to 17 days show no differences in sodium and potassium concentrations. Growth occurs only in the illuminated discs, however (Table V). It is important that ion retention and transport may be studied in the absence of growth simply by keeping the plants in the dark.

Anoxia.—Under a nitrogen atmosphere in the dark *Porphyra* loses potassium and gains a smaller amount of sodium (Fig. 3). Exogenous glucose does not prevent the potassium loss and sodium gain, although in some cases an

increase in the acidity of the medium was noted, suggesting fermentation (probably by bacteria present and not by *Porphyra*).

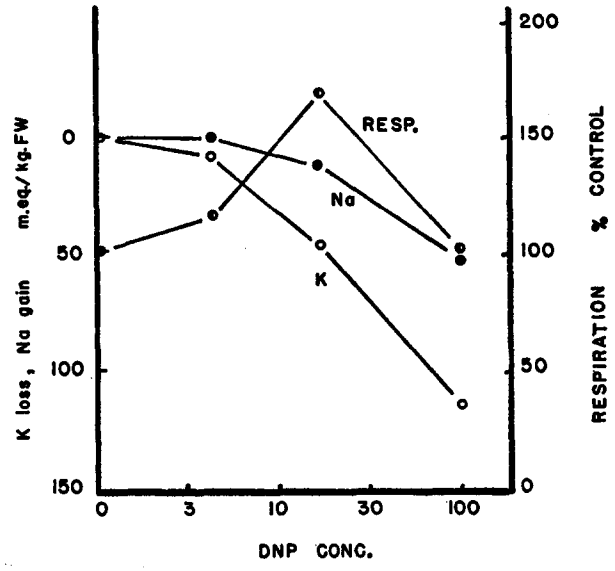

FIG. 2. Effects of dinitrophenol on sodium and potassium contents and rates of oxygen consumption of *Porphyra perforata*. DNP concentrations M $\times 10^{-5}$.

TABLE V

Sodium and Potassium Contents of Porphyra perforata Kept in the Light and Dark in Running Sea Water. Averages of Duplicates

Treatment	Time	K		Na	
		Total m.eq./sample	M.eq./.kg. FW	Total m.eq./sample	M.eq./kg. FW
	days				
Light	0	44.9	138	23.6	73.0
Light	5	70.0	145	31.7	66.4
Light	10	91.3	135	44.0	65.0
Light	15	112	141	61.8	73.0
Dark	5	56.3	152	22.2	64.2
Dark	10	50.1	159	24.9	77.1
Dark	15	45.0	124	21.4	58.9

Potassium Accumulation and Sodium Extrusion after Anoxia.—The effects of anoxia are reversed by passing air through the medium; potassium uptake and sodium extrusion take place against concentration gradients for both ions (Table VI). Both processes are stimulated by light, although light is not

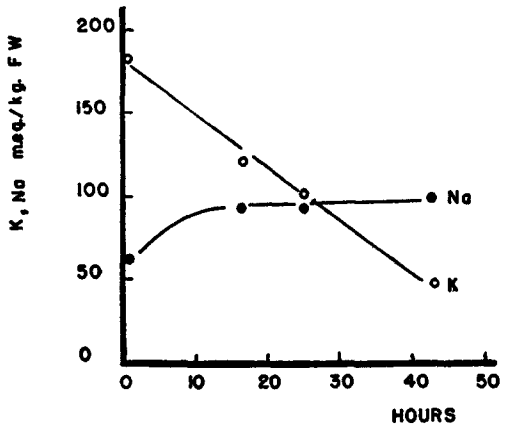

FIG. 3. Potassium and sodium contents of *Porphyra perforata* during anoxia in the dark. Average values.

TABLE VI

Potassium and Sodium Contents of Porphyra perforata Following Prolonged Exposure to Anoxia

Experiment in light, dark, and dark with 0.001 M NaCN. K and Na in units m.eq./kg. FW

Treatment	K	Average	Na	Average
After 48 hrs.' exposure to anoxia	51.6 56.1	53.8	93.0 106	99
After admitting air				
Light 3.75 hrs.	160 118	139	77.2 73.8	75.5
6.5 hrs.	145 161	153	62.9 66.0	64.4
Dark 3.75 hrs.	89.0 85.2	87.1	90.1 78.4	84.2
6.5 hrs.	93.0 102	97.5	85.2 92.2	88.7
Dark with NaCN 6.5 hrs.	46.1 45.1	45.6	94.6 102	98.3

absolutely necessary. Preliminary results suggest that the light stimulation is due to the increased removal of carbon dioxide from the cells in the light. Samples in the light with CO_2-free air bubbling showed rates of uptake and extrusion identical to those in the light with air bubbling, but 5 per cent CO_2–air completely inhibited transport in the dark.

Although sodium cyanide (0.001 M) had little effect on retention and exclusion, potassium uptake and sodium extrusion are largely abolished (Table VI).

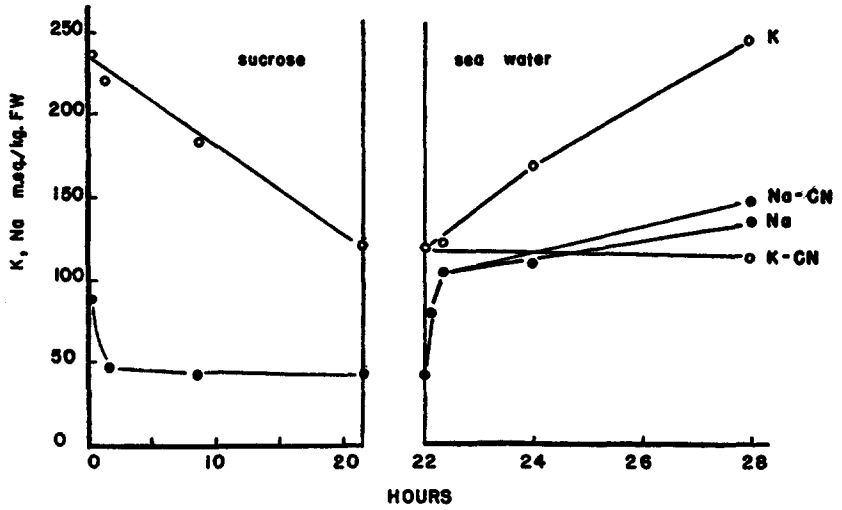

FIG. 4. Leaching of potassium and sodium from *Porphyra perforata* during a 22 hour exposure to 0.6 M sucrose and the subsequent accumulation of potassium and entry of sodium on returning the tissues to sea water. 0.001 M NaCN was added to the sea water in one series. Dark throughout.

Potassium Accumulation and Sodium Entry after Sucrose Leaching.—Cell potassium and sodium are reduced to low values by long exposure to 0.6 M sucrose (Fig. 4). The sodium remaining after a few hours (a constant value) is due to extracellular adsorption and occurs in living and killed tissues alike.

Samples leached in sucrose slowly accumulate potassium but show immediate increases in sodium on returning them to sea water (Fig. 4). Potassium entry, against the concentration gradient, is blocked by 0.001 M sodium cyanide, but sodium entry, with the concentration gradient, is not inhibited.

DISCUSSION

Several features were noted which make *Porphyra* especially good material for permeability studies. Both maintenance and the formation of ion concentration gradients may be studied, and these studies may be undertaken in

the absence of growth by keeping the plants in the dark. The morphological simplicity of the plant; its monostromatic nature, the separation of vegetative and fruiting cells on the blade, the large size of the cells, and their lack of a vacuole are also advantageous.

Unlike terrestrial and fresh water plants and vacuolated, coenocytic marine algae, *Porphyra* cells exclude chloride and sodium while they selectively accumulate potassium (see Table I). This situation may also exist in *Ulva* (3). The similarity in the distribution of ions and the concentration gradients maintained by *Porphyra* and many animal cells, such as nerve (11, 12), muscle (13, 14), and red blood cells (15) may be noteworthy.

The reduction in magnitude of the ionic gradients of *Porphyra* by inhibitors suggests that metabolic energy is required for their maintenance. Both potassium retention and sodium exclusion are dependent upon the presence of oxygen and upon oxidative-phosphorylating respiration. Potassium accumulation following anoxia or sucrose leaching, and sodium extrusion following anoxia, are also metabolically mediated as indicated by cyanide inhibition and by light stimulation.

Because of the possibility of a high non-diffusible cell anion content a Donnan equilibrium cannot be completely ruled out as a mechanism for potassium retention. The differences in Cl_o/CL_i and K_i/K_o ratios, the lack of a one-to-one exchange of sodium for potassium during exposure to inhibitors, and the slow entry of potassium into low potassium cells do not support this suggestion, however.

Clearly, either potassium accumulation or sodium extrusion takes place against both a concentration and a potential gradient (if one exists), and thus at least one of these ions may be actively transported. Active transport in *Porphyra* must be limited to entry or extrusion from the cytoplasm because no vacuoles are present.

Separate effects of light in stimulating potassium and sodium transport in *Ulva* have been suggested (3): light promotes potassium retention *via* photosynthesis, and promotes sodium exclusion *via* some more "direct reducing effect." The latter has been cited as evidence for the operation of a redox mechanism of sodium transport. No direct reducing effects of light have been observed in cobalt (22) or in cesium (23) uptake by the red alga, *Rhodymenia*, however. Cesium uptake occurs in the light only if CO_2 is present, and cobalt uptake, mediated by light, apparently involves light alteration of cell CO_2 concentrations. Preliminary experiments indicate that the latter situation may also be involved in the light stimulation of potassium uptake and sodium extrusion, following anoxia, in *Porphyra*. Since no experiments were performed with *Ulva* to determine the role of carbon dioxide concentration in the light effects observed, the suggestion of a "direct reducing effect" of light may not be justified, considering the known inhibitory effects of high CO_2 concentration on ion accumulation (24).

A role of sulfhydryl groups in ion transport suggested by Lewin (25) for silicon incorporation in diatoms, and in hemolysis of red blood cells (26) is corroborated by the inhibitory effects of arsenite and *p*-chloromercuribenzoate on potassium retention and sodium exclusion in *Porphyra*. LeFevre's suggestion (26) that a sulfhydryl-containing enzyme involved in phosphorylation may be inactivated by these inhibitors is given further importance by recent reports of low P/O ratios obtained in cell-free mitochondrial systems in the presence of sulfhydryl inhibitors (27).

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