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THE MOVEMENT OF H^+ AND OTHER IONS AT THE ONSET OF PHOTOSYNTHESIS IN ULVA

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SUMMARY

A rapid extrusion of Na^+ from Ulva, induced by light, also occurs with other cations, as evidenced by $^{86}Rb^+$ and $^{85}Sr^{2+}$ tracer studies; anion movement ($^{36}Cl^-$, $^{35}SO_4^{2-}$ and [^{14}C]acetate) is not affected by light. The Na^+ flux has a pH optimum of about pH 8, and has a time constant similar to that for the movement of H^+ and HCO_3^- concerned in photosynthesis. Substitution experiments indicate that the only externally added ion necessary for the short-term light-induced ion movements is HCO_3^-.

INTRODUCTION

Proton movement has been shown to have a particularly significant role in photosynthesis of chloroplasts[1,2]. However, the mechanisms need further study: *e.g.*, investigation of *Rhodospirillum rubrum* chromatophore-induced pH changes, rather than dye and electrode methods that require different interpretations[3]. Most other studies on ion movements have been made on chloroplasts[4], and more understanding is needed of the relationship of H^+ to movements of other ions within the plant cell. Ulva, because of its planar surface and two-cell thickness, is a particularly useful organism on which to study questions concerning the role of H^+ movement in photosynthesis.

Light has been shown to evoke rapid movements of Na^+ in Ulva[5]. In other algae, substitution in the ionic environment can affect metabolism[6,7], and light changes the net ion uptake over a period of several hours[8,9]. An alkaline pH will slow the photosynthetic activity of littoral marine algae, but Ulva shows more tolerance to pH 10 than other algae[10]. In an earlier study[5], it was suggested that the movement of Na^+ from Ulva as induced by light, had a relationship to the inward movement of protons that occurs with photosynthesis. In this paper, we will demonstrate that this movement is not specific to Na^+, but also occurs with several cations. Other data suggest that such H^+ changes might be coupled to the movement of HCO_3^-, which supplies CO_2 for the dark reactions of photosynthesis.

METHODS

Ulva lobata or *Ulva expansa* were obtained either in San Francisco Bay or Monterey Bay and maintained in a recirculation aquarium system described elsewhere[11]. The apparatus and methods used in determination of rate constants have been described in detail earlier[5]. Briefly, the tracer was added to one side of the apparatus, which holds the algae between two 100-ml compartments containing sea water, and cooled to 15°. Each compartment was sampled with a 100-μl pipette, and the activity determined in a Packard Series 410A γ-spectrometer for ^{22}Na, ^{86}Rb, ^{137}Cs, and ^{85}Sr, and in a Packard Model 4000 Tri-Carb for ^{36}Cl, ^{14}Cl, and ^{35}S using a scintillant mixture designed for sea water counting[12]. The rate constants for each isotope were calculated according to a standard equation for exponential appearance[5].

Rapid changes in H^+ movements were determined by wrapping the algae tightly around a Sargent Model S-30070-10 combination electrode and securing it with a rubber band. Experiments were performed in sea water, in a black plastic chamber with a window and with a hole on top for the electrode. Of numerous combinations tried, this procedure allowed for the most rapid and sensitive measurement of H^+ movement. Changes in H^+ were measured directly as a potential difference in mV across the combination electrode, rather than in pH units. Most experiments were monitored for 5 min, the time required to reach 95% of the steady-state response at constant pH of 7.8. For measuring diffusion of H^+ across Ulva, a pair of H^+ combination electrodes was placed on either side of the two compartment apparatus described above, and 100 ml of 4 M HCl were added to bring one side to approx. pH 3.5. Potential differences for each combination electrode were amplified by means of a Keithley Model 300 operational amplifier and monitored through a Honeywell digital voltmeter and digital recorder.

The light source for determination of rate constants was a 200-W quartz–iodine lamp with a calibrated irradiance (6500 Å) of 9.3 μW/cm^2, at the algae surface. For other purposes of illumination a 400-W Lucalox lamp was used at 20 cm.

RESULTS

Table I summarizes the results of compartment studies on the light and dark rates of some anions and cations. Three anions, $^{36}Cl^-$, $^{35}SO_4^{2-}$ and [^{14}C]acetate show no light effect, and their dark diffusion rates across the Ulva membrane are lower than that of the cations. SO_4^{2-} has significantly slower dark and light rates than does Cl^-, as would be expected if the stronger negative change of SO_4^{2-} were repelled by the negatively charged cell wall of Ulva. The cations in general move more rapidly across the algae than anions, as can be seen by comparing their dark rate constants of $15 \cdot 10^{-4}$ and $6 \cdot 10^{-4}$, respectively. Moreover, $^{86}Rb^+$ and $^{85}Sr^{2+}$ showed an increase in flux due to light similar to that of Na^+ reported previously[5]. As measured by the rate constant, this 3- to 4-fold increase in ion movement indicates that an active process is involved; however, the increase is not specific to a particular cation.

The empirically fitted rate constants above were useful in describing the appearance of tracer under dark or light conditions. One must note, however, that dark movement represents a diffusional transport of tracer, while light movement represents an extrusion of tracer from the tissue. Thus, movement of cations induced by

TABLE I

CHANGES IN THE RATE CONSTANTS FOR MOVEMENT OF SOME TRACER ANIONS AND CATIONS WITH LIGHT

Based on exponential appearance of tracer through an Ulva tissue membrane[5]. Tracer was injected on the distal side of the two-compartment chamber. Radioactivity was sampled at 15-min intervals during 60 min in dark, then at 1-min intervals during 5 min in light.

	n	*Rate constant* (min^{-1})	
		Dark *0–60 min*	*Light* *60–62 min*
Cations			
$^{22}Na^+$	18	$(15 \pm 1) \cdot 10^{-4}$	$(62 \pm 9) \cdot 10^{-4}$
$^{86}Rb^+$	4	$(18 \pm 2) \cdot 10^{-4}$	$(69 \pm 7) \cdot 10^{-4}$
$^{85}Sr^{2+}$	6	$(8 \pm 1) \cdot 10^{-4}$	$(21 \pm 4) \cdot 10^{-4}$
Anions			
$^{36}Cl^-$	10	$(6 \pm 10) \cdot 10^{-4}$	$(7 \pm 10) \cdot 10^{-4}$
$^{35}SO_4^{2-}$	5	$(2.5 \pm 2) \cdot 10^{-4}$	$(3.5 \pm 3) \cdot 10^{-4}$
[^{14}C]Acetate	4	$(1.2 \pm 2) \cdot 10^{-4}$	$(2.5 \pm 3) \cdot 10^{-4}$

TABLE II

FACILITATION BY LIGHT OF $^{137}Cs^+$ EXCHANGE FROM ULVA IN RESPONSE TO IONIC RINSE

Sections were incubated for 18 h at 15° in presence of $^{137}Cs^+$ tracer, rinsed for 30 sec in sucrose or ionic medium as indicated above, incubated again in sea water for 5 min in light or in dark, then counted.

		Radioactivity per disc (counts/min)	
		0.82 M sucrose rinse	*Sea water + 0.5 mM CsCl rinse*
Dark		20 100	15 100
		18 600	17 500
		17 500	17 600
	Mean	18 700	16 730
Light		16 400	8 240
		13 500	7 740
		19 600	9 760
	Mean	16 500	8 580

light differs fundamentally from through-transport in dark. This was shown previously by the observation that tracer was extruded transiently during light from the tissue to both baths[5]. The extrusion is better shown in Table II, where discs of Ulva were presoaked for 18 h in $^{137}Cs^+$, then eluted in light or in dark. Clearly brief light exposure significantly reduced the tissue content of $^{137}Cs^+$. As expected, there was little difference between light and dark for sucrose elution, since sucrose solution provided no exchangeable ions.

The extrusion of $^{22}Na^+$ from Ulva, affected by light, is pH dependent, as demonstrated by Fig. 1. From pH 3 to 9, the dark rate constant for Na^+ movement across Ulva remains the same. However, the short-term enhancement by light of Na^+ movement is drastically reduced on either side of a maximum at pH 8; the normal pH

for sea water. At pH 3 both the light and dark rate constants are the same. These results indicate that the cation extrusion is related to photosynthesis.

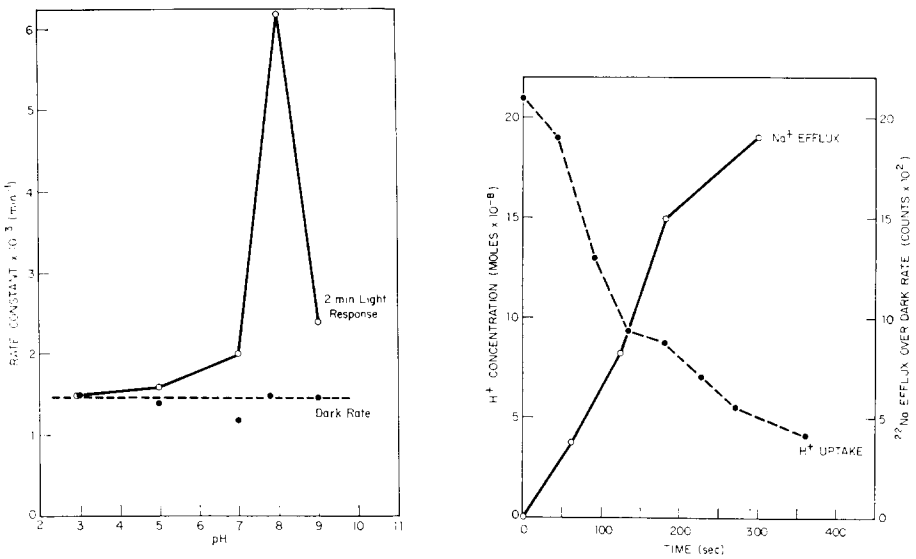

Fig. 1. pH optima of dark (●---●), and light (○—○) rate constants. Ulva was dark adapted for 18 h and pH was adjusted immediately before experiment. $^{22}Na^+$ was used as a tracer to determine the rate constant for two-compartment transfer.

Fig. 2. Comparison of H^+ (●---●) and $^{22}Na^+$ (○—○) movements in Ulva as induced by light. At each time interval, Na^+ increments represent mean of three determinations, as radioactivity measured *minus* radioactivity predicted for dark (diffusional) transport; the latter amounted to only ¼ of the radioactivity due to light. H^+ decrements were determined as the mV change in potential on a combination glass electrode, around which Ulva tissue was wrapped.

In Fig. 2, we examined the time courses for Na^+ extrusion and for proton uptake independently with onset of photosynthesis. This was done with a wrapped combination electrode, as described earlier. Although the experimental situation is quite different from that for determination of rate constants, both ion movements are rapid for 3 min, and decrease thereafter. Ordinarily the proton movement may go on for a longer time, but because Ulva is wrapped around the electrode, the amount of H^+ may become limiting after pH 9; beyond 9.5, the rate of proton movement is very slow. In results not presented, the H^+ movement is also inhibited at pH 5 and 3. Proton movement seems to show a pH relationship similar to that of Na^+ movement as was depicted in Fig. 1, *i.e.*, it is slower on either side of a pH optimum. One should note that our method of measuring H^+ concentration allowed small changes to be determined more or less independently of the pH of the surrounding medium. The most reasonable explanation for this is that buffering capacity of the sea water between the algae and the glass electrode is small compared to that of the Ulva surface, so that apparently we are measuring the pH in equilibrium with the outer cell wall. When the pH is above 6.3, CO_2 is not soluble, resulting in inhibition of the photosynthetic process. At a basic pH, either CO_2 or H^+ may be limiting. However, it is difficult experimentally to separate the movements of HCO_3^- and H^+ in Ulva by

the procedures employed in this paper, so that HCO_3^- movements could not be measured directly.

For through-transport of tracers under dark conditions, Fick's first law of diffusion ought to be reasonably applicable for this experimental situation because of the planar surface, and thinness of the Ulva tissue, and because of the experimental configuration. In Table III diffusion coefficients were calculated from concentration

TABLE III

COMPARISON OF DIFFUSION COEFFICIENTS FOR THROUGH-TRANSPORT OF PROTONS AND OTHER IONS ACROSS ULVA; DARK CONDITIONS

Diffusion coefficient: $D = (\mathrm{d}S_A/\mathrm{d}t)\ (1/A)\ (l/C_A)$ where $A = 11.3\ cm^2$; $l = 100\ \mu$; $\mathrm{d}S_A/\mathrm{d}t$ was based on time rate of change as shown in Fig. 2 for a 60-min interval. For protons: $\mathrm{d}S_A/\mathrm{d}t$ measured directly. For Na^+ and Cl^-: $\mathrm{d}S_A/\mathrm{d}t = (\mathrm{d}L_A/\mathrm{d}t) \cdot (1/X_A)$; *e.g.*, $(1.5 \cdot 10^{-3}$ counts/min $^{22}Na^+)/(1$ min$) \cdot (0.45$ moles $Na^+)/(1$ count/min $^{22}Na^+)$. Symbols as defined in ref. 5.

Ion	*Diffusion rate,* dS_A/dt *(μmoles/min)*	*Concn.,* (C_A) *(M)*	*D* *(cm²/min)*
Na^+	0.80	0.45	$1.3 \cdot 10^{-3}$
H^+	0.8	$0.71 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$
Cl^-	3.18	0.53	$0.5 \cdot 10^{-3}$

TABLE IV

HCO_3^- REQUIREMENT FOR PHOTOSYNTHETIC RELATED PROTON MOVEMENT

Three representative experiments with Ulva wrapped around glass electrode; in each experiment there were successive changes in bathing medium. Change in electrode potential (mV) corresponds either to removal by Ulva of H^+ from the medium during light (1st column) or to return of H^+ during dark (2nd column).

	Change in potential 5 min light (mV)	*Change in potential 5 min dark (mV)*
Expt. I mannitol substitution		
Sea water (pH 7.8)	+48	−46
0.5 M mannitol, 1 mM choline bicarbonate (pH 7.8)	+68	−83
0.5 M mannitol, 1 mM Tris (pH 7.8)	+17	−14
Expt. II choline substitution		
Sea water (pH 7.8)	+43	−40
0.5 M choline chloride, 1 mM choline bicarbonate (pH 7.8)	+51	−37
0.5 M choline chloride, 1 mM Tris (pH 7.8)	+16	−13
Expt. III artificial sea water		
Sea water (pH 7.8)	+50	−41
ASP2 (NTA)* (pH 7.8; 0.28 mM $NaHCO_3$)	+19	−18
Instant ocean** (pH 7.8; 2.4 mM $NaHCO_3$)	+45	−47
0.5 M NaCl (pH 6.7)	+14	−23

* Ref. 13.
** Ocean Systems Inc.

differentials, which were quite large. The net rate of change of material was determined by $^{22}Na^+$ tracer for stable Na^+, and by H^+ electrode for protons. There seemed to be no essential difference between dark rates of diffusion for either the ions or the protons. Thus both H^+ and Na^+ diffuse across Ulva by similar mechanisms, probably by exchange diffusion. The observation that Cl^- diffuses at a slower rate, further supports the inference that exchange diffusion is an appropriate model for H^+ and Na^+ movements.

H^+ uptake due to light can be lowered by substitutions in the media (Table IV). However, this effect seems primarily to be a depletion of a limiting concentration of HCO_3^- needed for photosynthesis. Both mannitol and choline can depress the H^+ response, but this effect can be overcome by adding HCO_3^-. Other externally added anions or cations appear not to be necessary for the rapid H^+ movement. It also appears that several artificial sea waters (only data for ASP2 are shown) have too little HCO_3^- for a satisfactory H^+ response; this, in turn, may limit culture of algae. Another artificial sea water (Instant Ocean) seems quite satisfactory. Movement of H^+ is reversed when light is turned off, as can be seen from the second column of Table IV; *i.e.*, the surface of Ulva returns to its original H^+ concentration.

Fig. 3 indicates that increasing graded concentrations of HCO_3^- in mannitol solution can result in a saturation response for proton uptake. In choline solution, increasing proportions of sea water also give a linear response obviously because of the HCO_3^- present in sea water. In mannitol solution, a saturation response is approached only above 5 mM concentrations of HCO_3^-. This, in part, may reflect diffusion of CO_2 from the medium. However, it is probable that in many ocean conditions, the concentration of HCO_3^- is rate limiting for Ulva growth. These experiments

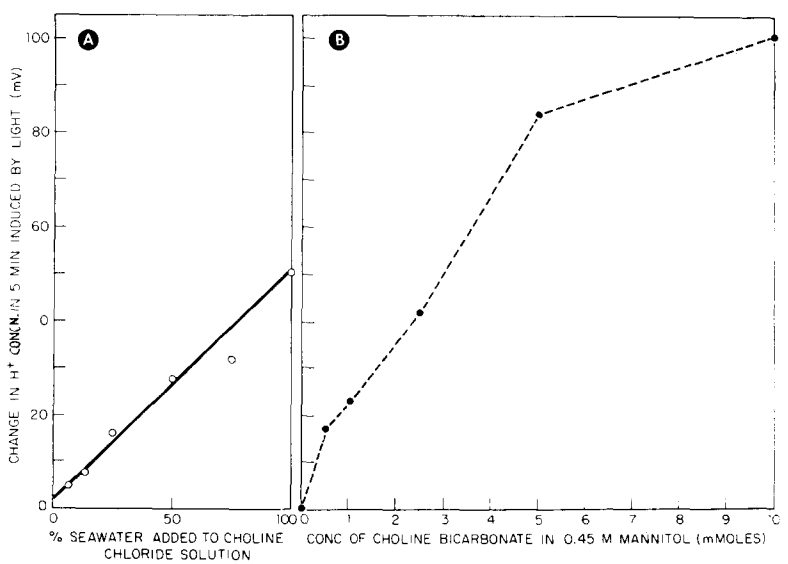

Fig. 3. Effect of HCO_3^- on the H^+ response: (A) substitution of choline chloride solution in lieu of normal sea water (containing HCO_3^-); (B) substitution of $NaHCO_3$ solution for pure mannitol solution (no HCO_3^-). Each point is the overall 5-min response to a Lucalox light source as measured in mV, at a particular concentration. The same wrapped algal preparation was used throughout A or B; the initial pH surrounding sea water was 7.8.

indicate three things: that HCO_3^- concentration can be rate limiting in some experimental situations with Ulva, that external HCO_3^- is needed for an inward movement of H^+ in response to light, and that both ions accompany each other in the light response.

DISCUSSION

Table I shows that the light-activated Na^+ flux reported previously[5], is not dependent on any particular cation studied. The lack of cation specificity may be the result of either a nonenzymatic process, probably cation exchange within the anionic mucopolysaccharide matrix of the cell wall of Ulva[14], or the movement of internal ions from the chloroplasts[4]. Either concept is reinforced by the fact that among the three anions studied (Cl^-, SO_4^{2-}, and acetate), there is no significant change in flux with the onset of light, and the demonstration that only another cation will remove absorbed Na^+ (ref. 5). It is probable, as seen by the comparison of rates in Fig. 2, that proton movement involved in photosynthesis is the active component of the light effect, and that as protons move to the interior of the cell, there is a requirement for HCO_3^-.

At the same time, Table II shows that there is an outer movement of ions, probably K^+ as shown by the $^{137}Cs^+$ tracer. However, it should be noted that since there is little exchange in the sucrose solution, an external exchangeable ion such as HCO_3^- is needed. Since chloroplasts have been shown to undergo conformational changes with light[15,16], it is also possible that the chloroplasts of Ulva, at the same time, undergo related changes[17].

Studies on chloroplasts[1,2] have shown that light induces H^+ movement into the chloroplast, producing a proton gradient. According to the chemiosmotic hypothesis of MITCHELL[18,19], the proton gradient maintains the chemical potential necessary for photophosphorylation. In Ulva, the chloroplasts cover the area immediately within the cell wall. When light is turned on, there may arise a proton gradient reaching equilibrium within a few minutes across, not only the chloroplast, but the entire cell wall. This corresponds to the time for maximum photosynthetic induction as measured by O_2 evolution[20]. The process is reversible, because when light is turned off, the surface of the algae returns to the original pH within the same time (Table IV), as has been shown previously[21].

In our determinations, we did not measure HCO_3^- directly. However, in cells of whole algae, CO_2 fixation seems a necessary part of the movement of protons, as evidenced by the requirement for HCO_3^- (Fig. 3 and Table IV). The cation movement induced by light is also HCO_3^- dependent. As seen from Fig. 1, there is inhibition at pH 3 and 5, where CO_2 is not very soluble. The reason for the inhibition of the Na^+ flux on the alkaline side is more difficult to elucidate, since it can be interpreted either as a lack of H^+ for chemiosmotic coupling, or as a loss of CO_2 for the dark reactions of photosynthesis. Ulva, at an alkaline pH, survives better than most marine algae, and this has been attributed to its ability to utilize HCO_3^- (ref. 10). Alternatively, this utilization may be due to the ability of Ulva to maintain a more efficient proton gradient across its cell wall.

The rapid H^+ movement does not require any other externally added ion besides HCO_3^-. All other ions can be replaced, as shown in Table IV by substitution of Tris–

mannitol, Tris–choline buffer, or pure NaCl. Only HCO_3^- as the major ion at pH 7.8, is necessary. Possibly the proton moves with HCO_3^- thus conserving charge and implying a function of the proton pump in co-transport, as has been proposed with other anions in chloroplasts[22]. This may also have some relevance to the observations that organic acids, stronger than carbonic, have been shown to affect conformational changes[23,24]. The movement of HCO_3^- does not conflict with the chemiosmotic coupling theory[19], since a proton gradient is maintained.

These results indicate that the effect of light on transport of ions in Ulva is concerned primarily with proton movement which is at least dependent on, and possibly coupled to movements of external HCO_3^-, concerned in CO_2 fixation. There is also a displacement of other cations from both within the cell (notably the chloroplast; ref. 4) and bound to the cell wall (see Table II), creating a proton gradient from the interior of the algae to the surrounding sea water. The relationship between cation and anion (HCO_3^-) movement seems to require further experimentation. Similar ion-exchange mechanisms, as indicated by the ability of other algae to absorb ions[25], could also be concerned with maintaining the algae against the high saline conditions of its environment, and the assimilation of nutrient ions.

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