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RELATION OF SWELLING AND PHOTOPHOSPHORYLATION TO LIGHT-INDUCED ION UPTAKE BY CHLOROPLASTS *IN VITRO*

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SUMMARY

1. The effect of chloroplast swelling on light-induced sodium and strontium uptake and the relation of the energy-transfer pathway to light-induced strontium uptake in spinach (*Spinacia oleracea*) chloroplasts was investigated.

2. Light-induced swelling of spinach chloroplasts was required for sodium uptake and greatly enhanced strontium uptake. Sodium uptake may occur by an exchange for potassium. A rapid light-stimulated and a gradual light-independent release of magnesium was observed.

3. No light-induced ion uptake or swelling was found for *Porphyra yezoensis* or *Euglena gracilis* var. bacillaris chloroplasts, the latter actually shrinking in the light.

4. Dicumarol, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, and desaspidin decreased strontium uptake primarily by inhibiting light-induced swelling; pentachlorophenol and Gramicidin J decreased strontium uptake both by inhibiting swelling and by uncoupling from electron flow; octylguanidine equally inhibited strontium uptake and photophosphorylation, while slightly enhancing swelling.

5. Cetylpyridinium bromide, phlorizin, chlorpromazine, and quinacrine appeared to uncouple chloroplasts at a site between that involved with ATP formation and the site involved with strontium uptake.

6. A heat-labile coupling-factor fraction prepared as a protein or by EDTA treatment of chloroplasts enhanced photophosphorylation and inhibited light-induced strontium uptake, suggesting that it interacted with the energy-transfer pathway between strontium uptake and ATP-formation sites.

INTRODUCTION

Light affects ion movements in isolated spinach chloroplasts. Light can cause the uptake of calcium[1,2], strontium[3,4], barium[5], phosphate[1], sodium[1], and hydrogen[6] ions, and a release of potassium[1,2] and magnesium[2]. This is an oversimplification, especially with regard to sodium where the fluxes can be in either direction[2]. These

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; PMS, phenazine methosulfate (*N*-methylphenazonium methosulfate).

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ion movements in spinach chloroplasts have been investigated from two points of view. First, ion fluxes that accompany and perhaps cause the reversible light-induced chloroplast shrinkage have been studied by DILLEY AND VERNON[2]. Second, the accumulation of certain ions in chloroplasts has been found under conditions favoring the hydrolysis of ATP[1,3–5], analogous to energy-dependent ion uptake in mitochondria[7] as PACKER[8] has speculated. Concerning this second type of ion movement, electron-opaque dots on the lamellae of chloroplasts incubated in the presence of calcium, strontium, or barium have recently been revealed by electron microscopy[4,5], indicating the accumulation of these ions. This form of ion accumulation may represent an active trapping process. The bivalent cation may form a complex with an anion, presumably phosphate, which becomes deposited locally and increases in size with time[4,5].

Since the ion fluxes observed by DILLEY AND VERNON[2] accompany light-induced chloroplast shrinkage, while the ATP-dependent ion uptake occurs at the same time as light-induced swelling[1,3–5], the importance of size changes for the latter ion uptake was investigated. Swelling was a necessary condition for maximum light-induced uptake of both strontium and sodium. It has been suggested that strontium uptake depends on the energy-transfer pathway[3,4]. Various inhibitors were therefore studied for their relative effect on strontium uptake and photophosphorylation. In addition the relation of a coupling factor[9–11] to these two processes was investigated. It is suggested that ion uptake occurs at a site prior to the formation of ATP.

METHODS

Spinach (*Spinacia oleracea*) was purchased commercially and chloroplasts isolated in 175 mM NaCl or KCl, 50 mM Tris–HCl (pH 7.9) as previously described[4]. For spinach chloroplasts isolated in 350 mM sucrose, 50 mM Tris–HCl (pH 7.9), the centrifugal forces employed during isolation were doubled. *Porphyra yezoensis* was provided by the Yamamoto Nori Research Laboratory, Tokyo, Japan; the thalli were ground with quartz sand in 60% Carbowax 4000 (polyethyleneglycol), 80 mM NaCl, 25 mM Tris–HCl (pH 7.9); after straining through four layers of cheesecloth, the suspension was centrifuged at 1000 $\times$ *g* for 1 min, and the supernatant fraction was centrifuged at 1000 $\times$ *g* for 5 min to obtain the chloroplast-containing pellet; chlorophyll was determined spectrophotometrically[12]. *Euglena gracilis* var. bacillaris was cultured for 5 days as described for the basal medium of CRAMER AND MYERS[13], except that the pH was 6.3; after harvesting by centrifugation, the cells were resuspended in 175 mM NaCl or KCl, 50 mM Tris–HCl (pH 7.9), washed, and broken by a single passage through a French pressure cell at 280 kg/cm^2; chloroplasts were then isolated by the same centrifugation procedure as described for spinach[4].

The reaction mixture for ion uptake and swelling studies contained the isolation medium and 5 mM $MgCl_2$, 10 μM PMS, 100 μM $SrCl_2$ (with ^{85}Sr obtained from the Union Carbide Corp., Oak Ridge, Tenn.) or 100 μM NaCl (with ^{22}Na obtained from The Radiochemical Centre, Amersham, England), and other additions as indicated. The chlorophyll concentration was 200 μg/ml for spinach, 10 μg/ml for Porphyra, and 50 μg/ml for Euglena. For ^{85}Sr studies, the media were made up with sodium salts. For ^{22}Na studies, all sodium salts, including those in the isolation medium,

were replaced by potassium salts. ^{42}K was obtained from the Japan Atomic Energy Inst., Tokyo.

5-ml aliquots of the reaction mixture were incubated at 25° for 30 min in transparent centrifuge tubes in the dark or at a light intensity of 30000 lux provided by tungsten lamps[4]. Next the tubes were centrifuged at 20000 × *g* for 10 min at 4°, the pellet was weighed, and the total number of counts per tube as well as the counts/ml in the supernatant fraction was determined. Ion uptake by the chloroplast fraction was calculated by multiplying the total amount of the ion added to the tube by the fraction of that ion removed from the reaction mixture by the chloroplasts (decrease in counts/ml in the supernatant fraction as compared to counts/ml in the initial reaction mixture). The chloroplast-associated volume was obtained by dividing the pellet volume (pellet weight was converted to volume by assuming a density of 1.000 g/ml) by the number of chloroplasts per tube using the factor of $15 \cdot 10^8$ chloroplasts per mg chlorophyll[3,14].

Magnesium was determined with a Perkin–Elmer atomic absorption spectrometer Model 303 with the kind assistance of Dr. K. TAKAHARA of the Japan Monopoly Corp. Hospital, Tokyo, Japan.

Photophosphorylation was measured by the method of AVRON[15]. [^{32}P]P_i was obtained from The Radiochemical Centre and purified on a Dowex 1 column according to SUELTER *et al.*[16]. The reaction mixture contained 35 mM NaCl, 10 mM Tris–HCl (pH 7.9), 5 mM $MgCl_2$, 10 μM PMS, 3 mM ADP, 3 mM sodium–potassium phosphate (pH 7.9), and [^{32}P]P_i. Incubation was for 1 min at 20° either with 100000 lux provided by tungsten flood lamps or in the dark. The chlorophyll concentration was 15 μg/ml.

A coupling-factor protein was isolated according to the description of VAMBUTAS AND RACKER[11] up to and including the ammonium sulfate precipitation steps. When necessary, NH_4^+ was removed by dialysis. Protein concentration was determined by the Folin technique, as described by LOWRY *et al.*[17].

Data are presented in the form mean ± standard error of the mean. Unless otherwise specified, results are for spinach chloroplasts.

Rutamycin, octylguanidine, carbonylcyanide phenylhydrazone derivatives, desaspidin, Gramicidin J, and chlorpromazine were generously provided by Dr. S. MINAKAMI (University of Tokyo, Tokyo, Japan), Dr. B. C. PRESSMAN (University of Pennsylvania, Philadelphia, Pa.), Dr. P. G. HEYTLER (E. I. DuPont de Nemours and Co., Wilmington, Del.), Dr. H. BALTSCHEFFSKY (University of Stockholm, Stockholm, Sweden), the Nikken Kagaku Co., Ltd. (Tokyo, Japan) and the Shionogi and Co., Ltd. (Osaka, Japan), respectively.

RESULTS

Influence of swelling

Various concentrations of formate and acetate were employed to study the relationship between light-induced chloroplast swelling and ion uptake (Fig. 1). These compounds greatly decrease the light-induced swelling of spinach chloroplasts, as shown by PACKER AND SIEGENTHALER[18]. The chloroplast-associated volume, which was determined for the same tubes in which ion uptake was measured, decreased as the anion concentration was increased, and both the strontium and the sodium

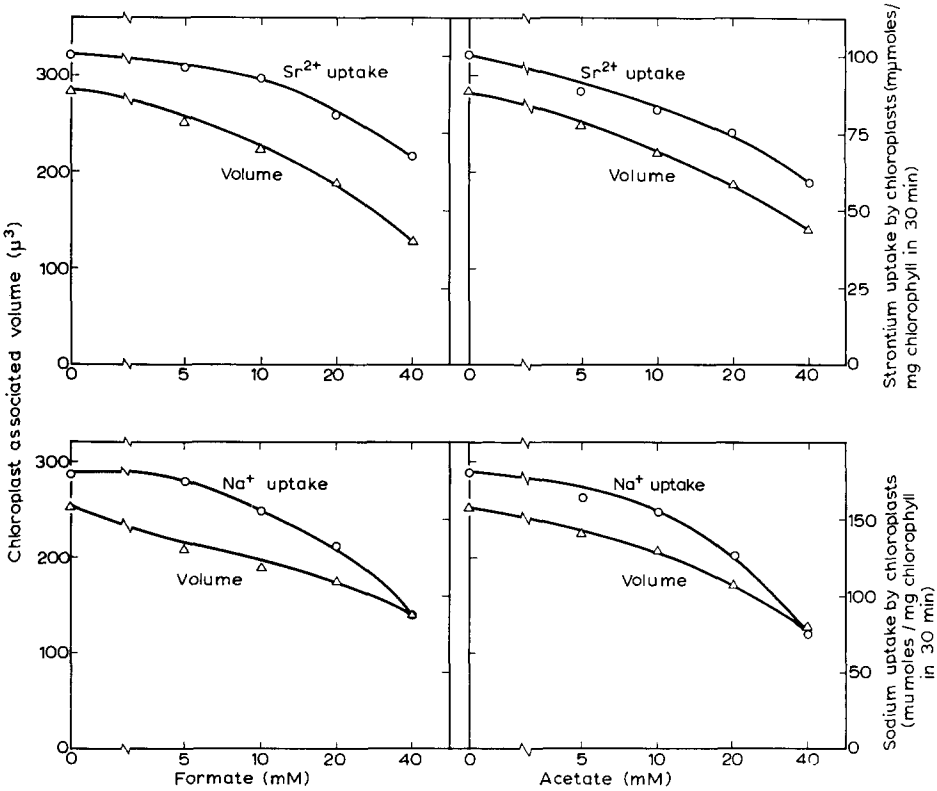

Fig. 1. Effect of formate and acetate on chloroplast volume and ion uptake. Spinach chloroplasts were incubated in the light as described in METHODS. The anion was added as the sodium salt for ^{85}Sr studies and as the potassium salt for ^{22}Na studies, always at pH 7.9. Each point is the average of two or three experiments.

uptake also continuously decreased. As the concentration of formate was raised from 0 to 40 mM, the light-induced (light *minus* dark) swelling of the chloroplasts was inhibited an average of 65 %, while the light-induced strontium uptake decreased by 43 % and sodium uptake by 51 %. For the same change of acetate concentration, the light-induced swelling was inhibited an average of 64 %, while the light-induced strontium uptake decreased by 49 % and sodium uptake by 58 %. In both cases, sodium uptake appeared to be more associated with swelling than strontium uptake. Light-induced chloroplast swelling is apparently necessary to obtain optimum uptake of sodium and strontium.

Spinach chloroplasts were also isolated and incubated in a medium containing sucrose. After 30 min, the chloroplast-associated volume was 27 μ^3 in both the light and the dark. The light-induced strontium uptake by the chloroplasts was 0.9 $\pm$ 0.2 % (percentage of ion removed from the initial reaction mixture by the chloroplast fraction) and the sodium uptake was 0.0 $\pm$ 0.1 %. For controls performed with the NaCl and KCl media, the light-induced strontium uptake was 13 % and for sodium was 36 %. Sodium uptake was clearly more dependent on swelling than strontium uptake.

The necessity of light-induced swelling for appreciable ion uptake was investi-

gated with chloroplasts from two algae. For 7 experiments with chloroplasts from Porphyra, the light to dark ratio of chloroplast volume was 1.03 ± 0.02 and the light to dark ratio of strontium uptake was 1.02 ± 0.03. Added acid or base (up to 0.16 ml 0.2 M HCl or NaOH per 5 ml) did not change the light to dark ratio for strontium uptake. For chloroplasts from Euglena, no light-induced swelling occurred and no light-induced uptake of strontium or sodium was observed at pH 6.2 (the pH optimum for O_2 evolution by these chloroplasts), 7.0, or 7.9. In fact, for 5 experiments at pH 7.9 in the NaCl medium without added strontium, the light to dark volume ratio was 0.87 ± 0.04, and upon adding 5 μM CCCP it was 0.98 ± 0.02. BELSKY, SIEGENTHALER AND PACKER[19] have also observed a light-induced shrinkage of Euglena chloroplasts that is present at pH 8 and is inhibited by CCCP. The light-induced absorbance changes of Euglena extracts observed by BROWN, BRIL AND URBACH[20] are consistent with these structural changes. Euglena chloroplasts thus apparently have a light-induced shrinkage under the same conditions which result in a light-induced swelling of spinach chloroplasts. Neither Porphyra nor Euglena chloroplasts manifest the ion uptake that depends on swelling for spinach chloroplasts.

Various plant hormones were tested for possible effects on strontium uptake or size changes of isolated spinach chloroplasts. No changes of strontium uptake or swelling in the light or the dark were found with indoleacetic acid (10^{-6} to $5 \cdot 10^{-5}$ M), kinetin (10^{-6} to 10^{-5} M), or gibberellic acid (10^{-4} M).

Na^+ uptake, K^+ release, Mg^{2+} efflux

The characteristics of sodium uptake differed from previous studies, perhaps owing to the type of soil in which the plants were grown (discussed below). For example, a time lag of 3 min had previously been found for sodium uptake in the light[3] and omission of magnesium had no effect[1], whereas here no time lag was evident (uptake paralleled in time the swelling curve) and omission of magnesium decreased sodium uptake in the light by 65 %. In agreement with previous results with calcium[1], sodium uptake was maximal from 1 to 3 mM ATP, the pH optimum was 7.9, and the ability to take up sodium in a dark period following a 10-min illumination decayed with a half-life of 1.5 min.

For electrical neutrality, either anion uptake or cation release must accompany the light-induced sodium uptake. Two possible anions, chloride and ATP, are apparently not taken up in sufficient quantity[1]. Moreover, the cation, H^+, is taken up, not released[6]. However, light can cause the efflux of potassium from spinach chloroplasts under different conditions[2]; consequently, the effect of added sodium on potassium release was investigated. Chloroplasts were isolated in the KCl medium. Preincubation was carried out in the reaction medium containing ^{42}K (half-life 12.4 h) for 30 min in the dark at 0° to allow for exchange labeling before the usual incubation in the light. Alternate tubes received 3 mM NaCl labeled with ^{22}Na (half-life 2.58 years; hence both isotopes could be determined for the same tube). Two experiments done in quadruplicate are averaged. In the tubes with sodium, its uptake was 2.2 ± 0.1 μmoles/mg chlorophyll in 30 min. In the presence of sodium, 2.7 ± 0.8 μmoles less potassium/mg chlorophyll in 30 min were found in the chloroplast fraction than in the absence of sodium. Hence, the light-induced sodium uptake occurred concomitantly with the release of potassium, suggesting that sodium accumulation may occur by an exchange for potassium.

DILLEY AND VERNON[2] have reported a light-induced magnesium efflux accompanying the light-induced shrinkage of spinach chloroplasts. In the present experiments, magnesium movements accompanying the light-induced chloroplast swelling were investigated (Fig. 2). A gradual release of magnesium occurred in the dark, amounting to approx. 0.76 μmole/mg chlorophyll in 30 min. Also, a light-stimulated (light *minus* dark) release of about 0.14 μmole magnesium/mg chlorophyll occurred within 30 sec. This latter component may be related to the rapid light-induced magnesium efflux observed by DILEY AND VERNON[2].

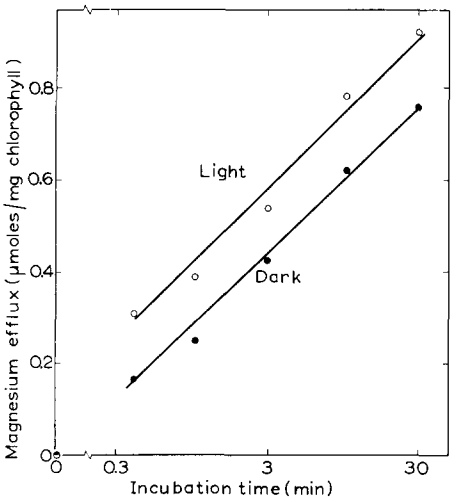

Fig. 2. Magnesium efflux from spinach chloroplasts. After incubation for various periods in the light or dark, the chloroplast suspension was centrifuged at 20000 $\times$ *g* for 10 min at 4° and the magnesium content of the supernatant fraction was determined by atomic absorption spectroscopy. The NaCl reaction medium (without added $SrCl_2$) was used. 5 mM $MnCl_2$ replaced the usually added $MgCl_2$, since this system also supports the light-induced chloroplast ATPase and ion uptake[1]. A magnesium release of 0.94 μmole/mg chlorophyll occurred during chloroplast isolation and centrifugation and has been subtracted. Three experiments are averaged.

Inhibitors of photophosphorylation

Since light-induced strontium uptake has been suggested to depend on the energy-transfer pathway[3,4], the relative effect of inhibitors of photophosphorylation[21–25] on strontium uptake and ATP formation was investigated. Marked differences in concentrations necessary for inhibition of strontium uptake and photophosphorylation were observed for these compounds, sometimes owing to effects on the light-induced swelling. Chloroplast swelling is greatly enhanced by light, especially in the presence of PMS (ref. 26), but it has not been directly demonstrated that swelling depends on electron flow. In fact, certain compounds (see below) inhibited swelling to a much greater extent than photophosphorylation, although under different chemical conditions.

Dicumarol, FCCP, and desaspidin all greatly decreased light-induced strontium uptake and swelling, but had much less effect on photophosphorylation (Fig. 3A–C). For example, 75 μM dicumarol decreased strontium uptake by 94 %, swelling 68 %, and photophosphorylation only 14 % (Fig. 3A). For 3 μM FCCP, strontium uptake was decreased by 97 %, swelling 81 %, and photophosphorylation 29 % (Fig. 3B),

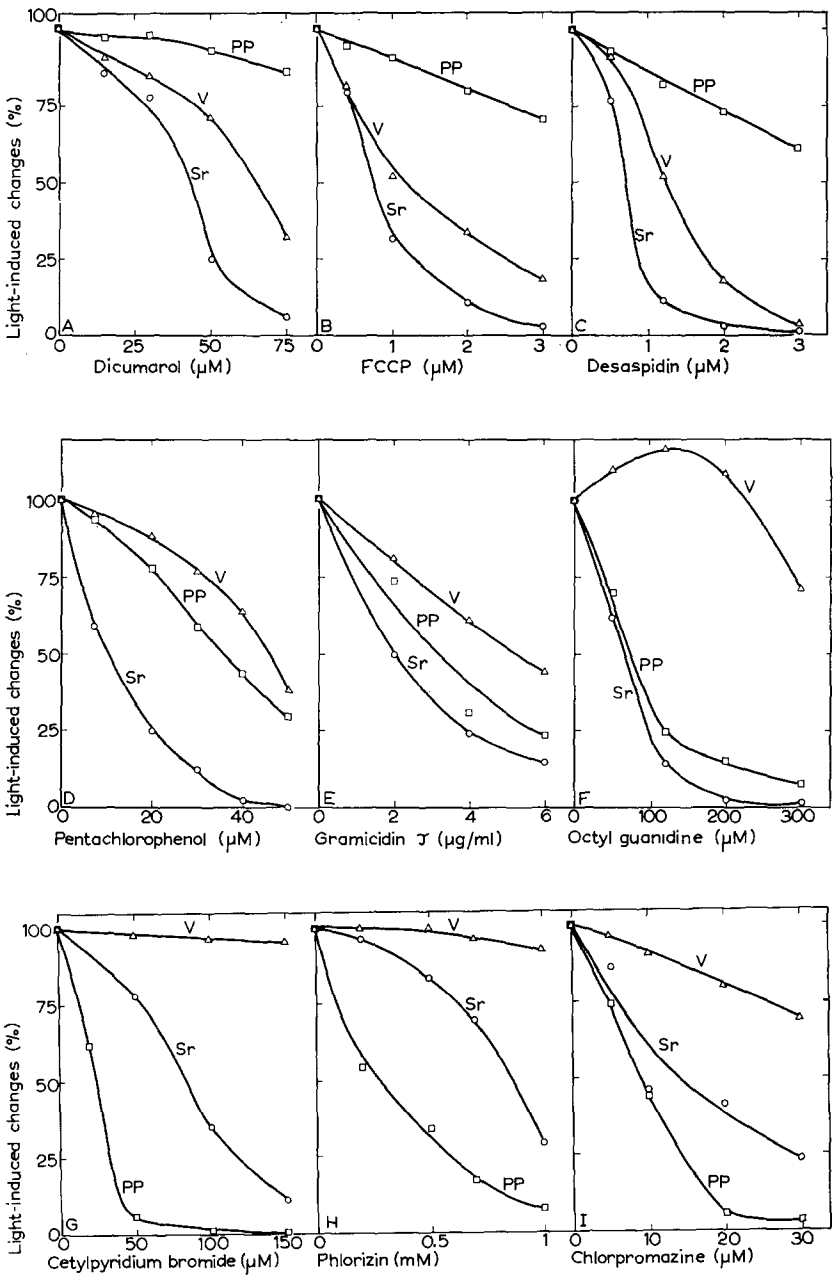

Fig. 3. Effects of various compounds on light-induced swelling, photophosphorylation, and strontium uptake. Light-induced (light *minus* dark) changes were measured for spinach chloroplasts as described in METHODS. Control values for chloroplast volume after 30 min averaged 260 μ^3 (range, 235 to 291) in the light and 59 (55 to 68) in the dark; controls for photophosphorylation averaged 722 μmoles ATP/mg chlorophyll per h (508 to 1010) in the light and 0 (−1 to +3) in the dark; controls for strontium uptake averaged 115 mμmoles/mg chlorophyll in 30 min (86 to 149) in the light and 14 (9 to 18) in the dark. Each point is the average of two or three experiments. V, volume (△); PP, photophosphorylation (□); and Sr, strontium uptake (○).

in agreement with photophosphorylation data of AVRON AND SHAVIT[22]. Desaspidin (2 μM) decreased strontium uptake by 97 %, swelling 82 %, and photophosphorylation 27 % (Fig. 3C), as reported for photophosphorylation under these conditions by GROMET-ELHANAN AND ARNON[27]. Strontium uptake always decreased more markedly than swelling, possibly owing to an uncoupling effect in addition to the inhibition of swelling.

Pentachlorophenol and Gramicidin J inhibited strontium uptake to the greatest extent, photophosphorylation to an intermediate extent, and swelling least (Fig. 3D, E). For example, 35 μM pentachlorophenol decreased swelling by 30 %, photophosphorylation 50 %, and strontium uptake 94 % (Fig. 3D). Gramicidin J (3.2 μg/ml) decreased swelling by 32 %, photophosphorylation 50 %, and strontium uptake 68 % (Fig. 3E). For these compounds, light-induced strontium uptake appeared to decrease owing both to an uncoupling and to the inhibition of swelling, stressing the importance of the energy-transfer pathway as well as swelling for ion uptake.

Octylguanidine uncoupled photophosphorylation 50 % near 75 μM (Fig. 3F), agreeing with data of AVRON AND SHAVIT[22] for PMS-supported photophosphorylation. The inhibition of light-induced strontium uptake closely paralleled that of photophosphorylation with 50 % decrease at about 70 μM. Octylguanidine enhanced the light-induced swelling with a maximum of about 17 % increase at 120 μM (Fig. 3F).

Three other compounds were more effective in decreasing photophosphorylation than strontium uptake. Cetylpyridinium bromide had very little effect on the light-induced swelling in the concentration range used (Fig. 3G). However, it decreased photophosphorylation at a much lower concentration than was necessary for equal inhibition of strontium uptake, *e.g.* 50 % inhibition of photophosphorylation occurred for 25 μM, whereas 85 μM cetylpyridinium bromide was necessary to inhibit light-induced strontium uptake to the same extent. Also phlorizin inhibited photophosphorylation at a lower concentration than strontium uptake (Fig. 3H). ATP formation was decreased 50 % for about 0.25 mM phlorizin, while 0.9 mM gave the same inhibition of light-induced strontium uptake. The inhibition of photophosphorylation by phlorizin closely agrees with the results of IZAWA, WINGET AND GOOD[25]. For the higher concentrations of chlorpromazine employed, photophosphorylation was apparently inhibited to a greater extent than light-induced strontium uptake, but there was considerable scatter in the data (Fig. 3I). Hence, photophosphorylation was more sensitive to inhibition than light-induced strontium uptake for cetylpyridinium bromide, phlorizin, and possibly chlorpromazine.

Quinacrine, another uncoupler of photophosphorylation, had a variable effect on strontium uptake in the light (Table I); inhibition by 50 μM quinacrine ranged from about 0 to 33 %. In the dark, it always decreased the small strontium uptake to approx. one-half.

Rutamycin (1 μg/ml), an inhibitor of oxidative phosphorylation, had no statistically significant effect on strontium uptake in the light and tended to decrease slightly (average 15 %) the low strontium uptake occurring in the dark. This is similar to results observed previously with oligomycin[3]. Although these two antibiotics may be inhibiting ATP-supported ion uptake by chloroplasts in the dark, more likely they are inhibiting ATP-supported ion uptake in mitochondria[7], which have been found in chloroplast preparations by electron microscopy[4].

TABLE I

EFFECT OF 50 μM QUINACRINE ON STRONTIUM UPTAKE BY SPINACH CHLOROPLASTS

Expt.	*Strontium uptake by chloroplast fraction (mμmoles/mg chlorophyll in 30 min)*			
	Light		*Dark*	
	Without quinacrine	*With quinacrine*	*Without quinacrine*	*With quinacrine*
1	57	58	10	5
2	124	100	9	4
3	129	86	11	6
4	117	78	10	5
5	150	136	10	3

Coupling factor

To investigate further the relation of the energy-transfer pathway to strontium uptake, the effect of a coupling factor was examined. The factor was isolated as described by VAMBUTAS AND RACKER[11]. The protein yield up to and including the ammonium sulfate precipitation steps averaged only 12 % of their values. In three experiments with 5 μg "coupling factor" protein/ml, photophosphorylation was increased 10 % and light-induced strontium uptake was decreased 6 % compared with the control values. When the chloroplasts were preincubated with 1 mM EDTA (see legend for Table II), the enhancement of photophosphorylation by 5 μg protein/ml was 28 %, *i.e.* EDTA may have stimulated the release from the chloroplasts of some component which is contained in the coupling-factor fraction.

Since many similarities exist between the coupling factor isolated by VAMBUTAS AND RACKER[11] and the heat-labile factor released by EDTA treatment of Swiss-chard chloroplasts as described by AVRON[9,10], coupling-factor experiments were also under-

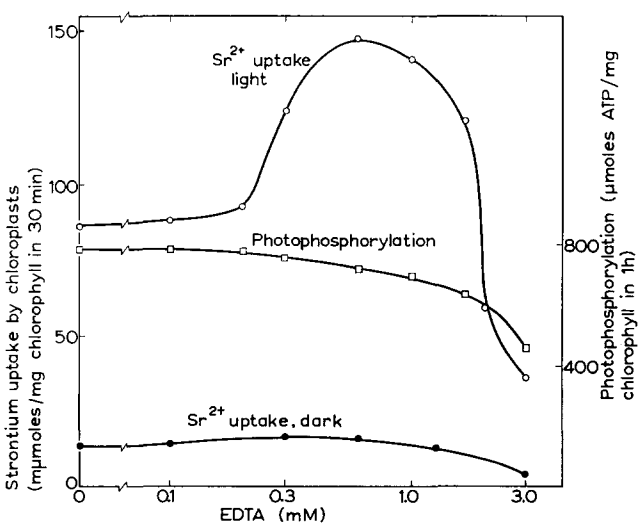

Fig. 4. Effect of EDTA on photophosphorylation and strontium uptake by spinach chloroplasts. Two experiments are averaged.

taken using the results of AVRON as a guide. First, EDTA was tested for its effects on strontium uptake and photophosphorylation (Fig. 4). The response of light-induced strontium uptake to EDTA was diphasic. At the lower EDTA concentrations, a dramatic increase of strontium uptake occurred in the light; at an optimum of about 0.6 mM EDTA, the light-induced uptake was approximately doubled. Inhibition of strontium uptake at high levels of EDTA may be due to chelation of strontium. Photophosphorylation was not enhanced by EDTA and was progressively decreased as the concentration was raised above 0.2 mM, *i.e.* for the same EDTA concentrations which increased strontium uptake. A great part of the EDTA inhibition of photophosphorylation may be due to chelation of magnesium, since adding an extra 3 mM $MgCl_2$ removed about 70 % of the inhibition of 3 mM EDTA (this addition removed only 15 % of the inhibition of strontium uptake occurring with 3 mM EDTA). In any case, 0.2–2 mM EDTA enhanced strontium uptake and inhibited photophosphorylation.

TABLE II

EFFECT OF EDTA PREINCUBATION ON STRONTIUM UPTAKE AND PHOTOPHOSPHORYLATION

Spinach chloroplasts (1 mg chlorophyll/ml) were preincubated in the NaCl isolation medium for 10 min in the dark at 0° with or without 1 mM EDTA. Chloroplasts were then obtained by centrifuging at 5000 × *g* for 5 min and were added to the usual reaction mixtures (see METHODS). 1 ml of the supernatant fraction from the preincubation was added per 5 ml as described and, where indicated, it was heated to 100° for 3 min. Seven experiments are averaged.

Description	*Strontium uptake (mμmoles/mg chlorophyll in 30 min)*	*Photophosphorylation (μmoles ATP/mg chlorophyll per h)*
Regular chloroplasts (no EDTA)	78 ± 6	739 ± 21
EDTA-preincubated chloroplasts	129 ± 11	616 ± 16
EDTA-preincubated chloroplasts + EDTA supernatant	74 ± 7	740 ± 15
EDTA-preincubated chloroplasts + heated EDTA supernatant	127 ± 14	615 ± 22

EDTA preincubation was studied for both strontium uptake and photophosphorylation (Table II). Preincubation of the chloroplasts with 1 mM EDTA increased strontium uptake in the light by 66 % and decreased photophosphorylation by 16 % compared with controls that had no EDTA in the preincubation medium. When 1 ml of the supernatant fraction from chloroplasts treated with EDTA was added to these chloroplasts in the incubation medium (hence reconstituting the original system), no enhancement of strontium uptake or decrease in photophosphorylation was observed compared to that without any EDTA treatment. In this case, the incubation medium contained 0.2 mM EDTA which would cause little effect according to the results shown in Fig. 4. Therefore the effect of EDTA preincubation on both processes seemed to depend on the release of some factor to the supernatant fraction. The enhancement of strontium uptake for EDTA-treated chloroplasts was decreased only 29 ± 12 % by adding 1 ml of the supernatant fraction from chloroplasts preincubated without EDTA, stressing the importance of EDTA for the release of the

substance. When either supernatant fraction was kept at 100° for 3 min and then added to the incubation medium, both the photophosphorylation and strontium uptake were the same as when no supernatant fraction was added. Heating the supernatant fraction apparently destroyed the factor which enhanced photophosphorylation and inhibited strontium uptake. In summary, EDTA preincubation appears to release into the supernatant fraction a heat-labile factor which can decrease strontium uptake and increase photophosphorylation.

DISCUSSION

Swelling of spinach chloroplasts appears to be required for sodium uptake and greatly enhances strontium uptake in the light. Formate and acetate inhibited swelling and ion uptake in the light. At a given anion concentration, sodium uptake was decreased to a greater extent than strontium uptake. In a sucrose medium, no light-induced swelling occurred and light-induced strontium uptake was decreased by 93 %, while sodium uptake was 100 % inhibited. No light-induced swelling of Porphyra chloroplasts was observed and also no light-induced uptake of strontium was detected. Finally, no light-induced uptake of sodium or strontium was found with Euglena chloroplasts whose volume was actually 13 % less in the light than in the dark. Hence it appears that appreciable light-induced ion uptake occurred only if there was chloroplast swelling. This swelling disrupts the chloroplast structure and increases the accessibility of the membranes to the ions[4,5]. This is supported by the finding that after a 3-min illumination in the presence of 1 mM $BaCl_2$, the mean diameter of electron-opaque particles on separated lamellae is 11.5 mμ, while for deposits in fairly intact grana it is 10.2 mμ (ref. 5). Light-induced swelling can expose sites on the lamellar membranes, a condition which may be necessary for optimum ion uptake.

Previously, phosphate uptake by spinach chloroplasts was shown to occur simultaneously with calcium uptake[1]. However, added phosphate did not affect the light-induced sodium uptake[1], suggesting that a different mechanism of maintaining electrical neutrality is involved. In the present experiments, light-induced sodium uptake can be fully accounted for by a concomitant release of potassium. As opposed to the formation of insoluble deposits on the lamellae, which is apparently the case for uptake of calcium, strontium, and barium[4,5], an exchange reaction involving potassium may underlie the sodium uptake.

The light-induced uptake of sodium reported here manifested different kinetics and magnesium dependency than has previously been observed[1,3]. DILLEY AND VERNON[2] have also encountered two types of commercial spinach with regard to sodium fluxes. The type of soil may be affecting the sodium movement. The spinach used in the present experiments was grown in acidic soils (near pH 5.3 before chemical treatment) of volcanic origin[28], while the previous experiments[1,3] were performed with spinach grown in neutral alluvial soils[29]. Furthermore, the relative content of sodium and potassium in spinach is extremely sensitive to the relative availabilities of these ions in the soil[30]. Since sodium uptake may be in exchange for potassium, the soil might well influence the light-induced sodium uptake by chloroplasts.

Quinacrine has been found by DILLEY AND VERNON[31], HIND AND JAGENDORF[23], and IZAWA[32] to enhance the light-induced light-scattering increase which is supposed to depend on a high-energy intermediate (see PACKER[8]). However, quinacrine (50 μM)

does not increase calcium and strontium uptake in the light, but rather has an effect varying from nearly complete inhibition[1] to no change (Table I). Although ion uptake by spinach chloroplasts appears to depend on a high-energy intermediate, the dependence is quite different from that involving light-scattering changes, contrary to preliminary speculation[1]. Another important difference between light-scattering changes and ion uptake involves ATP. Upon the omission of ATP from the reaction mixture, calcium and strontium uptake in the light is decreased 59–66 % (refs. 1, 4). On the other hand, the light-induced light-scattering increase does not require ATP, and can even be inhibited by ATP (refs. 31, 32). Quinacrine (50 μM) decreased the relatively small strontium uptake in the dark, perhaps by blocking an ATP-supported ion uptake. The importance of ATP for strontium uptake in the dark is clearly seen when ATP is omitted from the reaction mixture, since the uptake is then reduced over 70 % (ref. 4). ATP may provide phosphate as the basis of the ATP-dependent uptake of bivalent cations, perhaps by means of the magnesium-requiring light-triggered ATPase[1]. Also, ATP may enhance ion uptake by reducing the use of a high-energy intermediate to form ATP. In any case, light-induced strontium uptake seems to depend on both the energy-transfer pathway and the provision of an anion.

The relation of the energy-transfer pathway to strontium uptake was investigated by using inhibitors of photophosphorylation. Part of the inhibition of strontium uptake by some of these compounds was probably due to an inhibition of swelling. For example, with dicumarol, FCCP, and desaspidin, photophosphorylation was less than 20 % inhibited at concentrations which reduced light-induced swelling more than 50 %. In all cases, light-induced strontium uptake was inhibited more than light-induced swelling, supporting the contention that swelling is necessary for appreciable ion uptake. For pentachlorophenol and Gramicidin J, photophosphorylation was inhibited more than light-induced swelling. With these compounds, light-induced strontium uptake apparently decreased owing not only to inhibition of swelling, but also to uncoupling. For octylguanidine, both strontium uptake and photophosphorylation were inhibited to approximately the same extent. This compound probably interacts with the energy-transfer system with the result that ATP formation and ion uptake are simultaneously inhibited. Octylguanidine increased the light-induced swelling. Chloroplast swelling might be enhanced by a detergent effect (octylguanidine has a hydrophobic chain and a hydrophilic head) on the lamellar membranes as is the case with dodecylbenzene sulfonate[33] and Triton X-100 (ref. 4). Finally, cetylpyridinium bromide, phlorizin, and possibly chlorpromazine were all more effective inhibitors of photophosphorylation than of strontium uptake. IZAWA, WINGET AND GOOD[25] concluded that phlorizin might interact near the end of the energy-transfer sequence. In the present studies, different light intensities, chlorphyll concentrations, and chemical conditions were used for a comparison of ATP formation and strontium uptake. However, cetylpyridinium bromide, phlorizin, and perhaps chlorpromazine may interact with the energy-transfer pathway between a site involved with strontium uptake and one involved with photophosphorylation. GROMET-ELHANAN AND AVRON[34] have suggested that quinacrine interacts during the formation of ATP from a high-energy intermediate. This site may also be subsequent to a site involved in ion uptake, since quinacrine has only a moderate effect on strontium uptake in the light (Table I) at a concentration leading to nearly complete inhibition of photophosphorylation[22,31].

Hence it appears possible to interact with the energy-transfer sequence so that photophosphorylation is inhibited with little or no effect on strontium uptake.

The coupling-factor experiments also helped to locate the site in the energy-transfer pathway possibly involved with strontium uptake. VAMBUTAS AND RACKER[11] have isolated an enzyme from spinach chloroplasts which stimulated photophosphorylation in subchloroplast particles. They suggested that this coupling factor might catalyze the last step of transphosphorylation. Following their procedure, a fraction was prepared that slightly enhanced photophosphorylation, especially if the chloroplasts had been pretreated with EDTA. Uncoupling by an EDTA pretreatment was first noted by JAGENDORF AND SMITH[35]. Although this uncoupling effect can be lessened by NaCl[10,23,35], it still occurs under the conditions for these experiments (Table II). Addition of the coupling-factor fraction appeared to decrease the light-induced strontium uptake. Hence, it may act between a site involved with ion uptake and one involved with ATP formation. To investigate further the possible influence of the coupling factor on strontium uptake, another approach was tried. AVRON[9,10] has obtained a heat-labile coupling factor from the supernatant fraction of EDTA-treated Swiss-chard chloroplasts. In the present experiments, the supernatant fraction of EDTA-treated chloroplasts also contained a heat-labile factor enhancing photophosphorylation. This supernatant fraction markedly inhibited strontium uptake and the inhibition was eliminated by heating (Table II). That is, it again appears that a coupling factor might act at a site of the energy-transfer pathway between ATP formation and strontium uptake sites.

Certain conditions favor ion uptake by isolated spinach chloroplasts. Light-induced swelling is required for sodium uptake and greatly enhances strontium uptake. For electrical neutrality, cation uptake must be accompanied by anion uptake, as for strontium and calcium accumulation, or by exchange with other cations, as appears likely for sodium uptake. ATP enhances ion uptake under conditions favoring its hydrolysis, a process triggered by light. Although photophosphorylation *per se* is apparently not necessary, light leads to a high-energy state which supports ion uptake. It is not known whether, or to what extent, this type of ion uptake occurs in plants. However, older leaves do accumulate more calcium[36]. Moreover, calcium is not readily transferred from leaf to leaf[36,37]. These characteristics could be due to a light-induced calcium uptake and its deposition in chloroplasts in an insoluble state.

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REFERENCES

1 P. S. NOBEL AND L. PACKER, *Plant Physiol.*, 40 (1965) 633.
2 R. A. DILLEY AND L. P. VERNON, *Arch. Biochem. Biophys.*, 111 (1965) 365.
3 P. S. NOBEL, *Doctoral Thesis*, University of California, Berkeley, 1965.
4 P. S. NOBEL, S. MURAKAMI AND A. TAKAMIYA, *Plant Cell Physiol. Tokyo*, 7 (1966) 263.
5 P. S. NOBEL, S. MURAKAMI AND A. TAKAMIYA, *Proc. 6th Intern. Congr. Electron Microscopy, Kyoto, 1966*, Vol. 2, Maruzen, Tokyo, 1966, p. 373.
6 J. NEUMANN AND A. T. JAGENDORF, *Arch. Biochem. Biophys.*, 107 (1964) 109.

7 A. L. LEHNINGER, *The Mitochondrion*, Benjamin, New York, 1964, p. 157.
8 L. PACKER, *Photosynthetic Mechanisms of Green Plants*, Publ. 1145, Natl. Acad. Sci.-Natl. Res. Council, Washington, D.C., 1963, p. 587.
9 M. AVRON, *Biochim. Biophys. Acta*, 77 (1963) 699.
10 M. AVRON, *La Photosynthèse*, (Colloq. Intern. C.N.R.S.), Gif-sur-Yvette, 1962, C. N. R. S., Paris, 1963, p. 543.
11 V. K. VAMBUTAS AND E. RACKER, *J. Biol. Chem.*, 240 (1965) 2660.
12 G. MACKINNEY, *J. Biol. Chem.*, 140 (1941) 315.
13 M. CRAMER AND J. MYERS, *Arch. Mikrobiol.*, 17 (1952) 384.
14 A. B. TOLBERG AND R. I. MACEY, *Biochim. Biophys. Acta*, 109 (1965) 424.
15 M. AVRON, *Biochim. Biophys. Acta*, 40 (1960) 257.
16 C. H. SUELTER, M. DELUCA, J. B. PETER AND P. D. BOYER, *Nature*, 192 (1961) 43.
17 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
18 L. PACKER AND P.-A. SIEGENTHALER, *Plant Physiol.*, 40 (1965) 1080.
19 M. M. BELSKY, P.-A. SIEGENTHALER AND L. PACKER, *Plant Physiol.*, 40 (1965) 290.
20 J. BROWN, C. BRIL AND W. URBACH, *Plant Physiol.*, 40 (1965) 1086.
21 H. BALTSCHEFFSKY AND D. Y. DE KIEWIET, *Acta Chem. Scand.*, 18 (1964) 2406.
22 M. AVRON AND N. SHAVIT, *Biochim. Biophys. Acta*, 109 (1965) 317.
23 G. HIND AND A. T. JAGENDORF, *J. Biol. Chem.*, 240 (1965) 3202.
24 A. T. JAGENDORF AND J. NEUMANN, *J. Biol. Chem.*, 240 (1965) 3210.
25 S. IZAWA, G. D. WINGET AND N. E. GOOD, *Biochem. Biophys. Res. Commun.*, 22 (1966) 223.
26 L. PACKER, P.-A. SIEGENTHALER AND P. S. NOBEL, *J. Cell Biol.*, 26 (1965) 593.
27 Z. GROMET-ELHANAN AND D. I. ARNON, *Plant Physiol.*, 40 (1965) 1060.
28 I. KANNO, *Japanese Soil Types* (in Japanese), Noh-bun-kyo, Tokyo, 1964, p. 74.
29 R. E. STORIE AND F. HARRADINE, *Soil Sci.*, 85 (1958) 207.
30 J. J. LEHR, *Plant Soil*, 2 (1949) 37.
31 R. A. DILLEY AND L. P. VERNON, *Biochemistry*, 3 (1964) 817.
32 S. IZAWA, *Biochim. Biophys. Acta*, 102 (1965) 373.
33 M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 69 (1963) 130.
34 Z. GROMET-ELHANAN AND M. AVRON, *Plant Physiol.*, 40 (1965) 1053.
35 A. T. JAGENDORF AND M. SMITH, *Plant Physiol.*, 37 (1962) 135.
36 A. NASON AND W. D. MCELROY, in F. C. STEWART, *Plant Physiology*, Vol. III, Academic Press, New York, 1963, p. 509.
37 A. WALLACE, E. FROLICH AND O. R. LUNT, *Nature*, 209 (1966) 634.