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LIGHT-INDUCED CHANGES IN THE IONIC CONTENT OF CHLOROPLASTS IN *PISUM SATIVUM*

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SUMMARY

1. The content of chloroplasts isolated in two minutes from pea plants in the light or dark was ascertained both *via* osmotic responses in sucrose solutions interpreted by the Boyle–Van 't Hoff relation and from direct determination of the K^+, Na^+, Mg^{2+}, Ca^{2+} and Cl^- associated with the chloroplasts.

2. From packed-volume and Coulter-counter results, the chloroplasts contained about 3.52 μmoles of osmotically active particles per mg chlorophyll when isolated from plants in the dark and only 2.39 μmoles per mg chlorophyll when isolated from plants in the light. Likewise, the total amount per mg chlorophyll of the five ions determined was about 32 % lower for chloroplasts from plants in the light compared with the dark.

3. The concentration of ions in the osmotically responding space was calculated using the chloroplast volume measured at the same osmolality as the cell sap (about 288 mosmolal). Chloroplasts both from plants in the light and the dark contained about 99 mM K^+, 92 mM Cl^-, 16 mM Mg^{2+}, 15 mM Ca^{2+} and 10 mM Na^+.

4. Based on an extension of the conventional Boyle–Van 't Hoff relation using irreversible thermodynamics, the reflection coefficient of the chloroplast limiting membranes for internal solutes was greater than 0.7. Sucrose was essentially excluded from these chloroplasts.

5. The light-induced decrease of K^+ in the chloroplasts and its reversal when the plants were placed in the dark had half-times of about 4 min. This decrease occurring *in vivo* was 75 % inhibited by *p*-trifluoromethoxycarbonylcyanide phenylhydrazone and was completely abolished by nigericin, indicating that the light-induced K^+ efflux from chloroplasts required either ATP or a high-energy intermediate created by photosynthetic electron flow.

INTRODUCTION

A simple, gentle technique for isolating chloroplasts in 2 min has been described[1]. In addition to saving time, this procedure might prevent appreciable ion loss from the chloroplasts during their isolation as well as provide a means of assessing the ongoing photosynthetic ability of the plant. For example, chloroplasts isolated by this tech-

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; TES, *N*-tris (hydroxymethyl)methyl-2-aminoethane sulfonate; FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone.

nique from illuminated pea plants had an endogenous photophosphorylation rate of 24 μmoles ATP·(mg chlorophyll)$^{-1}$·h^{-1}, while those isolated from plants in the dark had half this rate[2]. Analogously, MILLER[3] found that the ability of filtered homogenates of young leaves of garden peas to reduce a dye, dichlorophenolindophenol, about doubled when the plants were placed in the light. NIKITINA[4] in studies with isolated pea chloroplasts using the same dye concluded that the Hill reaction was not simply a photochemical event, but was influenced by changing enzymatic activity in the plant during the day. Correlations between a light-induced chloroplast shrinkage *in vivo* and the increased efficiency of photophosphorylation *in vitro* for chloroplasts isolated from illuminated plants have been pointed out[2]. Clearly, the physiological state of chloroplasts in the plant cell can influence photosynthetic activities subsequently measured *in vitro*.

Chloroplasts isolated from illuminated plants were smaller than those from plants in the dark[2]. Both a Coulter counter and a centrifugation technique indicated that the volume of chloroplasts from plants in the dark was 39 μ^3, while for chloroplasts from plants in the light it decreased 20 % to 31 μ^3 (ref. 5). An action spectrum for the chloroplast shrinkage *in vivo* had a shoulder at 700–715 nm, suggesting that photosystem I alone can support the volume change[6]. The introduction into the plant of the uncouplers, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone (FCCP) and nigericin, markedly inhibited the light-induced shrinkage, while 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) had little effect. This light-induced chloroplast shrinkage *in vivo* depends either on ATP or a high-energy state created by electron flow[6]. The light-induced shrinkage of chloroplasts retained during their isolation and subsequent volume determination[2,5,6] appears to be the light-induced chloroplast flattening observed *in vivo* by both light[7–11] and electron[10,12,13] microscopy.

Here, attention is focused on the ions in chloroplasts. Since chloroplasts are larger when isolated from plants in the dark, they should contain more osmotically active particles than for chloroplasts from plants in the light. First the 'osmometric' behavior of these chloroplasts was measured and considered in terms of the Boyle–Van 't Hoff relation:

$$\pi\,(V - b) = n\,RT \tag{1}$$

where π is the osmotic pressure, V is the volume of the chloroplasts, b is the so-called "non-osmotic" volume, and n is the apparent number of osmotically active particles in $V - b$ (R and T have their usual meanings of the gas constant and absolute temperature). A determination of n for chloroplasts from plants both in the light and dark provided information on the quantity of solutes in the chloroplasts. Next, the amount of five ions in the chloroplasts was determined. Finally, the kinetics and metabolic basis of changes in the K^+ content of these rapidly isolated chloroplasts were investigated. The rapid and gentle isolation of these chloroplasts should prevent a major change in their ionic content and hence the results obtained may closely reflect the situation occurring *in vivo*.

MATERIALS AND METHODS

Pisum sativum Laxton's Superb was grown at 20° in moist vermiculite for 14 days. Daylight fluorescent tubes provided 2000 lux for 12 h each day. Chloroplasts were isolated in 0.2 M sucrose buffered with 0.02 M *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonate (TES)–NaOH at pH 7.9 using a procedure requiring only 2 min (ref. 1) (for Na^+ determinations, TES–NaOH was replaced by Tris–HCl). Chloroplast volume determinations for various sucrose concentrations were completed within 4 min of isolation using both the packing volume corrected for 'dead space' with $[^{14}C]$-dextran and a Coulter counter calibrated for each solution[5].

Ion concentrations in both the resuspended chloroplast pellet and the decanted supernatant fluid were determined by conventional methods. The ionic content of the resuspended pellet reflects the ions associated with the chloroplasts at the end of the centrifugation step used for their isolation and hence refers to a situation less than 2 min after harvesting the plants[1]. Cl^- was measured with a silver electrode coated with AgCl. The diluted chloroplast pellets and the supernatant fluid were stored for 1 h at 20° before measurement. Triton (0.1 %), which structurally disrupts the chloroplasts, did not affect the measured potential. Na^+ and K^+ were measured using a flame photometer, the samples being directly aspirated into the burner. Mg^{2+} and Ca^{2+} were determined with an atomic absorption apparatus (preliminary dry ashing of the samples did not change the results within experimental error, and hence the chloroplast-containing samples were used without pretreatment). Data were corrected for the Mg^{2+} contribution from chlorophyll. Ion standards were made using reagent grade chemicals dissolved in the chloroplast isolation medium.

The ion data were corrected for interstitial fluid in the chloroplast pellet as well as for chloroplasts in the supernatant fluid. The ion concentration measured for the pellet can be expressed as $I_p = f_{pc}\, I_c + f_{pe}\, I_e$, where f_{pc} is the fraction of the pellet which is chloroplasts, I_c is the average concentration of the ion in the chloroplasts, f_{pe} is the fraction of the pellet which is extra-chloroplastic ($f_{pe} = 1 - f_{pc}$), and I_e is the concentration of the ion in the extra-chloroplastic (interstitial) fluid. An analogous equation applies to the concentration of the ion measured in the supernatant fluid, *viz.* $I_s = f_{se}\, I_e + f_{sc}\, I_c$. The chloroplast volume (µl chloroplasts per mg chlorophyll) times the chlorophyll measurement (mg chlorophyll per µl pellet) gives f_{pc}. As a first approximation, it was assumed that f_{sc}/f_{pc} equaled the chlorophyll concentration of the supernatant fluid divided by that in the pellet, *i.e.* the chloroplasts remaining in the supernatant fluid make the same contribution per mg chlorophyll as those in the pellet (in practice, f_{sc}/f_{pc} was about 0.05 hence this correction was small). Finally $f_{se} = 1 - f_{sc}$ and so I_e and I_c could be calculated. From I_c (µmoles/µl) and the volume of the chloroplasts (µl per mg chlorophyll), the results of the ion determinations could be expressed in µmoles per mg chlorophyll.

FCCP and nigericin were generously provided by Dr. P. G. HEYTLER (du Pont de Nemours, Wilmington, Del.) and Dr. R. L. HARNED (Commercial Solvents, Terre Haute, Ind.), respectively.

RESULTS

Chloroplasts isolated from plants in the light or dark respond osmotically to variations in the external sucrose concentration (Fig. 1). Both the chloroplast packed volume corrected for trapped supernatant fluid (Fig. 1a) and the mean chloroplast volume determined with a Coulter counter (Fig. 1b) vary linearly with the reciprocal of the sucrose concentration from about 0.13 to 0.40 M, with some non-linearity suggested at 0.10 M. Moreover, chloroplasts isolated from plants in the dark have a larger volume than those isolated from plants in the light at all osmotic strengths employed. The equality of the intercepts on the ordinate in Fig. 1 indicates that chloroplasts both from plants in the light and the dark have the same non-osmotic volume as would be anticipated. The overall response of these rapidly isolated chloroplasts indicates that chloroplasts *in vivo* contain more osmotically active particles in the dark than in the light.

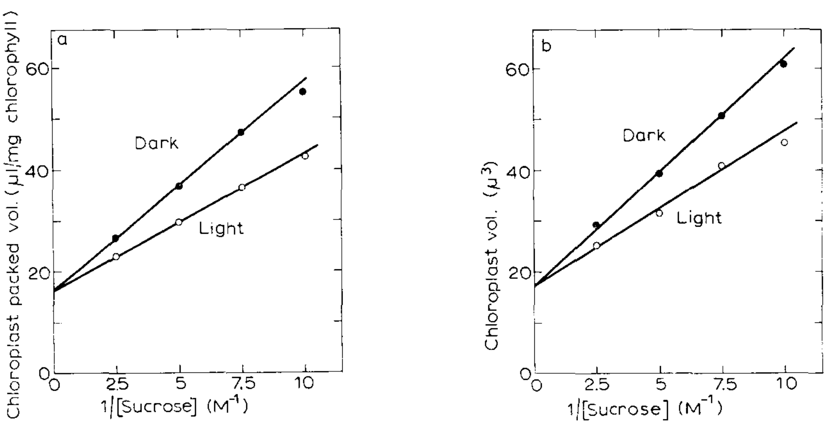

Fig. 1. Osmotic volume changes in sucrose media for chloroplasts isolated from plants in the light or dark. Light refers to chloroplasts isolated from plants kept 4 h in the light (2000 lux provided by daylight fluorescent tubes) while dark is for chloroplasts isolated from plants kept 4–12 h in the dark. In all experiments, TES–NaOH (pH 7.9) was present at 0.1 times the sucrose concentration. Two or three determinations under each condition are averaged. a, packed-volume determination corrected for 'dead space' with [^{14}C]dextran. b, mean chloroplast volume as determined with a Coulter counter.

To identify the light-dependent decrease in osmotically active particles in the chloroplasts (Fig. 1) as a light-induced shrinkage *in vivo* presupposes that the osmotic pressure of the cytoplasm is not markedly different for plants in the light compared with the dark. To check this supposition, the osmolality of the cell sap was measured. 10 g of leaves were firmly ground and the freezing point depression of the expressed sap determined with a commercial osmometer. For crushed leaves from plants kept 1 h in the light (2000 lux), the result was 288 ± 2 (S.E.) mosmoles per kg water, while the mosmolality of sap from crushed leaves of plants kept 1 h in the dark was 287 ± 2 mosmoles per kg water (7 determinations). Under the condition of these experiments, the osmotic pressure of the cytoplasm appears to be insensitive to the illumination status of the plants.

The limiting membranes of chloroplasts act as a barrier to sucrose. Using chloroplasts suspended in 0.2 M [^{14}C]sucrose buffered with 0.02 M TES–NaOH (pH 7.9), the supernatant fluid trapped in the pellet following a 3-min centrifugation at 10000 × *g* was determined. The ratio of the pellet radioactivity to that of an equal weight of supernatant fluid indicates the fraction of the pellet penetrated by the sucrose (see ref. 5 for procedure). For chloroplasts from plants in the light, this fraction was 0.337 ± 0.012 (S.E.), while for chloroplasts from plants in the dark it was 0.295 ± 0.010 (S.E.). This is only slightly higher than the values of 0.33 and 0.28

for the two types of chloroplasts obtained using the presumably non-penetrating [^{14}C]dextran[5]. Under the conditions of these experiments, very little sucrose enters the chloroplasts. The larger 'dead space' for chloroplasts from plants in the light compared with the dark suggests some conformational differences between the two types of chloroplasts as has already been pointed out[5].

TABLE I

IONIC CONTENT OF PEA CHLOROPLASTS ISOLATED FROM PLANTS IN THE LIGHT OR DARK

Chloroplasts were isolated from plants kept 4 h in the light (2000 lux) or 4–12 h in the dark. The ionic contents of the resuspended pellet and supernatant fluid were measured and the amount of the specific ion associated with the chloroplasts was calculated as described in MATERIALS AND METHODS. Data are presented as the average ± S.E. (number of determinations).

Ion	*µmoles/mg chlorophyll*	
	Light	*Dark*
K^+	1.214 ± 0.027 (15)	1.660 ± 0.018 (15)
Na^+	0.134 ± 0.003 (8)	0.155 ± 0.004 (8)
Mg^{2+}	0.192 ± 0.021 (7)	0.298 ± 0.022 (7)
Ca^{2+}	0.205 ± 0.010 (7)	0.242 ± 0.005 (7)
Cl^-	1.011 ± 0.071 (10)	1.704 ± 0.087 (10)

The content of certain ions in these rapidly isolated chloroplasts was determined and the results are summarized in Table I. The most prevalent cation was K^+. The amount of Mg^{2+} or Ca^{2+} in the chloroplasts was about 1/6 that of K^+, while Na^+ was about 1/10 as abundant. Cl^- was present to about the same extent as K^+ and probably was the major anion. The total amount of K^+, Na^+, Mg^{2+}, Ca^{2+}, and Cl^- associated with chloroplasts from plants in the light was 2.76 ± 0.08 (S.E.) µmoles/mg chlorophyll which increased to 4.06 ± 0.09 for chloroplasts from plants in the dark. Each of these five ions was less abundant per mg chlorophyll for chloroplasts isolated from plants in the light compared with the dark (Table I). As would be expected from osmotic considerations, the light-induced chloroplast shrinkage occurring *in vivo* (Fig. 1) is accompanied by a decrease in the number of ions present in the chloroplasts (Table I).

The light-induced shrinkage *in vivo* at 2000 lux has a half-time of about 3 min, while the chloroplast swelling when the plants are placed back in the dark has a half-time of about 5 min (ref. 5). Here, the K^+ content of the isolated chloroplasts was measured after the plants were kept in the light or dark for short periods (Fig. 2). When plants were placed in the light, the K^+ associated with the subsequently isolated chloroplasts decreased 28 % in 30 min. The half-time for this decrease was about 4 min. When the plants were placed back in the dark, the K^+ associated with the chloroplasts increased to the original level and the half-time was about 5 min. Hence, the kinetics of the changes in the amount of K^+ in the chloroplasts upon varying the illumination condition of the plants was very similar to the time course of the light-dependent volume changes.

Certain inhibitors can be introduced into the plant *via* the stems[6]. Although the actual concentration of the compounds at the site of the chloroplasts is not known, such an approach was used to study the metabolic basis of the K^+ changes occurring in the plant cell. The uncoupler FCCP (5 μM) in the bathing solution for the cut stems inhibited the light-dependent decrease in the K^+ content of subsequently isolated chloroplasts about 75 % compared with the control using water (Table II). The results with the uncoupler nigericin were more striking. Not only did 0.5 μM nigericin completely abolish the light-dependent decrease in the K^+ associated with the chloroplasts, but also it somewhat reduced the K^+ content of chloroplasts isolated from plants in the dark. The net result was that the chloroplast K^+ in the presence of nigericin was actually lower in the dark instead of the light. FCCP and nigericin markedly inhibit the light-induced shrinkage of chloroplasts *in vivo*[6], and they similarly abolish the accompanying light-dependent K^+ decrease.

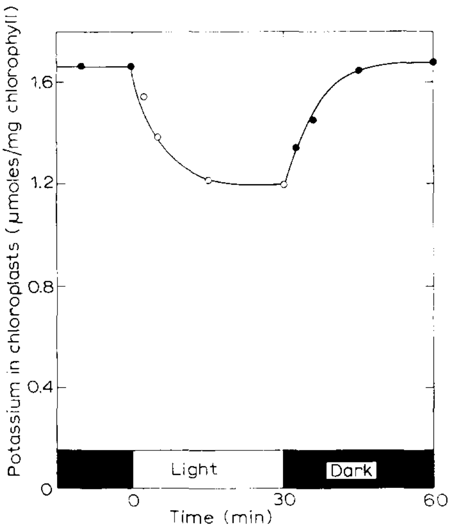

Fig. 2. Changes in K^+ content of chloroplasts upon rapid alteration of the illumination condition of the plants. Plants were illuminated with the usual growth light (2000 lux) or placed in the dark for the periods indicated after which chloroplasts were isolated within 2 min and their K^+ content evaluated as described in MATERIALS AND METHODS.

TABLE II

EFFECT OF UNCOUPLERS INTRODUCED THROUGH THE STEMS ON THE K^+ CONTENT OF CHLOROPLASTS ISOLATED FROM PLANTS IN THE LIGHT OR DARK

Plants were cut approx. 3 cm below the lowest foliage leaf and placed in beakers containing the indicated solution at a depth of 2 cm. After maintaining the cut plants for 4 h in the light (2000 lux) or dark, chloroplasts were isolated and their K^+ content was determined. Data are presented as average ± S.E. for 6–8 determinations under each condition.

Bathing solution for stems	*μmoles K^+/mg chlorophyll*	
	Light	*Dark*
Water	1.29 ± 0.03	1.65 ± 0.02
5 μM FCCP	1.55 ± 0.02	1.64 ± 0.03
0.5 μM nigericin	1.71 ± 0.02	1.48 ± 0.06

DISCUSSION

These rapidly isolated chloroplasts respond osmotically to variations in the external sucrose concentration. Such chloroplasts are 95 % Class I (regarded as intact) when isolated[5], suggesting that the barrier to sucrose penetration is the chloroplast limiting membranes. Direct measurement of the supernatant fluid in the pellet further indicated that sucrose does not readily enter the chloroplasts. The radioisotope penetrated 34 % of the pellet for chloroplasts from plants in the light and 30 % for chloroplasts from plants in the dark using [^{14}C]sucrose, and only 1 % less using [^{14}C]dextran[5] (presumed not to enter the chloroplasts). In 1955, MERCER *et al.*[14] presented osmotic evidence suggesting that the external membranes of Nitella chloroplasts are differentially permeable. Subsequent studies with isolated poke[15] and spinach[15–17] chloroplasts have further described osmotic responses, although it was not always clear whether the properties of the two chloroplast limiting membranes or the properties of the internal lamellar membranes were being measured. Furthermore, the inner one of the chloroplast limiting membranes invaginates during the development of the organelle to form the internal lamellae[18] and hence might be expected to have special properties. Nevertheless, the various studies on the osmotic properties of chloroplasts consistently indicate that the limiting membranes can act as a barrier to certain solutes such as sucrose.

The number of osmotically active particles in the chloroplasts can be calculated *via* the Boyle–Van 't Hoff relation (Eqn. 1). The volume of the chloroplasts, V, is expressed in μl/mg chlorophyll (for the Coulter counter data, the conversion factor of $0.93 \cdot 10^9$ pea chloroplasts per mg chlorophyll was used[5]). If π/RT is expressed in moles/l ($\equiv$ μmoles/μl), then n is in μmoles/mg chlorophyll. For chloroplasts from plants in the dark, n was 3.48 μmoles/mg chlorophyll from the packed volume data (Fig. 1a) and 3.57 from the Coulter counter data (Fig. 1b). For plants in the light, the number of osmotically active particles in the chloroplasts had decreased to 2.34 μmoles/mg chlorophyll from the packed volume results and 2.44 for the Coulter counter. This represents a light-induced decrease *in vivo* of 32–33 % in the osmotically active particles in the chloroplasts. This prediction of fewer particles in the chloroplasts isolated from plants in the light compared with the dark was borne out by direct measurement of five ions associated with the chloroplasts (Table I). The overall light-induced decrease in the number of these ions in the chloroplasts was 32 % with decreases in the individual ions as follows: K^+, 27 %; Na^+, 16 %; Mg^{2+}, 34 %; Ca^{2+}, 15 %; and Cl^-, 40 %. Hence, the light-induced decrease of solutes in the chloroplasts *in vivo* predicted from the Boyle–Van 't Hoff relation and the overall light-induced decrease in the five ions determined were both about 32 %.

As is well known, the Boyle–Van 't Hoff relation is an equilibrium expression derived assuming the membranes are semi-permeable. Considerable success in describing deviations from semi-permeability has been achieved by the theory of irreversible thermodynamics[19]. The appropriate expression underlying the Boyle–Van 't Hoff relation for the case where solute molecules can cross the membrane is $\sigma^0\pi^0 = \sigma^1\pi^1$ where σ^0 and σ^1 are the reflection coefficients of the chloroplast limiting membranes for external and internal solutes, respectively, and π^0 is the external osmotic pressure (π in Eqn. 1), while π^1 is the osmotic pressure inside the chloroplasts. The reflection coefficient describes the permeability behavior of the membrane to solutes. For example, if a certain solute can not penetrate, then $\sigma = 1$ and the full osmotic pressure of that solute is achieved. If on the other hand the solute can penetrate somewhat, then σ is less than one and $\sigma\pi$ represents the apparent or effective osmotic pressure ($\sigma = 0$ if the solute passes through as freely as water in which case no osmotic pressure would be exerted). Based on the consideration of reflection coefficients, the conventional Boyle–Van 't Hoff relation can be re-expressed as follows:

$$\pi(V-b) = \frac{\sigma^{i} n RT}{\sigma^{0}} = \frac{\sigma^{i} \Sigma \gamma_{s} n^{i}_{s}}{\sigma^{0}} RT \tag{2}$$

where γ_s is the activity coefficient of species s, n^i_s is the number of moles of solute s within $V-b$ and $n = \Sigma\gamma_s n^i_s$ has been used as a convenient first approximation. Since fairly dilute solutions appear to be involved in chloroplasts, γ_s will be set equal to 1 for the purposes of calculation. In the present experiments, all the ions were not determined and in addition, some of the ions may be bound and not contribute osmotically. Considering the monovalent (Na^+,K^+) and divalent (Mg^{2+}, Ca^{2+}) cations *plus* the monovalent anion (Cl^-) and an assumed additional divalent anion to balance the charge, chloroplasts from plants in the light had 3.28 μmoles solutes per mg chlorophyll, while those from plants in the dark had 4.66 μmoles per mg chlorophyll and these values will be used as estimates for Σn^i_s. From the finding that sucrose does not penetrate the chloroplast limiting membranes, σ^0 will be set equal to 1 ($\sigma^0 = 1$ if the membrane completely excludes sucrose and TES–NaOH). Using these approximations and the values of $\pi(V\text{-}b)/RT$ determined from the osmotic responses, σ^i was 0.73 for chloroplasts from plants in the light and 0.76 for chloroplasts from plants in the dark (σ^i would be even higher if the activity coefficients are less than 1). Such high values for σ^i indicate that these chloroplasts are quite intact and that the limiting membranes can act as a permeability barrier.

The expressed cell sap was about 288 mosmoles per kg water both for plants in the light and in the dark. It was previously argued[1] that the maximum rate of endogenous photophosphorylation *in vitro* in sucrose solutions might occur at a tonicity similar to that in the plant cell. This maximum occurred near 0.23 M sucrose, 0.023 M TES–NaOH (pH 7.9) which has a measured mosmolality of 280, close to the value actually determined for the cell sap (results with NaCl as the osmoticum[1] are difficult to interpret due to the effect of Cl^- on photosynthesis and the light-induced chloroplast swelling in NaCl media). Based on interpolations from Fig. 1, the volume of chloroplasts at the same osmolality as the cell sap is 29.0 μ^3 when isolated from plants in the light, and 35.3 μ^3 in the dark. These should be reasonably close estimates of pea chloroplast volumes *in vivo*.

For the calculation of ionic concentrations in pea chloroplasts, the amount of the ions (Table I) in the osmotically responding volume ($V-b$) was determined. The total volume, V, was that for the same osmolality as the cell sap, while the non-osmotic volume, b, was 16.2 μl/mg chlorophyll as determined from both the packed volume and Coulter counter data (Fig. 1). Implicit and perhaps invalid assumptions are that the ions were unbound and in one compartment. Nevertheless, the conclusion is reached that although the amount of these ions per mg chlorophyll varies markedly with the illumination condition of the plant from which the chloroplasts are isolated, the concentration in the chloroplasts is quite similar in the light and dark. There was about 99 mM K^+, 92 mM Cl^-, 16 mM Mg^{2+}, 15 mM Ca^{2+}, and 10 mM Na^+. MENKE[20] was the first to indicate that spinach chloroplasts contain K^+, Mg^{2+}, and Ca^{2+}. Later, STOCKING AND ONGUN[21] documented that chloroplasts represent a major site for ion accumulation in plant cells; averaging their data for bean and tobacco leaves indicates that of the ions determined, the amount of K^+ in the chloroplasts was the highest, Na^+ the lowest, with Mg^{2+} and Ca^{2+} being intermediate. In 1963, SALTMAN, FORTE AND FORTE[22] reported that Nitella chloroplasts accumulate potassium (about 84 mM) and virtually exclude Na^+ (only 4 mM), resulting in concentrations similar to the values obtained for pea chloroplasts. For supposedly intact pea chloroplasts, HARVEY AND BROWN[23] found only 6.4 μg K^+ per mg chlorophyll which gives a concentration of about 11 mM using the osmotically responding volume of pea chloroplasts determined here. Ionic concentrations in *Tolypella intricata* chloroplasts isolated nonaqueously have been calculated by LARKUM[24]. The Mg^{2+} and Ca^{2+} concentrations are similar to the values for peas. Although the concentrations of K^+ and Na^+ were much higher than observed here, the ratio of 10:1 was similar. General conclusions from these various observations are: (1) chloroplasts have a high concentration of K^+; (2) Na^+ is often present at about one-tenth the extent of K^+; (3) the principal anion appears to be Cl^-; and (4) chloroplasts contain appreciable Mg^{2+} and Ca^{2+}, perhaps both near 15 mM when averaged over the osmotically responding volume.

Next, some observations on light-induced changes in chloroplast ion content *in vitro* will be considered with respect to the changes occurring *in vivo* retained by these rapidly isolated chloroplasts. A light-induced K^+ and Mg^{2+} efflux (and in some cases also Na^+) accompanying the shrinkage of spinach chloroplasts *in vitro* has been described by DILLEY AND VERNON[25]. They envision that following a light-induced proton uptake by the chloroplasts, Mg^{2+} and K^+ flow out to maintain charge neutrality. This results in an osmotic imbalance which leads to water efflux and the observed shrinkage. From their data, the light-induced efflux of K^+ is 0.20 μmole per mg chlorophyll, while that for Mg^{2+} is 0.13 μmole per mg chlorophyll. NOBEL[26] also using isolated spinach chloroplasts has observed a light-induced efflux of 0.14 μmole Mg^{2+} per mg chlorophyll. CROFTS, DEAMER AND PACKER[27] have described a light-induced efflux of 0.2–0.3 μmole K^+ per mg chlorophyll. This change was in general not reversible in the dark and depended markedly on the chloroplast isolation conditions as would be expected. Although comparing these various results for isolated and generally fragmented chloroplasts with the effluxes determined here which occur *in vivo* is not really justified, it is interesting to note that the light-induced K^+ efflux was 0.45 μmole per mg chlorophyll while that of magnesium was 0.11 μmole per mg chlorophyll for pea chloroplasts *in vivo*.

Finally, the metabolic basis for the light-induced ion movements *in vivo* will be considered. The reversible light-induced K^+ efflux from chloroplasts (Fig. 2) had the same kinetics as the light-induced chloroplast shrinkage and its reversal in the dark[5] (half-times near 4 min). Of the cations in chloroplasts *in vivo*, K^+ is the most abundant and undergoes the greatest variation in μmoles per mg chlorophyll during the chloroplast volume changes. Although a mechanism for the light-induced chloroplast shrinkage involving K^+ extrusion as the step which osmotically leads to water loss can not be stated with certainty, it is clear that factors affecting K^+ and water loss from the chloroplasts must be interrelated. Based on the results with the uncouplers FCCP and nigericin (Table II), the light-induced decrease in K^+ content of chloroplasts *in vivo* appears to require either ATP or a high-energy state created by electron flow.

Nigericin led to a slightly higher amount of K^+ in the chloroplasts in the light compared with the dark. SHAVIT, DILLEY AND SAN PIETRO[28] have observed that nigericin in the presence of KCl can reverse the usual light-induced chloroplast shrinkage and can cause a small light-induced swelling *in vitro*. PACKER[29] had shown that fairly high concentrations of nigericin can lead to a light-induced K^+ uptake by isolated chloroplasts. It is perhaps surprising that both the light-induced K^+ efflux from chloroplasts and the effects of nigericin are so similar *in vivo* and *in vitro*. Chloroplasts are surrounded by two closely apposed membranes which may have quite different permeability properties. In this regard, nigericin may influence the inner of these two membranes. Since this inner membrane invaginates to form the stromal and granal lamellae[18], some similarity between processes occurring *in vivo* and results obtained *in vitro* using naked lamellar systems might be expected. The following conclusions can be made regarding the metabolic basis of the chloroplast ion movements occurring *in vivo*: (1) both the light-induced chloroplast shrinkage and the light-induced efflux of K^+ depend on a high-energy intermediate or on ATP, (2) the kinetics of water and K^+ efflux are similar at a time resolution of about 1 min, (3) there is no evidence for the chloroplasts being maintained far from osmotic equilibrium, and (4) K^+ may play a key role in regulating chloroplast volume in that it is the principal chloroplast cation and also its light-induced efflux from chloroplasts *in vivo* can be markedly affected by uncouplers.

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REFERENCES

1 P. S. NOBEL, *Plant Physiol.*, 42 (1967) 1389.
2 P. S. NOBEL, *Biochim. Biophys. Acta*, 153 (1968) 170.
3 J. H. MILLER, *Am. J. Botany*, 47 (1960) 532.
4 A. N. NIKITINA, *Dokl. Akad. Nauk Tadzh. SSR*, 10 (1967) 44.
5 P. S NOBEL, *Plant Physiol.*, 43 (1968) 781.
6 P. S. NOBEL, *Plant Cell Physiol. Tokyo*, 9 (1968) 499.
7 E. BÜNNING, *Z. Botan.*, 37 (1942) 433.
8 G. BUSCH, *Biol. Zentr.*, 72 (1953) 598.
9 J. ZURZYCKI, *Protoplasma*, 58 (1964) 458.
10 H. HILGENHEGER AND W. MENKE, *Z. Naturforsch.*, 20b (1965) 699.
11 T. VANDEN DRIESSCHE, *Exptl. Cell Res.*, 42 (1966) 18.
12 H. KUSHIDA, M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 79 (1964) 201.
13 L. PACKER, A. C. BARNARD AND D. W. DEAMER, *Plant Physiol.*, 42 (1967) 283.
14 F. V. MERCER, A. J. HODGE, A. B. HOPE AND J. D. MCLEAN, *Australian J. Biol. Sci.*, 8 (1955) 1.
15 K. NISHIDA, *Plant Cell Physiol. Tokyo*, 4 (1963) 247.
16 A. B. TOLBERG AND R. I. MACEY, *Biochim. Biophys. Acta*, 109 (1965) 424.
17 R. A. DILLEY AND A. ROTHSTEIN, *Biochim. Biophys. Acta*, 135 (1967) 427.
18 D. H. NORTHCOTE, *Brit. Med. Bull.*, 24 (1968) 107.
19 J. DAINTY, *Advan. Botan. Res.*, 1 (1963) 279.
20 W. MENKE, *Z. Physiol. Chem.*, 263 (1940) 104.
21 C. R. STOCKING AND A. ONGUN, *Am. J. Botany*, 49 (1962) 284.
22 P. SALTMAN, J. G. FORTE AND G. M. FORTE, *Exptl. Cell Res.*, 29 (1963) 504.
23 M. J. HARVEY AND A. P. BROWN, *Biochem. J.*, 105 (1967) 30P.
24 A. W. D. LARKUM, *Nature*, 218 (1968) 447.
25 R. A. DILLEY AND L. P. VERNON, *Arch. Biochem. Biophys.*, 111 (1965) 365.
26 P. S. NOBEL, *Biochim. Biophys. Acta*, 131 (1967) 127.
27 A. R. CROFTS, D. W. DEAMER AND L. PACKER, *Biochim. Biophys. Acta*, 131 (1967) 97.
28 N. SHAVIT, R. A. DILLEY AND A. SAN PIETRO, *Biochemistry*, 7 (1968) 2356.
29 L. PACKER, *Biochem. Biophys. Res. Commun.*, 28 (1967) 1022.