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# ACID TO BASE PHOSPHORYLATION AND MEMBRANE INTEGRITY IN PLASTIDS OF GREENING MAIZE

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SUMMARY

Fully or partially green chloroplast fragments from maize are capable of synthesizing ATP upon transfer from an acidic to an alkaline environment. It has not been possible to demonstrate this activity in membranes of maize etioplasts. Evidence is presented which shows the development of the capacity to perform acid to base phosphorylation can occur without parallel chlorophyll formation. Acid to base phosphorylation capacity can develop in darkness after brief illumination.

The acid to base capacity seems to have at least two prerequisites: (a) the presence of osmotically responsive internal spaces; and (b) a lamellar component which can be removed by treatment with EDTA. Etioplast fragments possess the EDTA-removable component, but they cannot respond osmotically to changes in the sucrose concentration of their environment. It is suggested that the etioplast's failure to catalyze acid to base phosphorylation is at least in part related to its lack of capacity to exhibit osmotic responsiveness.

## INTRODUCTION

Plastids in angiosperms germinated and grown in darkness fail to form chlorophyll and become functional in photosynthesis. These etioplasts typically possess a small amount of protochlorophyllide and some membraneous material, most of which is arranged into one or two characteristic prolamellar bodies. Since they lack chlorophyll, the immature plastids are incapable of performing at least the light-dependent reactions of photosynthesis. Upon illumination of whole etiolated plants, or of leaves detached therefrom, the plastids become green, and attain the capacity to carry out photosynthesis.

The work described here is part of an attempt to determine steps in the acquisition of photosynthetic competence by membranes of developing chloroplasts. The immediate objective of the present study was to determine whether etioplast internal membranes are able to perform two processes which seem to be related to photosynthesis but which appear neither to depend upon light as an energy source, nor require the presence of chlorophyll. These two responses are: (a) the ability to synthesize ATP

Abbreviations: PMS, phenazine methosulfate; CMU, *p*-chlorophenyl-1,1-dimethyl urea; Tricine, *N*-tris(hydroxymethyl)-methylglycine; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid.

from added ADP and inorganic phosphate upon transition from an acid environment to a basic one (as was shown in green spinach plastid fragments by JAGENDORF AND URIBE[1]); and (b) the capacity of plastid fragments to respond to changes in their osmotic environment.

The maize plant provides excellent material for studying the development of the capacities described above. Unlike Spinacea or Phaseolus, usually favorite organisms for biochemical inquiry, maize can be grown easily and practically in the dark to yield copious quantities of leaf tissue. The work reported here on maize demonstrates that green chloroplast fragments from this plant, like those from spinach, are capable of ATP synthesis upon acid to base transition, and have the ability to respond osmotically. Plastid membranes from etiolated material, however, are incapable of performing either of these functions. It is shown that a coupling factor (or factors) for photosynthetic phosphorylation[2,3] is probably required for acid to base activity. Maize etioplasts contain such a factor[4]. Consequently, it is suggested that the inability to respond osmotically is, at least, one factor in the proplastid's failure to carry out acid to base phosphorylation. The relevance of these findings to plastid maturation is discussed.

## MATERIALS AND METHODS

### *Isolation of plastid fragments*

*Zea mays* (L.), strain WF9TMS × B 37 (Illinois Foundation Seeds, Inc., Champaign, Ill.)., was used exclusively in this study. The seeds were sown, after approx. 15 h of soaking in tap water on vermiculite equilibrated with water for a similar period of time. Plants were grown for 6 days at 28° in either a dark room where occasional brief green safelight illumination may have been experienced or under white fluorescent lights. Greening of etiolated tissue took place under cool white fluorescent lights at an intenstity of 200 ft-candles.

All steps in plastid isolation were carried out at or near 4°. The tissue (usually about 800 g of etiolated or partially green or 200 g of fully green material) was harvested, chilled, and macerated in a Waring Blendor at full line voltage for approx. 10 sec in the presence of 0.5 M sucrose–0.001 M $MgCl_2$–0.05 M *N*-tris(hydroxymethyl)-methylglycine (Tricine), pH 7.9 (2 ml of medium per g of fully light-grown tissue or 1.5 ml per g of etiolated or partially green material). The macerate was filtered through 8 layers of cheesecloth and centrifuged in a Sorvall GSA rotor for 5 min at 121 × *g*. The precipitate from this centrifugation was discarded and the supernatant recentrifuged at 1085 × *g* for 10 min. The precipitate from the second centrifugation, which generally had a volume of about 0.5 ml, was resuspended in approx. 40 ml of 0.01 M NaCl and allowed to stand for 10 min to rupture the outer chloroplast membranes. The fragments thus obtained were centrifuged for 10 min at 3020 × *g* in a Sorvall SS-34 rotor. The final precipitate was resuspended in approx. 5 ml of 0.01 M NaCl, and passed through a Ten Broek hand homogenizer to destroy clumps and ensure random distribution of the fragments.

### *Assay of acid to base phosphorylation*

The acid stage of the reaction contained in 1.35 ml: 16.5 μmoles of succinate (pH 4.0) 16.5 mμmoles of *p*-chlorophenyl-1,1-dimethyl urea (CMU), an appropriate amount of sucrose or water, and the plastid fragments.

The alkaline solution contained in 1.0 ml: 120 μmoles of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 8.15; 6.7 μmoles of ADP; 13 μmoles of $MgCl_2$; 17 μmoles of NaOH; 82 nmoles of $K_2HPO_4$; and approx. $2 \cdot 10^7$ counts/min of carrier-free $^{32}P$ (as $Na_2HPO_4$).

The succinic acid and CMU were added to the plastid fragments in the presence of the osmotic agents and this mixture was incubated for 30 sec at 0°; the alkaline solution was then added. After 15 sec, 0.2 ml of 20 % trichloroacetic acid was added to terminate the reaction. In controls, succinic acid and CMU were mixed with the alkaline solution. Plastid preparations were then added, and after 15 sec the reaction was terminated with trichloroacetic acid. The reaction tubes were centrifuged for 10 min at 12000 × *g* in an SS-34 rotor, and 1.0 ml of the clear supernatant was removed to determine the amount of $^{32}P$ esterified to ADP (ADP + $P_i \longrightarrow$ ATP). The assay procedure is essentially that of AVRON[5]. Cerenkov radiation caused by $^{32}P$ was estimated in distilled water on a Packard TriCarb liquid scintillation counter according to the method of CLAUSEN[6].

*Pigment determinations*

Chlorophyll or chlorophyllide (arising from protochlorophyllide in etiolated tissue which was routinely given 1 min of white illumination immediately before plastid isolation) was estimated by extracting aliquots of the plastid suspension with 80 % acetone, determining the optical density of the extracts at 649 and 665 nm, and applying the extinciton coefficients of VERNON[7]. Pigment determination on intact leaves were carried out by macerating the tissue in 80 % acetone with a small amount of $MgCO_3$, filtering and employing the coefficients cited above.

*Packed pellet volumes*

In determining the packed pellet volume of plastid fragments, the following mixture was placed in a Stafford-Shevsky and McNaught tube: 0.53 ml of the plastid suspension in 0.01 M NaCl, 0.27 ml sucrose solution or water, and 0.55 ml of the succinate–CMU solution used in the acid to base acid stage. The Stafford-Shevsky and McNaught tubes were centrifuged in an I.E.C. Model UV centrifuge at room temperature using the 240 rotor at 2550 × *g* for 10 min; no further decrease in pellet volume was seen with longer times of centrifugation. The packed pellet volume was read directly from the graduations on the tube.

*EDTA-removable factor(s) in acid to base phosphorylation*

Plastids from etiolated and green maize were isolated as described above through the second centrifugation. The precipitates were resuspended in 0.01 M NaCl and 0.15 mM EDTA (pH 8.0) and allowed to stand for 15 min at room temperature. Centrifugation for 15 min at 19000 × *g* in an SS-34 rotor ensued. The supernatants from this centrifugation were used in the reconstitution experiment. The precipitates, consisting of partially depleted membranes were dispersed in 0.01 M NaCl with a Ten Broek homogenizer.

To reconstitute the acid to base activity of depleted green lamellae, the following mixture was allowed to stand for 10 min at room temperature: 2.5 ml of depleted green lamellae in 0.01 M NaCl; 6.0 ml green or etiolated EDTA supernatant or 0.01 M NaCl containing 0.15 mM EDTA, pH 8.0; 0.30 ml 0.01 M EDTA, pH 8.0; 1.0 ml 0.10 M

$MgCl_2$. Centrifugation for 15 min at 19000 × *g* followed. The supernatant was discarded, and the precipitate resuspended in 0.01 M NaCl, and recentrifuged at 19000 × *g* for 10 min. The precipitate from this centrifugation was resuspended in 0.01 M NaCl, homogenized, and used for the acid to base reaction as described earlier. Pigment samples were taken at this point. Green plastids isolated as above, but in the absence of EDTA, were used to determine the acid to base activity levels of untreated lamellae.

All chemicals employed were of the highest purity commercially available. Carrier-free $Na_2H^{32}PO_4$ was purchased from Tracerlab, Waltham, Mass.

## RESULTS

### *Acid to base phosphorylation by maize plants*

Spinach chloroplast fragments esterify ADP to form ATP when they are shifted from an acidic to an alkaline environment[1]. The data of Table I show that maize chloroplast membranes can do so as well. Etioplast membranes, on the other hand, show no significant activity under conditions where 1 % of the activity of green chloroplasts would have been detectable (see DISCUSSION). A series of experiments was carried out to establish how long dark-grown plants must be illuminated before the capacity for acid to base phosphorylation can be detected. In some experiments slight activity could be found in plastids from plants illuminated continuously for 90 min, but this was not consistent. Significant activity was regularly observable in plastids from plants illuminated continuously for 150 min. Typical data are given in Table I.

TABLE I

THE ACID TO BASE PHOSPHORYLATING ACTIVITY OF PLASTIDS ISOLATED FROM MAIZE PLANTS ILLUMINATED FOR VARYING PERIODS OF TIME

Plastids were isolated as described in MATERIALS AND METHODS from maize plants illuminated with white light as indicated. The acid to base reaction was as in MATERIALS AND METHODS.

| *Illumination* | *Counts/min incorporated per g fresh wt.* leaf tissue* |
|---|---|
| Etiolated | 443 |
| 150 min | 1 482 |
| Fully green | 292 015 |

* Estimated from pigment concentrations in the plastid fragments and in the leaves: counts per min per μg chlorophyll added × μg chlorophyll/g tissue = counts/min per g tissue.

### *The development of acid to base reaction capacity*

The most obvious difference between the etioplast and the chloroplast is the presence of chlorophyll in the latter. Dark-grown maize plants of the type used here accumulate comparatively small amounts of chlorophyll during their first 1–3 h of illumination; after this time rapid pigment synthesis commences and continues for many hours. The acid to base reaction itself is not driven by light energy, but does the development of the acid to base reaction capacity depend in whole or in part upon the accumulation of chlorophyll? Chlorophyll synthesis in angiosperms stops very soon after plants are returned to darkness even during a period of rapid chlorophyll synthe-

sis[8]. Thus, it is possible to study the acid to base reaction in plastids from plants with approximately equal concentrations of chlorophyll but with different histories. Etiolated plants were illuminated for 5 h; one group was harvested and processed immediately, and two others were returned to darkness for either 13 or 18 h before harvest and assay. The time spans of the 5th to the 13th and the 5th to the 18th h were chosen, because they were within the period of most rapid chlorophyll accumulution if plants are illuminated continuously.

As shown in Table II, there is little or no increase in chlorophyll per g of fresh weight of leaf tissue after plants are returned to darkness, but the capacity to carry out the acid to base reaction approximately triples during the dark period illumination.

Similar results were obtained when plastids from plants exposed to light for 1 min and returned to darkness for 24 h were compared, with regard to acid to base capacity, with plants illuminated continuously for 24 h. (During 1 min exposure the protochlorophyllide present is converted to chlorophyllide and during the subsequent period of darkness this is esterified to chlorophyll. New protochlorophyllide is formed during the dark period following illumination but this is not converted to chlorophyllide in darkness by angiosperms. The total amount of protochlorophyllide and chlorophyll present after 1 min illumination and 24 h in darkness is approx. 1/100 of the chlorophyll present per g of leaf tissue from plants grown in the light for 24 h.) As shown in Table III, the plastids from plants exposed to light for 1 min and returned to darkness are capable of acid to base phosphorylation unlike plants which have been

TABLE II

EFFECT OF A DARK PERIOD AFTER 5 h OF ILLUMINATION ON THE DEVELOPMENT OF ACID TO BASE CAPACITY

Plastids were isolated from plants illuminated as indicated. Isolation and acid to base procedure as described in MATERIALS AND METHODS, Fragments containing 34.8 μg chlorophyll used per tube.

| *Illumination* | *Chlorophyll (μg/g fresh wt. of leaf tissue)* | *Counts/min incorporated per ml reaction supernatant* |
|---|---|---|
| 5 h light | 79.5 | 2265 |
| 5 h light + 13 h dark | 78.2 | 5613 |
| 5 h light + 18 h dark | 87.9 | 6330 |
| 23 h light | 601.2 | 5004 |

TABLE III

THE DEVELOPMENT OF ACID TO BASE CAPACITY AFTER 1 min OF ILLUMINATION WHEN FOLLOWED BY A DARK PERIOD

Plastids were isolated from plants illuminated as indicated. Isolation and acid to base procedures as described in MATERIALS AND METHODS.

| *Illumination* | *Pigment added (μg/reaction mixture)* | *Counts/min incorporated per ml supernatant* | *Counts/min incorporated per μg chlorophyll added* |
|---|---|---|---|
| 1 min light + 24 h dark | 6.3 | 2 606 | 415 |
| 24 h light | 389.0 | 39 100 | 101 |

exposed for 1 min just before harvest. The activity per $\mu g$ of chlorophyll in the reaction mixture is about 4 times greater than that for plastids from leaves illuminated for 24 h.

The experiments for which data are shown in Tables II and III thus show that the development of acid to base capacity does not necessarily parallel chlorophyll accumulation either in the lag or rapid accumulation phase of pigment deposition. Once dark-grown maize plants have been illuminated, their acid to base capacity can continue to increase even in darkness and in the absence of further accumulation of chlorophyll.

*EDTA-removable factor(s) for acid to base phosphorylation*

In 1963 AVRON[2] showed that photophosphorylation by swiss chard plastid fragments could be inhibited by EDTA treatment, subsequent centrifugation of the fragments, and removal of the supernatant. This inhibition could be partially reversed by adding back the EDTA supernatant in the presence of $Mg^{2+}$. In the same publication it was demonstrated that the EDTA supernatant contained a non-dialyzable component (or components) which was operationally termed a coupling factor; it presumably linked electron transport to phosphorylation.

JAGENDORF AND URIBE[1] demonstrated that acid to base phosphorylation in spinach plastid fragments was also very sensitive to EDTA, but no reversal experiments were attempted.

A clear indication of the necessity for some EDTA-removable membrane component(s) for acid to base phosphorylation in maize is given by Table IV. Plastid membranes incubated in the presence of their own EDTA extract under conditions which promoted reconstitution of photophosphorylation (in this case an excess of EDTA and the presence of $Mg^{2+}$, *cf.* MCCARTY AND RACKER[9]) exhibited enhanced acid to base incorporation when compared to controls. There was also a considerable

TABLE IV

EDTA-REMOVABLE FACTOR(S) FOR ACID TO BASE PHOSPHORYLATION

Green plastids were isolated as described in MATERIALS AND METHODS through the second centrifugation. Resuspension in 0.01 M NaCl–0.15 mM EDTA (pH 8.0) followed. Incubation for 15 min at room temperature partially removed the factor(s). Etioplast membranes were incubated in a similar manner to obtain the etiolated EDTA extract. Subsequent reconstitution took place in the presence of 0.01 M EDTA–0.1 M $MgCl_2$, and one of the following: green EDTA extract, etiolated extract, or 0.15 mM EDTA in 0.01 M NaCl; incubation: 10 min at room temperature. Acid to base reaction is as described in MATERIALS AND METHODS. Plastid fragments containing 680 $\mu g$ of chlorophyll were used per reaction mixture. Lamellae not treated with EDTA incorporated 125000 counts/min.

| *EDTA-removable factor(s)* | *Counts/min incorporated per ml reaction supernatant* | *Stimulation (%)* |
|---|---|---|
| *Depleted green lamellae incubated with:* | | |
| EDTA in 0.01 M NaCl (control) | 42 206 | — |
| Etiolated EDTA extract | 50 909 | 21 |
| Green EDTA extract | 60 375 | 43 |
| Supernatants alone: | | |
| Etiolated EDTA extract + $Mg^{2+}$ | 545 | |
| Green EDTA extract + $Mg^{2+}$ | (−436) | |

increase in the activity of chloroplast fragments when they were incubated with an EDTA extract of prolamellar body membranes of etioplasts. The conclusion may be drawn from these observations that etioplasts contain at least one of the components necessary for acid to base phosphorylation. The data of LOCKSHIN *et al.*[4] show that maize etioplasts contain a dissociable factor for photophosphorylation.

*Osmotic experiments*

If neither the availability of the coupling factor nor chlorophyll is the limiting factor, what differences between chloroplasts and etioplasts could account for the failure of the latter to carry on acid to base phosphorylation? Plastid fragments from green and etiolated plants differ in their responses to the osmotic properties of their environment.

Fig. 1 shows the effect of increasing molarities of sucrose on the packed pellet volume of green and EDTA-treated green lamellar fragments. It is apparent that both preparations respond in essentially the same manner, suggesting that EDTA has no major effect on membrane integrity or permeability to sucrose. The sucrose concentration of the acid stage also has a clear and marked influence on the acid to base activity of the green fragments (Fig. 1). This effect parallels the osmotic response. Other experi-

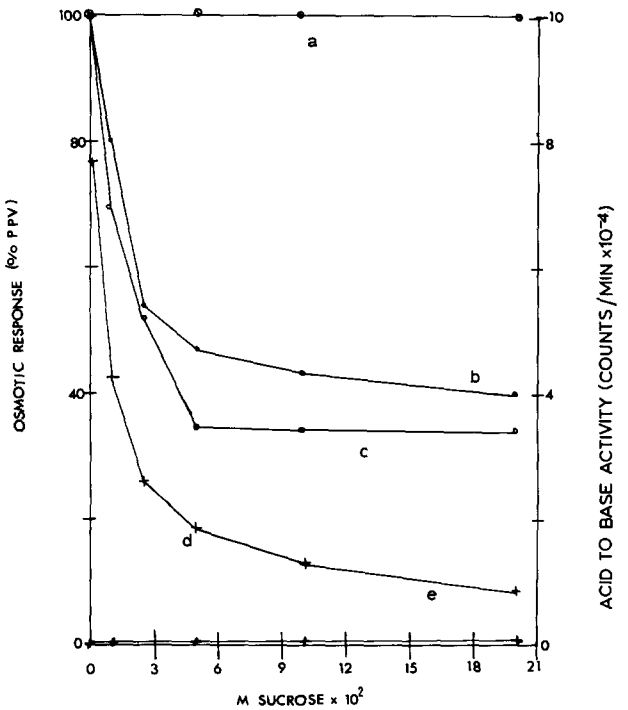


Fig. 1. The effect of sucrose concentration on the acid to base activity and packed pellet volume of green and etioplast fragments. % PPV: the packed pellet volume of an aliquot of plastids resuspended at the indicated molarity of sucrose before centrifugation compared with the packed pellet volume of an equal aliquot of plastids resuspended in 0.01 M NaCl before centrifugation. Plastid isolation, acid to base reaction and packed pellet volume procedures as in MATERIALS AND METHODS. a, etiolated packed pellet volume; b, green packed pellet volume; c, green–EDTA packed pellet volume; d, green acid to base activity; e, etiolated acid to base activity.

ments in this series demonstrate that high concentrations of sucrose do not completely suppress acid to base phosphorylation. At a concentration of 0.5 M sucrose about 10 % as much ATP is still made as when sucrose is omitted during the acid stage.

Fig. 1 also shows that there is no effect of molarity on the volume of etioplast membranes in the range of 0–0.2 M sucrose. Data from other experiments show that etioplast packed pellet volume is the same in 0.01 M NaCl as in 0.01 M NaCl and 0.5 M sucrose. As was shown earlier, the proplastids are incapable of making ATP to any measureable extent by acid to base transition; therefore, as expected, the inclusion of up to 0.5 M sucrose in the acid stage is without effect.

## DISCUSSION

### *Limitations of the method for detecting acid to base phosphorylation in etioplasts*

Are the proplastids completely inactive in catalyzing acid to base phosphorylation, or do they carry out the reaction at reduced levels? This question can be approached only indirectly. The mature green leaves we have usually used contain about 1.5 mg of chlorophyll per g fresh weight of tissue. Typical values obtained for etiolated leaves illuminated for a short period of time (only long enough to convert protochlorophyllide to chlorophyllide), on the other hand, are about 5 $\mu$g pigment per g fresh weight of tissue. Since little if any plastid division is likely to occur during the greening of maize (BOGORAD, unpublished observations), it is not unreasonable to conclude that the average fully green chloroplast contains on the order of 300 times as much pigment as the average proplastid. In studies of acid to base phosphorylation by etioplast membranes, the plastid material employed per reaction mixture usually contained approx. 2.5 $\mu$g of chlorophyllide. This would then be equivalent to assaying green plastid fragments containing 750 $\mu$g of chlorophyll. The latter amount is close to that normally employed in these studies for green fragment acid to base experiments, and would be expected to bring about the incorporation of approx. 100000 counts/min of labeled phosphate into ATP. Therefore, values of 1000 counts/min over background in etioplast fragment assays would have indicated that the plastids were phosphorylating at 1 % of the rate of green fragments. Values this high were observed occasionally but not consistently. Thus, from these considerations, we conclude that if etioplasts can carry on acid to base phosphorylation at all, the rate is less than 1 % of that of green plastid fragments.

It is of interest to compare the development of acid to base activity in greening maize leaves with the development of the capacity to do photophosphorylation. Using the artificial photosynthetic cofactor phenazine methosulfate (PMS)[1] in the absence of inhibitors such as *p*-chlorophenyl-1,1-dimethyl urea (CMU) and with the aid of high levels of radioactivity, we have observed marginal photophosphorylation after 1 h of greening time and clear ATP synthesis after 90 min of light. As would be expected, completely etiolated proplastids show no activity.

The 90 min figure cited for the commencement of photophosphorylation stands in marked contrast to the 150 min figure cited above (*e.g.* Table I) as the time for the earliest reliable manifestation of acid to base activity. The two types of phosphorylation appear to have some of the same prerequisites, (*e.g.* the coupling factor and intact membrane systems). The disparity in the earliest time of appearance of these activities probably results from the far greater sensitivity of the PMS assay. It is

practically impossible to saturate this response with light so, in principle, slight activity can be amplified by using high light intensities and long reaction times. These conditions obtained in the work described above. It seems very likely that plastids from dark-grown maize plants illuminated for 90 min can carry on acid to base phosphorylation, although our data do not show this.

*Final considerations*

URIBE AND JAGENDORF[10,11] have shown in spinach chloroplast fragments that the amount of ATP made in acid to base transition experiments is correlated with the amount of organic acid taken up by the fragments during the acid stage into spaces inaccessible to inulin. Also, acid to base activity and organic acid uptake were affected in the same manner by the tonicity of the medium in which the reaction took place. Furthermore, they found that damaging the plastid membranes by freezing destroyed both acid to base activity and osmotic responsiveness as measured by the packed pellet volume method. The sucrose concentration effects on acid to base activity and packed pellet volume have been corroborated here in green maize plastid fragments (Fig. 1). Also, it has been shown in the present experiments that the effect of sucrose concentration on packed pellet volume is not affected by EDTA, even though EDTA markedly inhibits acid to base activity. Thus, treatment with EDTA does not inhibit acid to base activity by abolishing the osmotic integrity of the plastid membranes. Both osmotic integrity and the presence of an EDTA-removable factor(s) are needed for acid to base activity. Furthermore, light–dark study shows the development of acid to base capacity is not obligatorily linked to chlorophyll deposition in the plastid.

Considering these observations, one may deduce that a system which lacks either the EDTA-removable factor or membrane integrity, or both of these attributes, would be incapable of acid to base phosphorylation and that the presence or absence of chlorophyll in the system may have no bearing on acid to base activity. The etioplast is not capable of measurable acid to base phosphorylation. Changing the concentration of sucrose has no effect on the packed pellet volume of etioplast fragments; perhaps no osmotically responsive enclosed areas are present. An EDTA-removable factor is, however, present in dark-grown plastids in sufficient quantities to reconstitute acid to base activity in green fragments depleted of it. Therefore, assuming that the lack of chlorophyll does not affect the acid to base reaction, one can conclude that the absence of osmotic responsiveness, as measured by packed pellet volume studies, is at least one reason for the etioplast's failure to carry out acid to base phosphorylation.

In considering the lack of response of the etioplast environment, it is useful to delineate two possibilities: (a) in 0.01 M NaCl the etioplast internal membranes are present as closed vesicles which are either completely permeable or completely impermeable to sucrose or water; or (b) under the conditions of our experiments, only open membrane fragments or ruptured vesicles are present and thus no osmotic response is possible. If possibility (b) obtains, then permeability cannot be defined in any meaningful way. On the basis of the data given, it is impossible to distinguish between the alternatives presented above. Preliminary electron micrographs show that etioplast preparations used here consist principally of prolamellar bodies, either naked or partially enclosed by remnants of the plastid outer membrane. If the internal membranes are indeed intact under the conditions of the experiment as suggested by the electron micrographs, then this would argue for proposition (a) above.

The important point which emerges from these observations is that etioplasts treated in the same manner as plastids which are partially or fully green respond differently with respect to acid to base capacity and osmotic sensitivity. As the plastid matures in response to illumination, the osmotic and acid to base properties develop. These developments entail unknown changes in the nature of the plastid membranes but appear to be unrelated to chlorophyll deposition. Acquisition of the capacity for osmotic responsiveness could come about by 'filling holes' in proplastid membranes, by stabilizing them in some way or by synthesis of entirely new membranes. Alternatively, the etioplast membranes might be completely disassembled and new different membranes might be formed; this possibility is remote in view of electron microscopic studies of plastid development (see *e.g.* ref. 12).

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REFERENCES

1 A. T. JAGENDORF AND E. URIBE, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 170.
2 M. AVRON, *Biochim. Biophys. Acta*, 77 (1963) 699.
3 V. K. VAMBUTAS AND E. RACKER, *J. Biol. Chem.*, 240 (1965) 2660.
4 A. LOCKSHIN, R. H. FALK AND L. BOGORAD, 226 (1971) 366.
5 M. AVRON, *Biochim. Biophys. Acta*, 40 (1960) 257.
6 T. CLAUSEN, *Anal. Biochem.*, 22 (1968) 70.
7 L. VERNON, *Anal. Chem.*, 32 (1960) 1144.
8 M. GASSMAN AND L. BOGORAD, *Plant Physiol.*, 42 (1967) 774.
9 R. E. MC CARTY AND E. RACKER, *Brookhaven Symp. Biol.*, 19 (1966) 202.
10 E. G. URIBE AND A. T. JAGENDORF, *Plant Physiol.*, 32 (1967) 697.
11 E. G. URIBE AND A. T. JAGENDORF, *Arch. Biochem. Biophys.*, 128 (1968) 351.
12 J. T. O. KIRK AND R. A. E. TILNEY-BASSETT, *The Plastids*, W. H. Freeman and Co., London, 1967, pp. 489–499.