Regulation of Photosynthetic Electron Transport in Intact Spinach Chloroplasts

I. INFLUENCE OF EXOGENOUS SALTS ON OXALOACETATE REDUCTION\textsuperscript{1,2}

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ABSTRACT

Relatively high concentrations of monovalent salts (150 millimolar) stimulated light-saturated uncoupled rates of O\textsubscript{2} evolution linked to oxaloacetic acid (OAA) reduction by intact chloroplasts 2- to 3-fold. In contrast, monovalent salts partially inhibited light-saturated rates of O\textsubscript{2} evolution coupled to CO\textsubscript{2} fixation and uncoupled rates of nitrite reduction. If the presence of high salt concentration, light-saturated rates of electron transport were about equivalent for all three terminal electron acceptors. It is inferred that exogenous monovalent salts have at least two effects on photosynthetic electron transport, independent of photophosphorylation and CO\textsubscript{2} metabolism: a partial inhibitory effect common to OAA, NO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{2} reduction and a marked stimulatory effect unique to the photosynthetic reductase of OAA.

The stimulation of electron transport to OAA was effected by certain exogenous monovalent salts (KCl or NaCl, but not LiCl). Divalent salts (MgCl\textsubscript{2} or CaCl\textsubscript{2}) and high osmotic strength were ineffective. The salt-induced stimulation was eliminated by low concentrations of phosphate or sulfate (\textlessthanorequalto 6 millimolar) and by higher concentrations of magnesium (\textgreatequalto 30 millimolar). These results suggest that the ion content of the medium (or cytosol) is potentially important in modulating photosynthetic electron transport events in intact chloroplasts.

Studies with isolated intact chloroplasts have shown that certain inorganic and organic substances, normally present in a cell, e.g. Pi and PGA,\textsuperscript{3} can have dramatic effects on photosynthetic metabolism. Thus far, attention has focused primarily on the transport and/or exchange of these substances via specific translocator proteins across the inner chloroplast envelope membrane (9) and on their direct or indirect effects on carbon metabolism (26). Despite the recognized importance of ion fluxes across the thylakoid membrane to primary photochemical processes (3, 11), relatively little is known about the influence of exogenous ions on photosynthesis in intact chloroplasts. An exception has been the observation that Mg\textsuperscript{2+} severely inhibits net O\textsubscript{2} evolution coupled to CO\textsubscript{2} fixation (1, 2, 4, 12, 13). Recent reports suggest that Mg\textsuperscript{2+} specifically inhibits light activation of several enzymes involved in both the Calvin cycle and C\textsubscript{3} dicarboxylic acid pathways. The inhibition was attributed to an indirect effect of Mg\textsuperscript{2+} on K\textsuperscript{+}/H\textsuperscript{+} or phosphate exchange across the chloroplast envelope (4, 12, 13, 15).

The limited interest in the influence of exogenous salts on photosynthesis in intact chloroplasts probably reflects the general assumption that the chloroplast envelope is relatively impermeable to most cations and to a number of anions (5, 7, 14, 25). One might assume that the reactions of intact chloroplasts would be relatively insensitive to the ion content of the medium. There have been several recent reports, however, of cation transport mechanisms (facilitating H\textsuperscript{+} movement or K\textsuperscript{+}/H\textsuperscript{+} exchange) operating across the chloroplast envelope (4, 5, 7, 15).

Here, we report that exogenous salts significantly alter light-saturated rates of photosynthetic electron transport in intact chloroplasts. The nature of the effect (stimulation or inhibition) depends upon the particular electron acceptor employed. Most of the experiments described deal with the 2- to 3-fold stimulation of the light-saturated uncoupled rate of electron transport from water to OAA by certain monovalent salts. A preliminary account of some of this work has been presented (20).

MATERIALS AND METHODS

Intact (type A) chloroplasts were isolated from greenhouse-grown spinach as described previously (23). Chloroplasts (13 \textmu g Chl/ml) were assayed in a medium containing 0.33 M sorbitol, 50 mM Hepes-KOH buffer (pH 8.0), 5 mM Na\textsubscript{2}SO\textsubscript{4}, 2 mM EDTA, 0.25 mM K\textsubscript{2}HPO\textsubscript{4}, and catalase (100 units/ml). Other additions to this assay medium are indicated in the figure legends. O\textsubscript{2} evolution was measured polarographically (19) at 20 C using broad band-pass filters (Schott OG530 and KG3 filters). Isolated chloroplast preparations were \textgreatequalto 75\% intact, as determined by the ferricyanide reduction method (8) and evolved O\textsubscript{2} at rates between 120 and 170 \textmu mol mg Chl\textsuperscript{-1} h\textsuperscript{-1} (uncorrected for broken chloroplasts in the preparations).

RESULTS

In our experience, isolated intact chloroplasts displaying good light-saturated rates (>100 \textmu mol O\textsubscript{2} mg Chl\textsuperscript{-1} h\textsuperscript{-1}) of net O\textsubscript{2} evolution coupled to CO\textsubscript{2} fixation invariably have lower maximum (uncoupled) net O\textsubscript{2} evolution rates under conditions where nitrite is the terminal electron acceptor (Fig. 1). Saturated (uncoupled) rates of O\textsubscript{2} evolution linked to OAA reduction are considerably lower than either nitrite or CO\textsubscript{2}-dependent rates (Fig. 1). In all cases, concentrations of NaHCO\textsubscript{3}, KnO\textsubscript{2}, or OAA in the assay medium were saturating. These saturated rates of O\textsubscript{2} evolution are markedly affected by addition of relatively high concentrations of

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\textsuperscript{3}Abbreviations: PGA: 3-phosphoglyceric acid; OAA: oxaloacetic acid; MA: methylvamine.
KCl to the assay medium (Fig. 1). In general, rates of CO₂ or NO₃⁻ reduction are inhibited (~30-40%) while rates of OAA reduction are stimulated 2- to 3-fold by KCl. In the presence of 150 mM KCl, saturated rates of electron transport are approximately the same whether CO₂ nitrite, or OAA is serving as the terminal electron acceptor.

A number of inferences may be drawn from the data of Figure 1. (a) Both nitrite and OAA seem to be inherently poorer terminal electron acceptors than bicarbonate in intact chloroplasts. This could reflect one or more factors, such as enzyme turnover times, rates of substrate transport across the chloroplast envelope, etc. (b) Exogenous KCl has at least two effects on intact chloroplasts: an inhibitory effect seen during CO₂ and NO₃⁻ reduction, and a stimulatory effect seen during OAA reduction. (c) The observed inhibitory or stimulatory effects are not related to steps involved in photophosphorylation or carbon metabolism. (d) Saturated rates of OAA reduction observed in the presence of KCl probably reflect a summation of an inhibitory effect common to all three terminal acceptors and a stimulatory effect unique to the reduction of OAA by intact chloroplasts.

Several features should be noted regarding the salt-induced increase in the rate of OAA reduction. First, stimulation is observed only at high (near saturating and saturating) light intensities (data not shown). The absence of a salt effect (either stimulatory or inhibitory) at limiting light intensities suggests that exogenous salts are not affecting primary photochemical events; e.g. light absorption, energy distribution, etc. Second, salt stimulation was observed only in the presence of uncouplers, suggesting that the rate of OAA reduction at high light and in the absence of uncouplers was probably limited by the turnover of ATP (note that ATP is not utilized for the reduction of OAA). Finally, KCl stimulation was observed only at OAA concentrations (>0.3 mM) sufficient to saturate O₂ evolution in the absence of KCl. In the presence of KCl, higher OAA concentrations (~2.5 mM) were required to support maximal rates of O₂ evolution.

Figure 2 shows the effect of external KCl concentration on the saturated rate of O₂ evolution (linked to OAA reduction) in intact chloroplasts. Increasing concentrations of KCl result in a progressive increase in the rate of O₂ evolution; saturation occurs at approximately 150 mM. Very high concentrations (~250 mM) of KCl produce less than optimal stimulation (data not shown).

An increase in osmotic potential (high sorbitol concentration) is without effect on uncoupled rates of OAA reduction (Table I). MgCl₂ and CaCl₂ (data not shown) are similarly ineffective, indicating both that external ionic strength is not a primary factor and that stimulation requires a monovalent cation. NaCl and KCl produce about equivalent stimulation, and we have used these salts interchangeably. KNO₃ is reasonably effective. Note, however, that Na⁺ added as Na-gluconate (the gluconate ion is impermeant [7]) and Cl⁻ added as a salt of the impermeant choline cation have marginal effects on rates of OAA reduction. The monovalent salt, LiCl, is also relatively ineffective. It seems that the stimulation of OAA reduction requires the presence of both a permeant anion and certain (permeant?) monovalent cations (see under “Discussion”).

The results in Table I indicate that exogenous divalent cations neither increase nor decrease light-saturated rates of OAA reduction in intact chloroplasts. This observation is confirmed by the results shown in Figure 3 where the effect of various concentrations of MgCl₂ on rates of OAA reduction were compared. In the presence of 150 mM KCl, lower concentrations of MgCl₂ (~50 mM) had no effect, while high concentrations were partially inhibitory. Similar results recently have been reported (4). In contrast, KCl-stimulated rates of OAA reduction were quite sensitive to MgCl₂, being inhibited completely by 30-40 mM MgCl₂. KCl-stimulated rates of OAA reduction were not affected by relatively low concentrations of MgCl₂ (~10 mM), and at higher MgCl₂ concentrations (~30-40 mM) rates were approximately the same in the presence and absence of exogenous KCl. Thus, increasing concentrations of MgCl₂ (~10 mM) appear to inhibit selectively the KCl-induced increase in the rate of OAA reduction. MgCl₂ (2 mM) has recently been reported to inhibit pyruvate + OAA or OAA + MA-dependent O₂ evolution 80-100% in intact mesophyll chloroplasts isolated from the C₃ plant Digitaria sanguinalis (12).

We have found that relatively low concentrations of sulfate and

![Fig. 1. Light-saturated rates of electron transport in intact chloroplasts using different terminal electron acceptors in the presence (E) or absence (C) of added KCl. Where indicated, final concentrations of additions were 10 mM NaHCO₃, 1 mM KNO₃, 2.5 mM OAA, and 30 mM MA.](image1)

![Fig. 2. Effect of KCl concentration on light-saturated uncoupled rates of OAA reduction. Assay medium contained 2.5 mM OAA and 30 mM MA.](image2)

**Table I. Influence of Salts on Light-saturated, Uncoupled Rates of OAA Reduction**

<table>
<thead>
<tr>
<th>Additions to Standard Assay Medium*</th>
<th>Net O₂ Evolution (μmol mg Chl⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
</tr>
<tr>
<td>KCl, 150 mM</td>
<td>86</td>
</tr>
<tr>
<td>NaCl, 150 mM</td>
<td>73</td>
</tr>
<tr>
<td>KNO₃, 150 mM</td>
<td>62</td>
</tr>
<tr>
<td>LiCl, 150 mM</td>
<td>41</td>
</tr>
<tr>
<td>Choline chloride, 150 mM</td>
<td>48</td>
</tr>
<tr>
<td>Na gluconate, 150 mM</td>
<td>28</td>
</tr>
<tr>
<td>MgCl₂, 75 mM</td>
<td>36</td>
</tr>
<tr>
<td>Sorbitol, 300 mM</td>
<td>29</td>
</tr>
</tbody>
</table>

* Assay medium contained 2.5 mM OAA and 30 mM methylamine; other additions as indicated.
phosphate also selectively inhibit salt-induced increases in the rate of OAA reduction. Unstimulated rates of OAA reduction (minus NaCl) were insensitive to external phosphate concentrations up to 10 mM (Fig. 4). Salt-stimulated rates of OAA reduction were, however, progressively inhibited by increasing phosphate. At high phosphate concentrations, the rates approached those observed in the absence of NaCl. Comparable results were obtained with increasing concentrations of sulfate, i.e., a selective inhibition of the monovalent salt-induced increase in the rate of OAA reduction (Fig. 5). A similar inhibition was observed whether sulfate was added as the sodium or magnesium salt (Fig. 5).

Saturated rates of OAA reduction in intact chloroplasts have been reported (6) to be relatively insensitive to external pH. We found a similar lack of pH dependence between pH 7.2 and 8.4 when OAA reduction was measured at low exogenous salt concentrations (Fig. 6). Salt-stimulated rates of OAA reduction, however, were affected by the pH of the assay medium. In particular, salt-induced stimulation was significantly reduced at higher pH.

**DISCUSSION**

The role of ions in the regulation of a wide variety of photosynthetic processes has been well documented. Studies conducted principally with broken chloroplast preparations have shown that photosynthetic yields, quantum distribution, photophosphorylation, thylakoid structure, etc., are markedly affected by the ionic composition of the medium (3, 11). Earlier experiments with isolated intact (CO2-fixing) chloroplasts suggested that ionic com- positional of the cytosol may also be important in modulating photosynthetic activity (1, 2).

Interpretation of studies on ion control of photosynthesis in intact chloroplasts is complicated by the necessity of considering events not normally encountered in experiments with broken chloroplast preparations, especially carbon metabolism and exchange of materials across the chloroplast envelope. In this respect, it should be emphasized that the marked ion-induced stimulation in the rate of OAA photoreduction reported here is not a result of salt effects on photophosphorylation or CO2 metabolism. Stimulation was observed only in uncoupled intact chloroplasts and was unique to the photoreduction of OAA. In contrast, uncoupled rates of nitrite reduction (like coupled rates of CO2 fixation) were partially inhibited by relatively high salt concentrations. Thus, we conclude that net electron transport from water to ferredoxin (the proposed site of nitrite reduction [21]) is partially inhibited by exogenous monovalent salts in intact chloroplasts, and that the observed stimulation of OAA reduction is a result of an effect on steps peculiar to the utilization of OAA as a terminal electron acceptor. These steps include the transport of OAA across the chloroplast envelope via the dicarboxylate translocator (9), light activation (16) of chloroplast NADP-malate dehydrogenase (the...

**FIG. 3.** Influence of MgCl2 concentration on light-saturated uncoupled rates of OAA reduction measured in the presence (●) or absence (○) of added KCl. Other conditions as in Figure 2 except that Na2HPO4 was omitted from the assay medium.

**FIG. 4.** Influence of Pi concentration on light-saturated uncoupled rates of OAA reduction measured in the presence (●) or absence (○) of added NaCl. Other conditions as in Figure 2.

**FIG. 5.** Effect of sulfate concentration on light-saturated uncoupled rates of OAA reduction measured in the presence (●) or absence (○) of added NaCl. Note that the single data point (X) refers to the effect of 6 mM MgSO4 on the NaCl-stimulated rate of O2 evolution. Other conditions as in Figure 2.

**FIG. 6.** Influence of assay medium pH on light-saturated uncoupled rates of OAA reduction measured in the presence (●) or absence (○) of added NaCl. Other conditions as in Fig. 2.
envelope in transfer of reducing equivalents from NADPH to OAA), and the rate of catalysis by this enzyme. The influence of salts on these phenomena will be the subject of a subsequent report.

The mechanism(s) by which exogenous salts affect photosynthetic electron transport in intact chloroplasts is, at present, uncertain. An important consideration is that the chloroplast envelope, in particular the inner membrane, represents a barrier to the free exchange of neutral and charged species between the cytosol (or medium) and the chloroplast stromal compartment (9). Yet the observed stimulatory and/or inhibitory effects of certain exogenous salts, e.g. KCl, on electron transport events (Fig. 1) imply a direct or an indirect effect of external KCl on stromal ion composition. Although several envelope translocator systems (the proton translocator, a dicarboxylate translocator, and an adenosine nucleotide translocator) have been characterized (9), relatively little information exists concerning fluxes of other ions across the chloroplast envelope. Several studies have suggested that the inner envelope membrane is relatively impermeable to inorganic cations, e.g. Mg2+, K+, etc., but quite permeable to a number of anions, e.g. Cl−, NO3−, and acetate (5, 7). Cation exchange systems have, however, been proposed to operate across the chloroplast envelope. A light-induced H+ efflux associated (in part) with K+ influx was shown earlier to occur in intact chloroplasts (5). Recently, evidence for a (dark) diffusion-driven K+ (Na+)/H+ exchange system, modulated by Mg2+ has been reported (4, 15). We infer that the effects of exogenous KCl (or NaCl) observed in this study may reflect altered cation distribution between the chloroplast stroma and the external medium (or cytosol). In this regard, the importance of stromal K+ concentration in regulating photosynthetic electron transport to O2 has recently been suggested from studies of slow fluorescence quenching in intact chloroplasts (24).

The data presented regarding the stimulation of OAA reduction by exogenous KCl (or NaCl) do not enable us to dismiss the possibility that salt effects are exerted at the level of the envelope. Dicarboxylic acid translocator function might be quite sensitive to ionic environment. They are, however, consistent with the idea that stromal K+ (or Na+) levels are significantly altered (possibly by means of the reported K+/H+ exchange systems [4, 15]) in the presence of high external KCl. This is supported by the observation that some monovalent cations, but no divalent cations, elicit the effect. Furthermore, the decrease in stimulatory effect at high pH would be consistent with K+ uptake limited by the availability of H+ for exchange (note that an increase in medium pH produces an increase in stromal pH [9]).

We have found that Mg2+ (>10 mM), inhibits the monovalent cation-induced stimulation in the rate of OAA photoreduction (Fig. 3). Phosphate and sulfate have a similar inhibitory effect although at lower concentrations (Figs. 4 and 5). This could result from an effect of phosphate, sulfate, and Mg2+ on the transport of OAA via the dicarboxylate transporter on the proposed envelope K+/H+ exchange systems (see above) or on both. In this regard, it should be noted that Pi has been reported not to affect the dicarboxylate translocator (18) and that divalent cations, at least at relatively low concentrations (≤1 mM) only marginally (≤30%) affect light-induced K+/H+ exchange in intact chloroplasts (5). Likewise, the Mg2+ concentrations we found to be inhibitory (>10 mM) are severalfold higher than those reported to modulate the (dark) K+/H+ exchange systems (4, 15). We have difficulty equating our results with the proposed inhibition (directly by phosphate or indirectly by Mg2+) of light activation of NADP-malate dehydrogenase as suggested from studies with the C4 plant D. sanguinaca (12). First, with spinach chloroplasts, inhibition (by Mg2+) was observed only at relatively high divalent cation concentrations. Second, inhibition (by Mg2+, Pi, or sulfate) was observed only when rates of OAA reduction were stimulated by the addition of monovalent cations.

It should be emphasized that the salt concentrations that mark-

edly alter saturated rates of photosynthesis are within reported physiological levels in the cytosol (17, 22). The importance of the chemical composition of the cytoplasm to understanding photosynthesis in vivo has already been discussed with respect to the potential modulation of certain enzymes involved in CO2 metabolism (4, 10, 12, 13, 15). Our results suggest that photosynthetic electron transport events in intact chloroplasts (independent of photophosphorylation and CO2 metabolism) likewise may be influenced by exogenous electrolytes. Since electron transport to different terminal electron acceptors in intact chloroplasts shows different sensitivities to exogenous salt, ion concentrations in the medium (or cytosol) may also be important in regulating the partitioning of reducing equivalents within the chloroplast.

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