Structure and Activity of Chloroplasts of Sunflower Leaves Having Various Water Potentials

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Summary. Changes in membrane integrity, conformation and configuration, and in photosystem II (PS II) activity (measured as dichloroindophenol photoreduction) of sunflower (Helianthus annuus L.) chloroplasts were studied after leaf tissue had been desiccated to various water potentials (ψw). Fixatives for electron microscopy were adjusted osmotically to within 1 bar of the ψw of the tissue to prevent rehydration during fixation. PS II activity decreased to 50% of the control activity at a ψw of −26 bar. At this ψw, leaf viability was being lost but there was virtually no loss of integrity of the thylakoid lamellar system. Even at extreme ψw (below −100 bar), thylakoids retained much structural detail but were less stained. At −26 bar, intrathylakoid spacing (configuration) and lamellar thickness (conformation) were decreased in vivo. Upon isolation of the plastids, the differences in configuration disappeared but the differences in conformation remained. The decreases in membrane conformation and PS II activity both, in vivo and in vitro suggest that alterations in conformation may cause decreases in chloroplast activity at ψw as low as −26 bar. Since structural detail was maintained, however, previous observations of altered membrane integrity, which involved tissue fixed without osmotic support, may have been affected by tissue rehydration during fixation.

Introduction

Chloroplasts decrease in activity when leaves are subjected to low water potentials (ψw). Although most of the partial reactions of photosynthesis are affected (Nir and Poljakoff-Mayber, 1967; Fry, 1970, 1972; Boyer and Bowen, 1970; Potter and Boyer, 1973; Keck and Boyer, 1974), electron transport by PS II (Boyer and Bowen, 1970; Potter and Boyer, 1973; Keck and Boyer, 1974; Mohanty and Boyer, 1976) and photophosphorylation (Nir and Poljakoff-Mayber, 1967; Keck and Boyer, 1974) are particularly inhibited. The effects can be observed with chloroplasts lacking the outer membrane (Potter and Boyer, 1973; Keck and Boyer, 1974), which suggests that the changes occur at the level of the thylakoid membranes. Ultrastructural alterations also have been observed in thylakoids of leaves having low ψw (Aliciava et al., 1971; Kurkova and Motorina, 1974; Da Silva et al., 1974; Giles et al., 1974, 1976) and consist of thylakoid unstacking (Aliciava et al., 1971), thylakoid vesiculation (Aliciava et al., 1971; Kurkova and Motorina, 1974), and loss of contrast in thylakoid lamellae (Da Silva et al., 1974). Although comparisons between ultrastructure and activity have been made (Aliciava et al., 1971; Kurkova and Motorina, 1974), they have not been detailed enough to show whether changes in chloroplast activity are consistently accompanied by changes in chloroplast ultrastructure, and consequently the relationship remains uncertain. In the following study, we have examined chloroplast ultrastructure and the low ψw-induced inhibition of chloroplast activity over a range of ψw. Our objective was to determine whether changes in chloroplast structure could cause losses in chloroplast activity at low ψw.

One of the problems in a structural study of desiccated tissue is the possibility of rehydration during fixation when standard aqueous fixatives are used. Chloroplasts can recover activity in a few minutes when tissue rehydrates (Potter and Boyer, 1973) and rehydration has been observed in desiccated root tissue during aqueous fixation for observation under
the electron microscope (Nir et al., 1969). We avoided this problem by fixing tissue in the presence of osmotic support that was always within 1 bar of the leaf $\psi_w$.

Materials and Methods

Plant Material and $\psi_w$ Measurement

Sunflower plants (*Helianthus annuus* L., cv. Russian Mammoth) were grown from seed in soil in a controlled environment (temperature, day = $30 \pm 1^\circ$; night = $24 \pm 1^\circ$; irradiation = 0.19 cal cm$^{-2}$ min$^{-1}$ [fluorescent lamps, daylight-type]; photoperiod = 14 h). When plants were 3–3.5 weeks old, leaves from the third and fourth nodes were used for the experiments. Leaf $\psi_w$ was varied either by withholding water from the soil for up to 4 days (slow desiccation), or by excising one-half of the leaf blade and allowing it to dry in the controlled environment room (rapid desiccation). The other half of the leaf remained attached to the plant and served as the control. As shown previously (Potter and Boyer, 1973), excision of the leaf tissue does not alter chloroplast activity. Leaf $\psi_w$ was measured with a thermocouple psychrometer by the isopiestic technique (Boyer and Knipping, 1965).

Electron Microscopy

Samples for measuring $\psi_w$ and for electron microscopy were obtained rapidly from portions of the leaf blade immediately adjacent to each other. The tissue for microscopy required ca. 30 s to be transferred from the growth environment at high irradiance to the fixative at low irradiance. Control tissue was fixed in 1.5% glutaraldehyde (v/v) in 0.05 M phosphate buffer, pH 7.2, at 4 $^\circ$C. Postfixation was in 2% aqueous OsO$_4$ (w/v) overnight in the cold. Other samples were stored at -20 $^\circ$C for 1–3 days before fixation, in which case no loss of activity was observed. The tissues were immersed in fixative for 2 h at 4 $^\circ$C. Portions of the tissue were dehydrated in acetone and embedded in Epon 812. Thin sections were cut on an LKB-Huxley ultramicrotome using a diamond knife. The grids were stained with 2% uranyl acetate (w/v) and lead citrate, and were viewed with an RCA-EMU-4 electron microscope.

Measurements of Chloroplast Activity

Immediately after sampling for $\psi_w$ and electron microscopy, the remaining tissue was used to isolate chloroplasts for measuring PS II activity. The assay consisted of determining the initial rates of DCIP photoreduction by the chloroplasts according to the procedures for chloroplast isolation and assay reported previously (Keck and Boyer, 1974). Upon completion of the assays, glutaraldehyde was added to the chloroplast suspension to a final concentration of 1.5% (v/v) to prepare the isolated chloroplasts for electron microscopy. The chloroplasts were centrifuged at 10,000 x g for 5 min and the remaining post-fixation procedure was as above for electron microscopy. To achieve an accurate representation of the composition of the in vitro population, 9–10 samples were taken from various levels of the isolated pellet for each experiment.

Results

1. Leaf Water Potential, Chloroplast Structure and Activity

If fixative having $\psi_w$ of -6.5 bar was used to fix leaf tissue from plants adequately supplied with water (Fig. 1a, tissue $\psi_w$ = -5.3 bar), chloroplasts contained distinct grana with moderately tight stacking of the thylakoid lamellae. The limiting membranes of the chloroplasts were intact (Fig. 1a). When the same fixative was used after the tissue had been desiccated, thylakoid lamellae were loosely stacked (Fig. 1b, tissue $\psi_w$ = -21.9 bar). On the other hand, if sucrose was added to the fixative to provide osmotic support for the desiccated tissue, virtually all grana lamellae were tightly stacked (Fig. 1c; Table 1). Since the osmotically supported fixation was most likely to preserve cell structure, all further fixation was done with osmotic support.

Table 1. Thickness of intrathylakoid spaces and thylakoid lamellae of control and desiccated chloroplasts in vivo fixed with and without osmotic support

<table>
<thead>
<tr>
<th>Sample ($\psi_w$)</th>
<th>Fixative ($\psi_w$)</th>
<th>Intrathylakoid space (Å)</th>
<th>Thylakoid lamellae (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-5.3 bar)</td>
<td>Glutaraldehyde (-6.5 bar)</td>
<td>84 ± 8</td>
<td>151 ± 16</td>
</tr>
<tr>
<td>Desiccated (-21.9 bar)</td>
<td>Glutaraldehyde (-6.5 bar)</td>
<td>62 ± 17</td>
<td>140 ± 6</td>
</tr>
<tr>
<td>Desiccated (-21.9 bar)</td>
<td>Glutaraldehyde plus sucrose (-21.1 bar)</td>
<td>42 ± 7</td>
<td>118 ± 6</td>
</tr>
</tbody>
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