Photosynthesis under osmotic stress

Inhibition of photosynthesis of intact chloroplasts, protoplasts, and leaf slices at high osmotic potentials

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Abstract. 1. Photosynthesis of leaf slices, mesophyll protoplasts, and intact chloroplasts of spinach was inhibited in hypertonic sorbitol solutions. Sorbitol could be replaced by other nonpenetrating osmotica such as sucrose or glycinebetaine. As a penetrating solute, ethyleneglycol was also inhibitory, but osmolarities required for inhibition of photosynthesis were considerably higher than in the case of non-penetrating osmotica. - 2. With leaf slices and protoplasts, 50% inhibition by sorbitol was usually observed at osmotic potentials between 25 and 40 bar. With isolated intact chloroplasts, the osmotic potentials producing 50% inhibition varied considerably. Depending on the growth conditions of the plant material, 50% inhibition occurred between 14 and 40 bar. The integrity of the chloroplast envelope as measured by the accessibility of the thylakoid system for ferricyanide was not affected by osmotic stress. - 3. Quantum requirements for CO₂ assimilation and reduction of 3-phosphoglycerate or nitrite by intact chloroplasts increased under osmotic stress. The increase was larger for CO₂ reduction than for reduction of 3-phosphoglycerate or nitrite. - 4. In intact chloroplasts, electron transport to methylviologen was not much affected by osmotic stress. Basal electron transport was not stimulated, suggesting absence of uncoupling. - 5. The increase in ATP/ADP ratios on illumination of intact chloroplasts was slower at an osmotic potential of 36 bar than at 11 bar. - 6. The results indicate that inhibition of photosynthesis is not caused by the sensitivity of a single photosynthetic reaction to increased osmotic potentials. Rather, several reactions are sensitive to water stress. Osmotic stress acts on the photosynthetic apparatus mainly at the level of dark reactions and ATP synthesis, and much less on primary photoreactions or electron transport between water and the primary oxidant of photosystem I. - 7. The different sensitivity of chloroplasts to penetrating and non-penetrating solutes and the observed variability of chloroplast sensitivity to stress suggests that the reduction in water potential is not directly responsible for damage to the photosynthetic apparatus during osmotic stress. Rather, the composition of the chloroplasts appears to be a decisive factor which determines sensitivity or resistance to osmotic stress.

Key words: Chloroplast - Photosynthesis (under stress) - Protoplast - Spinacia - Water stress.

Introduction

Under water stress, leaves are known to close their stomata, and this is generally believed to be the cause of reduced carbon gain under periods of drought. Stomatal oscillations have been shown to be accompanied by similar oscillations of CO₂-assimilation. Oscillations of water potential were out of phase (Barrs 1968; Hopmans 1971). This suggests that photosynthesis is under stomatal control. However, there are also indications that direct inhibition of photosynthesis by water stress can decrease stomatal conductivity (Wong et al. 1979). Under moderate or severe stress photosynthesis appears to be limited not only by increased stomatal resistance but also by damage at the chloroplast level (Boyer and Bowen 1970; Boyer 1971; Boyer and Potter 1973; Jones 1973; Keck and Boyer 1973; Plaut 1971; Plaut and Bravdo 1973; Santarius and Ernst 1967; Santarius 1967; Santarius and Heber 1967).

Many of the earlier investigations on this subject used as their experimental material chloroplasts isolated by a standard procedure from wilted leaves with decreased water potentials (Boyer and Bowen 1970; Boyer 1971; Boyer and Potter 1973; Keck and Boyer
Since during wilting, leaf water potentials often decrease far below the water potentials of the standard isolation media (about -10 bar), chloroplasts obtained in this way are exposed to hypotonic stress during isolation, and this has recently been shown to severely affect photosynthesis of intact chloroplasts (Kaiser et al. 1981).

Further, only those effects of low water potential can be expected to be observed under these conditions, which are essentially irreversible. Even more important, if leaves are wilted in the light, it will be difficult to distinguish between direct effects of water stress on the photosynthetic apparatus and secondary damage caused by photoinhibitory processes which may be favored under these conditions (Krause et al. 1978; Osmond et al. 1980). We have therefore investigated effects of water stress on the physical integrity and function of isolated intact chloroplasts by isolating chloroplasts capable of high rates of photosynthesis by addition of non-penetrating polyols (Plaut 1971; Plaut and Bravdo 1973). Since extrapolation of data obtained by these methods to the situation in vivo is difficult, but of crucial importance, some key experiments were not only performed with intact chloroplasts, but also with isolated mesophyll protoplasts or with leaf slices. The latter are close to the in vivo situation, but are nevertheless independent of limitations caused by stomatal resistance.

In the following paragraphs, inhibition of photosynthesis of chloroplasts, protoplasts, and leaf slices by osmotic stress is compared, and attempts are made to reveal main targets of osmotic stress on the photosynthetic apparatus.

Materials and methods

Intact chloroplasts were isolated from freshly harvested spinach leaves (Spinacia oleracea L. cv. “Monatol”), according to Cockburn et al. (1968), with the following modifications: The blending medium contained 0.33 M sorbitol, 0.05 M MES-KOH (pH 6.5), 1 mM MgCl₂, 1 mM MnCl₂, 2 mM EDTA. After the first centrifugation, the chloroplasts were suspended and centrifuged again in a medium containing 0.05 M HEPES-KOH, pH 7.6, instead of MES-KOH. In some experiments, chloroplasts were isolated according to Heber (1973), with essentially similar results. The reaction medium for CO₂-dependent O₂ evolution contained in addition 2 mM or 4 mM KHCO₃, 0.2 to 0.5 M KH₂PO₄, and 1,000 U ml⁻¹ catalase. The percentage of intact chloroplasts was routinely determined in 0.5-ml aliquots of neutralized HC₁₀₄-extracts by the ferricyanide method (Heber and Santarius 1970). It varied between 70 to 95%.

Spinach mesophyll protoplasts were obtained by a modification of the technique of Edwards et al. (1978). After removing the lower epidermis, the leaves were incubated in a digestion medium at 28°C in the dark for 3 h. The protoplast suspension was purified by centrifugation on a discontinuous gradient (5 ml 500 mM sucrose + 2 ml 400 mM sucrose with 100 mM sorbitol + 1 ml 0.5 M sorbitol, all in 5 mM MES-KOH, pH 6.0). The basic constituents of the digestion medium were 1 mM CaCl₂, 500 mM sorbitol, 5 mM MES-KOH, pH 5.5. In addition, this medium contained 2.5% cellulase Onozuka SS and 0.5% macerozym R 10, 0.1% bovine serum albumine and 0.05% polyvinylpyrrolidone (molecular weight 10,000). The assay medium was identical with the chloroplast assay medium, but contained the sorbitol concentrations given in the legends.

For preparation of leaf slices, discs (12 mm diameter) were cut from the leaves and sliced to 500-μm broad strips with razor blades (compare Jones 1973; Jones and Osmond 1973). Slices from 4 discs (containing approximately 150 μg chlorophyll) were collected and floated in 4 ml infiltration medium, which was identical to the chloroplast resuspension medium (see above) and contained various sorbitol concentrations. The slices were infiltrated for about 2 min by gently evacuating and releasing the vacuum several times. Effective infiltration was indicated by a change in light-reflecting properties of the slices which became transparent green and sedimented readily. After infiltration, KHCO₃ and NaH₄CO₃ (10 mM, 6.29 Bq μmol⁻¹) were added and after 10 min in the dark, the slices were placed on a shaker, covered with a large petri dish containing a 1 cm-layer of water as a heat filter, and illuminated from the top with 160 W m⁻² white light. After incubation, the slices were removed from the medium, frozen in liquid nitrogen, and ground to a fine powder. Two ml 4.5% HClO₄ were added, and the homogenate was extracted at 0°C. Then, 0.2 ml of the homogenate were acidified with glacial acetic acid, flushed with air until dry, and counted by liquid scintillation.

O₂ evolution of chloroplasts and protoplasts was measured polarographically under illumination with 600 W m⁻² red light at 20°C.

The osmolarity of various solutions was calculated from the freezing point depression which was determined with a Knauer Semi-Microosmometer Typ M 21.20. ATP and ADP were determined in 0.5-ml aliquots of neutralized HClO₄-extracts by the luciferin-luciferase assay (Strehler 1970). Chlorophyll contents of chloroplasts, protoplasts, and leaf slices were obtained by Arnon's method (1949).

Results

The envelope of intact chloroplasts is rather impermeable to polyols such as sorbitol or sucrose. Isolated intact chloroplasts respond to increased concentrations of sorbitol as near-perfect osmometers (Kaiser et al. 1981), and similar behavior should be expected for isolated mesophyll protoplasts. Thus, the main effect of increasing the polyol concentration in a suspension of chloroplasts or protoplasts is an increase in osmotic potential of the external solution which causes a decrease in the osmotic volume and a corresponding increase in the internal solute concentration. The internal composition of chloroplasts and protoplasts and their physical integrity should, at least at the first observation, not be expected to be altered. The term “osmotic potential” refers to the potential osmotic pressure (π) of a solution in equilibrium with water and should not be confused with the osmotic component of the water potential (ψπ), which has a negative value.

Figure 1 compares the response of CO₂-dependent O₂ evolution of isolated chloroplasts with their integ-