Chloroplast acclimation to low osmotic potential

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ABSTRACT

Photosynthetic potential of isolated chloroplasts was investigated during in situ water deficits. An eight day stress cycle imposed on spinach plants reduced leaf ψw by 0.57 MPa, and leaf νw by 0.50 MPa, resulting in partial turgor maintenance during the stress cycle. Pressure/volume curves confirmed the occurrence of osmotic adjustment. Leaf ψw depression was associated with an altered response of chloroplasts to low νw in vitro. Optimum reaction medium νw for photosynthesis shifted from -1.04 to -1.57 MPa, and low νw was not as inhibitory to photosynthesis of plastids pre-exposed to stress in situ. These data indicate that plastids acclimate to low external νw in response to leaf water deficits. This response was still evident four days after a stress cycle ended, but was nearly reversed eight days after stress. Repeated stress cycles in situ did not increase the degree of chloroplast acclimation to low νw in vitro. Fast dehydration of leaves did not induce this apparent chloroplast acclimation.

ABBREVIATIONS

νw - osmotic potential; ψw - water potential; PEG - polyethylene glycol 8000; MPa - Megapascals

INTRODUCTION

Chloroplasts isolated from well-watered plants are routinely suspended in isotonic medium (-0.0 MPa) consisting of 0.33 M sorbitol when used for photosynthetic, and other metabolic studies. Berkowitz and Gibbs (1982a,b; 1983a,b) have investigated the effects of decreasing reaction medium νw on chloroplast metabolism. These studies indicate that exposure of plastids to higher concentrations of a non-penetrating solute such as sorbitol inhibited photosynthesis by 40 to 60%, respectively, at -1.57 MPa and -2.09 MPa. Significantly, lowering the medium νw to -2.09 MPa with a solute (ethylene glycol) which penetrates the chloroplast limiting membrane inhibited photosynthesis by only 15%. It was concluded from these data that when chloroplasts are exposed to low νw, they dehydrate until the stromal νw equilibrates with the external νw. It is not low stromal νw per se which inhibits the activity of photosynthetic carbon reduction cycle enzymes, but rather the stromal volume reduction which occurs during in vitro dehydration. It should be noted that the level of stress (ψw depression of up to 1 MPa) used in the cited studies is commonly encountered by field grown crop plants. Boyer (1985) has suggested that stromal volume reduction may cause inhibitions of chloroplast photosynthetic potential in situ, during water stress episodes. The goal of the research presented in this report is to determine whether in situ leaf water deficits modulate the response of chloroplasts to low ψw in vitro. The rationale was to induce osmotic adjustment (which lowers cell ψw at any hydration state) in plants exposed to a water stress cycle and to monitor the photosynthetic rate of chloroplasts isolated from this plant material at varying ψw in vitro.

MATERIALS AND METHODS

Week old seedlings (4/pot) of spinach (Spinacia oleracea L., cv. 'Winter Bloomdale') were transplanted to 21-cm clay pots of sand and grown with complete fertilizer (Berkowitz and Whalen, 1985) for six weeks under well watered conditions in a growth chamber (14 h daylength) at 250-290 umol·m⁻²·s⁻¹ light, at constant 21°C and 50% rh. Pots were then either irrigated with water or subjected to stress cycles (eight days without water). Fully expanded, nonsenescing leaves sampled after 5-7 h light were used for all studies. Leaf ψw and νw (i.e., νw of frozen and thawed leaf discs) were measured with a pressure bomb and thermocouple psychrometers, respectively (plants from three pots were sampled as replicates). Leaf turgor was calculated from the difference. Leaves were rehydrated (petioles cut twice under water and then kept at 5°C for 4 h) prior to pressure/volume determinations using the techniques of Cutler et al. (1979). Intact chloroplasts were isolated from water stressed and control plants in grind medium consisting of 0.33 M sorbitol, 50 mM Hepes-NaOH (pH 6.8), 2 mM Na₂EDTA, 1 mM MnCl₂, and 50 mM NaCl, using a 40% Percoll cushion as described previously (Berkowitz and Gibbs, 1982a). Photosynthetic rates in medium containing 50 mM Hepes-NaOH (pH 7.6), 2 mM Na₂EDTA, 1 mM MnCl₂, 0.25 mM KH₃PO₄, 5 mM NaHCO₃, 1000 u catalase and varying reaction medium νw (i.e., sorbitol) were ascertained as described previously (Berkowitz and Gibbs, 1982b) by measuring O₂ evolution or ¹⁴CO₂ fixation.

RESULTS AND DISCUSSION

Leaf νw of plants exposed to an eight day stress cycle declined from -0.67 to -1.24 MPa, and then rose to -0.91 MPa one day after rewatering (data not shown). Leaf ψw also declined after the first six days of this cycle, thus maintaining positive turgor. ψw dropped from an initial value of -1.00 to -1.50 MPa towards the end of the stress cycle, and rose to -1.22 MPa one day after rewatering. The decline in...
were determined to have an osmotic strength of -0.90 MPa at 100% relative water content, while this value was -1.138 at the end of a stress cycle. Pressure/volume analysis confirmed medium chloroplasts isolated from plants under this. Control leaves (mean of three replications) relative water content, while this value was -1.138 at the end of a stress cycle.

Chloroplasts isolated from plants during the first were isolated from water stressed plants in medium increased sorbitol. The osmotic strength of chloroplasts from -1.04 MPa decreased to -1.57 MPa still resulted in a stimulation of the photosynthetic rate (data not shown), as shown in Figure 1 for chloroplasts isolated from stressed plants. This suggests that chloroplast acclimation to low \( V_f \) in vitro (Fig. 1) was likely not due to changes in stromal \( V_f \) occurring during the isolation procedure. The experiment shown in Figure 1 was repeated again with a second set of plants; the results were similar. It appears, then, that in situ stress cycles shifted photosynthesis response to in vitro \( V_f \) depression. Matthews and Boyer (1984) have recently demonstrated that photosynthesis acclimation to low leaf \( V_f \) can involve physiological changes at the chloroplast level. It is interesting to note that leaf \( V_f \) was depressed by -0.5 MPa at the end of the stress cycle (day 8, insert of Fig. 1) and the optimum \( V_f \) in vitro shifted by 0.5 MPa. These data, then, indicate that in-leaves that are osmotically adjusting in response to water deficits, chloroplasts became somewhat less sensitive to low \( V_f \) in vitro.

Leaf osmotic adjustment is well known to allow for maintenance of cell turgor, which can result in increased cell growth and stomatal conductance during leaf water deficits. Stress acclimation, as evidenced in Figure 1, which could possibly reduce these adversarial effects of water deficit-induced low leaf \( V_f \) on chloroplast photosynthesis is, then, an additional way in which osmotic adjustment could maintain metabolite functions in leaves of droughted plants. One possible explanation for the effects shown in Figure 1 is an accumulation of solutes in the stroma of chloroplasts during dehydration and osmotic adjustment. It can be hypothesized that if solutes are accumulated and/or produced within the chloroplast during in situ leaf osmotic adjustment, the most severe effects of exposure to low \( V_f \) in vitro (induced due to stromal volume reduction upon exposure to low \( V_f \) ) on chloroplast photosynthesis could, then, be reduced. An alternate explanation for the stress induced chloroplast acclimation to low \( V_f \) shown in Figure 1 could be that during in situ leaf osmotic adjustment, the level of a stromal solute which is inhibitory to chloroplasts exposed to low \( V_f \) in vitro may be reduced. Boyer (1983) has presented preliminary data which indicate that stromal Mg level interacts with chloroplast dehydration inhibition of photosynthesis. Turner and Jones (1983) have indicated that the rate of water stress imposition affects the capability of plants to osmotically adjust; fast dehydration does not allow for the altered cell metabolism which results in solute accumulation and/or production. Therefore, experiments were conducted to determine if chloroplast acclimation to low \( V_f \) occurred when leaf \( V_f \) was lowered at a much faster rate. In order to induce a level of leaf \( V_f \) depression similar to the eight day stress cycle, chloroplasts were placed in PEG solutions of -1.2MPa. Initial leaf \( V_f \) of -0.56MPa declined to -1.11MPa after 80 min. Concomitant with this leaf \( V_f \) decline, leaf \( V_f \) declined only 0.10MPa over the incubation period. This rapid leaf \( V_f \) decline, without substantial \( V_f \) depression, had different effects on the photosynthetic response of subsequently isolated chloroplasts to in vitro stress than the 8 day stress cycle. Chloroplasts prepared from the PEG treated plants did not demonstrate any acclimation to low \( V_f \) (Fig. 2). In fact, in the experiment shown in Figure 2, photosynthesis of chloroplasts isolated from plants exposed to the PEG, 'fast dehydration' treatment was inhibited extent to a greater extent than the rate of the controls. These data indicate that an in situ water deficit induced too quickly to allow for leaf osmotic adjustment does not lead to chloroplast acclimation.