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Effects of Temperature on the Hill Reaction and Photophosphorylation in Isolated Cactus Chloroplasts[1]

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ABSTRACT

Chloroplasts isolated from *Opuntia polyacantha* Haw. (Cactaceae) are capable of noncyclic electron transport and ATP synthesis. Hill reaction rates, measured by O_2 evolution or by ferricyanide reduction, increase with increasing temperature to approximately 40 C. The temperature optimum of NADP reduction is 42 C while the optimum for noncyclic photophosphorylation is 35 C. NADP-linked phosphorylation exhibits a higher coupling ratio (P/e_2) than ferricyanide-linked photophosphorylation. The temperature optima for photochemical energy production correlate with photosynthetic properties of Crassulacean acid metabolism (CAM) plants and are discussed in relation to the operation of CAM at high tissue temperature.

Crassulacean acid metabolism is characterized by an initial nonautotrophic fixation of CO_2 into organic acids at night (13). The acids are decarboxylated during the day and the CO_2 generated is refixed into sugars via the pentose phosphate pathway (11). The operation of this pathway is dependent upon the photochemical production of both NADPH and ATP. There is little information available on photochemical processes in CAM[2] plants and in particular how these processes may complement this form of carbon metabolism. To investigate this relationship we have chosen the cactus *Opuntia polyacantha* from a plant community characterized by warm temperatures. In this paper we report the effects of temperature on the ability of chloroplasts isolated from *O. polyacantha*, to evolve O_2, photoreduce ferricyanide and NADP, and generate ATP.

MATERIALS AND METHODS

Plant Material. Plants of *O. polyacantha* Haw. were collected at the IBP field station in Colorado (shortgrass prairie) and transported to Washington State University. Plants were pretreated in a growth chamber programmed for a 15-hr photoperiod and a 25/15 C thermoperiod for a minimum of 2 weeks prior to chloroplast extraction. Quantum irradiance at plant height varied from 300 to 600 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Plants were grown under well watered conditions and were supplied with nutrient solution weekly.

Isolation of Chloroplasts. Young, actively expanding stems were collected 6 to 7 hr into the light period. After this material was obtained all further operations were performed in dim light and at 2 C. Stems were cut into small pieces while immersed in ice-cold extraction medium (50 mM Tricine [pH 8.1], 0.5 M sorbitol, 50 mM NaCl, 5 mM EDTA, mM $MgCl_2$, and 0.5 mg/ml BSA) in a ratio of 20 ml of extraction medium/g of plant material. The cut pieces were infiltrated in this medium under vacuum for 5 min to maximize chloroplast stability (7), then ground for 12 sec at half-line voltage and 8 sec at full line voltage in a Sorvall Omnimixer. To remove whole cells, the preparation was accelerated to 1,000*g* and allowed to decelerate in a Sorvall RC2-B centrifuge. The supernatant was transferred to new tubes and centrifuged for 5 min at 3,000*g* at 2 C. The chloroplast pellet was resuspended in extraction medium without EDTA. Chloroplasts prepared in this manner were stable for over 2 hr in their capacity for both electron transport and photophosphorylation and all experiments were completed within this time period. Measurements of O_2 evolution, ferricyanide and NADP reduction, and phosphate esterification were repeated on two to four separate chloroplast extractions. Chl concentrations were determined using the method of Arnon (1).

Isolated chloroplasts were prepared for electron microscopy by fixing in 2% glutaraldehyde buffered with 100 mM K-phosphate buffer (pH 7.3). They were subsequently postfixed with OsO_4, dehydrated through a standard series of ethanol, and embedded in Ladd Plastic. The chloroplasts were stained with uranyl acetate and lead citrate and examined using a Hitachi HU-125 electron microscope.

O_2 Evolution. O_2 evolution was monitored over a temperature range of 15 to 45 C by a Clark-type O_2 electrode. Light, provided by a projector bulb, was filtered through 12 cm of copper sulfate solution and an additional 8 cm of water. The reaction mixture (5.8 ml), containing 86 mM sorbitol, 50 mM Tricine (pH 8.1), 50 mM NaCl, 5 mM $MgCl_2$, 2 mM ferricyanide, and chloroplasts (20 μg Chl/ml), was allowed to equilibrate for 5 min at the desired temperature prior to illumination. O_2 evolution was monitored on a strip chart recorder and rates were calculated from an initial linear portion of the recording.

Ferricyanide and NADP Reduction. Ferricyanide and NADP reduction were determined by the spectrophotometric methods of Trebst (18), and over similar temperature ranges as O_2 evolution. Ferricyanide reduction was measured using a mixture (1.45 ml) containing chloroplasts (8-12 μg Chl/ml); 86 mM sorbitol, 50 mM Tricine (pH 8.1), 50 mM NaCl, 5 mM $MgCl_2$, 2 mM K_2HPO_4, 2 mM ADP, and 1 mM ferricyanide. Immediately following 1 min of saturating irradiance, trichloroacetic acid was added to a final concentration of 2%. Chloroplasts were pelleted by centrifugation and the absorbance of the supernatant determined at 420 nm. Dark controls showed no ferricyanide reduction.

The experimental conditions for measuring NADP reduction were identical except for the deletion of ferricyanide from the reaction medium and the addition of 3 μM purified spinach ferredoxin (Sigma) and 0.66 mM NADP. After 1 min of irradiance the reaction mixture was centrifuged and *A* determined at 340 nm.

Phosphate Esterification. Rates of ATP formation were deter-

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[2] Abbreviation: CAM: Crassulacean acid metabolism.

mined with either ferricyanide or NADP as the electron acceptor by following the incorporation of $^{32}PO_4$ into ATP. The amount of incorporation was determined according to Avron (2). Phosphorylation did not proceed in the absence of light.

RESULTS

Electron microscope examination of isolated chloroplasts confirmed the presence of a normal internal membrane structure. The lack of a limiting membrane indicates that chloroplasts were class II (16).

Electron transport as measured by O_2 evolution (Fig. 1) and the reduction of either ferricyanide (Fig. 2) or NADP (Fig. 3) has a temperature optimum in the range of 37 to 42 C. Photophosphorylation with either ferricyanide or NADP as the electron acceptor has a lower temperature optimum of 35 C (Figs. 2 and 3). All of the above reactions show approximately a 3-fold increase between the lowest analysis temperature (approximately 15 C) and the respective temperature optima with the exception of ferricyanide reduction. This latter process increases more than 4-fold between 15 and 40 C.

The ratio of phosphate esterified to electron transport (P/e_2) declines progressively with temperature with ferricyanide as the electron acceptor (Fig. 2). With NADP as the electron acceptor the P/e_2 remains close to unity up to 35 C above which uncoupling occurs (Fig. 3).

DISCUSSION

Electron transport in *O. polyacantha* is maintained at higher temperatures than photophosphorylation. In other plants photophosphorylation shows greater sensitivity to heat than electron transport (8, 10, 15), and enhanced electron transport at high temperatures reflects thermal uncoupling (6, 8). Our results also indicate that isolated cactus chloroplasts exhibit a differential coupling of electron transport to phosphorylation dependent upon the electron acceptor. NADP-linked phosphorylation exhibits a higher P/e_2 than ferricyanide-linked phosphorylation, especially at high temperatures (Figs. 2 and 3). This difference could reflect a relative increase in reduction of ferricyanide at PSII (19) as membranes become heat-damaged.

The temperature response of O_2 evolution, NADP reduction, and ferricyanide reduction indicates an optimum temperature for electron transport in cactus chloroplasts at approximately 40 C. This optimum is higher than reported for specific photochemical processes in other plants. Temperature optima for the Hill reaction among geographically diverse *Verbascum thapsus* populations range from 10 to 35 C (20). Arctic populations of *Deschampsia caespitosa* exhibit a 20 C optimum while alpine populations show a 30 C optimum (17). These studies reporting temperature optima for the Hill reaction do not address the temperature response of ATP synthesis, limiting interpretation of the adaptive significance. The 40 C temperature optimum for electron transport and the 35 C optimum for photophosphorylation in cactus chloroplasts clearly indicate the potential for photochemical energy production necessary for CO_2 fixation at high temperatures, and are consistent with optima demonstrated for photosynthetic processes associated with CAM. Rouhani *et al.* (14) report maximum CO_2 fixation of *Sedum* protoplasts between 35 and 40 C. In *Aloe* leaf slices, O_2 evolution was found to increase with temperature to the highest analysis temperature of 35 C (4). In intact plants, maximum CAM activity, as determined from diurnal changes in acidity, is found when days are warm and nights are cool (9). Osmond *et al.* (12) report that in

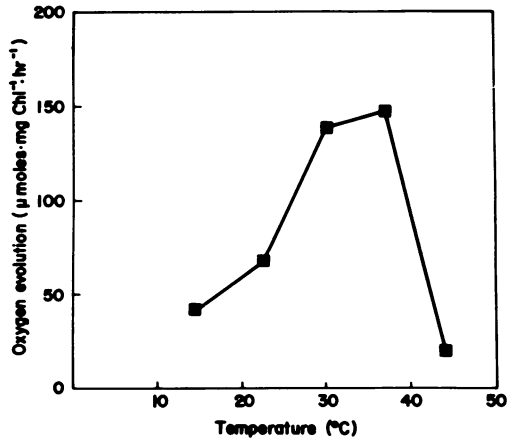

FIG. 1. Effect of temperature on O_2 evolution. Data points are means of two replicates and are typical of all experiments.

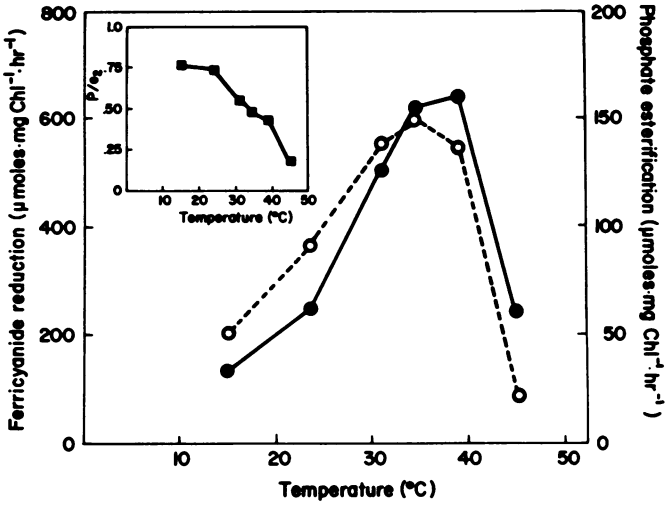

FIG. 2. Effect of temperature on ferricyanide reduction and photophosphorylation. O- - -O: phosphate esterification; ●——●: ferricyanide reduction. Inset is ratio of photophosphorylation to electron transport (P/e_2). Both ferricyanide reduction and phosphate esterification data points are means of two replicates and are typical of all experiments.

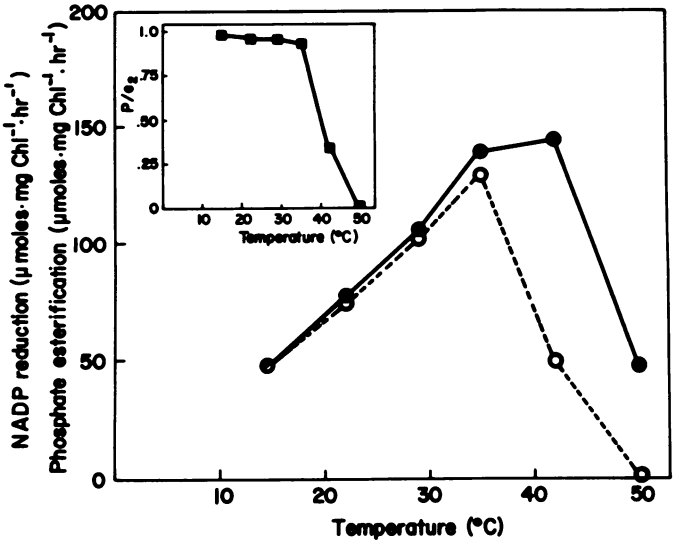

FIG. 3. Effect of temperature on NADP reduction and photophosphorylation. O- - -O: phosphate esterification; ●——●: NADP reduction. Inset is ratio of photophosphorylation to electron transport (P/e_2). Both NADP reduction and phosphate esterification data points are means of two replicates and are typical of all experiments.

addition to the low nighttime temperature requirement for optimal growth of *Kalanchoë daigremontiana*, high daytime temperatures are required. Rates of malate decarboxylation and the concomitant generation of a CO_2 source for autotrophic fixation in CAM plants have been shown to increase with temperature in the light (3, 5). The increase in photochemical energy production to relatively high temperatures would help provide for maximum carbon flow through the energy-dependent pentose phosphate pathway.

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