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Letter to the editor

The effect of chilling in the light on photophosphorylation

Analysis of discrepancies between in vitro *and* in vivo *results*

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Photosynthesis in chilling-sensitive plants is swiftly and severely inhibited when exposure to low temperatures ($0 < T < 12°C$) is accompanied by illumination (e.g., Long et al. 1983). In the search for those steps in photosynthesis which may account for this low temperature lability, many of the component processes of photosynthesis have been investigated. Photophosphorylation, appropriately, has received attention because of the central role that ATP plays in photosynthesis in general and chloroplast metabolism in particular. There has emerged a divergence of views regarding the effect of a low temperature and light treatment on photophosphorylation in chilling-sensitive species. In a recent comprehensive study, Terashima et al. (1989a) provide strong evidence that photophosphorylation is significantly inhibited in thylakoids isolated from cucumber leaves chilled in the light. A contemporary study by Wise and Ort (1989), on the other hand, demonstrates that the in situ activity of the coupling factor in the same species treated in essentially the same manner remains fully competent. Our intent here is to highlight the pertinent data and provide a unifying explanation for the seemingly contradictory results reported in the literature and by our two laboratories.

Kislyuk and Vas'kovskii (1972) were the first to look directly at the question of photophosphorylation competence after a chilling and light treatment. They reported that, coincident with a large reduction in $^{14}CO_2$ fixation by cucumber cotyledons, chilling in the light caused a precipitous decline in both cyclic and non-cyclic photophosphorylation in thylakoids isolated from chill-treated tissue. Garber (1977) extended this approach showing that cyclic photophosphorylation and proton uptake activity declined in proportion to a decreased capacity of the thylakoids to perform trypsin-activated ATP hydrolysis. Although somewhat indirect, these data implicated the catalytic portion of the coupling factor (i.e., CF_1) in the inhibition of photophosphorylation. Peeler and Naylor (1988a,b) chilled whole cucumber plants in the light then measured photosynthesis and the effect that an added uncoupler had on electron transport in thylakoids isolated from these plants. They found that CO_2 assimilation by intact leaves was severely inhibited (1988a) and that uncoupler treatment of isolated thylakoids failed to further stimulate the rate of electron transport (1988b). Thus, they concluded, as had Kislyuk and Vas'kovskii (1972) and Garber (1977) before them, that the chilling and light treatment of cucumber plants uncoupled ATP formation from electron transport in isolated thylakoids.

Terashima and co-workers (1989a,b) recently reinvestigated this issue using direct and definitive procedures to define the involvement of CF_1 in this inhibition of photophosphorylation. Although electron transport was not significantly affected in thylakoids isolated from cucumber

leaf discs immediately after they had been chilled in the light, photophosphorylation and proton uptake were obliterated. The addition of DCCD, an inhibitor that blocks proton efflux through the transmembrane proton conducting portion of the coupling factor complex (i.e., CF_0), restored most of the coupling and proton uptake, providing very strong evidence that the CF_1 complex had been lost from the photosynthetic membranes. Indeed, analysis of the membrane preparations by gel electrophoresis showed that the α and β subunits of CF_1 were all but lost after the chilling and light treatment.

Thus, there can be little doubt that, in cucumber thylakoids isolated from plants immediately after a simultaneous exposure to chilling temperatures and illumination, ATP formation is uncoupled from electron transport due to release of CF_1. However, there remains the important question of whether the uncoupling induced by CF_1 release is the cause of the persistent, largely irreversible inhibition of photosynthesis in cucumber plants after chilling in the light. Wise and Ort (1989) investigated this issue by measuring the effect of chilling and light on photophosphorylation competence in intact cucumber leaves which had been allowed to rewarm for 30 minutes after the chilling treatment.

The principle of the intact leaf measurement is that the electric field formed across the thylakoid membrane as a consequence of proton accumulation can be monitored through the effect that this electric field has on a special subset of the photosynthetic pigments within the membrane; the so-called 'electrochromic shift' (Witt 1979). The relaxation kinetics of this absorption change can provide direct information about coupling factor activity and ATP formation since the proton efflux which drives ATP synthesis is the dominant ionic current involved in thylakoid membrane depolarization. Fairly complex kinetics, indicative of the catalytic activation state of the coupling factor, can be resolved in whole leaves using these procedures (Kramer and Crofts 1989, Ort et al. 1990) as can effects of environmental stress on photophosphorylation (Ortiz-Lopez et al. 1987, Wise and Ort 1989, Ortiz-Lopez et al. 1990, Wise et al. 1990).

Following a chilling and light treatment sufficient to inhibit photosynthesis by 50%, the decay kinetics of the electrochromic shift were unchanged from that of the control cucumber plants and indicative of rapid ATP formation capability (Wise and Ort 1989). This study provided strong evidence that there is no uncoupling, or inactivation of coupling factor, in light-chilled cucumber plants that exhibit a large and persistent inhibition of net photosynthesis.

Based on the data already summarized, as well as some unpublished observations to be mentioned briefly below, the consensus interpretation of the data from our two laboratories is that:

i) Chilling in the light induces a reversible (see below) condition in cucumber that permits the release of CF_1 during thylakoid purification but,
ii) The inhibition of net photosynthesis that persists after rewarming is not directly due to any effect of chilling and light on photophosphorylation competence.

An important factor in explaining the difference between the in vitro data of Terahsima et al. (1989a,b) and the in vivo measurements of Wise and Ort (1989) appears to involve the different length of rewarming used in the two studies. Terashima and coworkers have extended their earlier work on thylakoids by isolating chloroplasts with intact outer envelopes. Consistent with the electrochromic change measurements taken on intact leaves by Wise and Ort (1989), intact chloroplasts isolated after leaves were rewarmed for thirty minutes showed good coupling between electron transfer and ATP formation (Terashima and Katoh, unpublished). An intriguing additional preliminary observation of Terashima's is that even intact chloroplasts display uncoupling unless chilled and irradiated leaves are given a minimal rewarming period (e.g., 30 min) prior to plastid isolation, possibly suggesting that CF_1 reassociates during the short incubation at warm temperatures.

Thus, a long standing polemic in the literature has been resolved; uncoupling of electron transport is not the direct cause of inhibited net photosynthesis in chilling-sensitive cucumber. It now remains to be determined if the transient uncoupling observed in isolated, intact chloroplasts at low temperatures is indicative of a first

step in a cascade of events that occurs in intact leaves and leads to an inhibition of photosynthesis that persists even after coupling has been restored.

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