Regular paper

Reduced levels of cytochrome \( b_6f \) in transgenic tobacco increases the excitation pressure on Photosystem II without increasing sensitivity to photoinhibition in vivo

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Abstract

We have examined tobacco transformed with an antisense construct against the Rieske-FeS subunit of the cytochrome \( b_6f \) complex, containing only 15 to 20% of the wild-type level of cytochrome \( f \). The anti-Rieske-FeS leaves had a comparable chlorophyll and Photosystem II reaction center stoichiometry and a comparable carotenoid profile to the wild-type, with differences of less than 10% on a leaf area basis. When exposed to high irradiance, the anti-Rieske-FeS leaves showed a greatly increased closure of Photosystem II and a much reduced capacity to develop non-photochemical quenching compared with wild-type. However, contrary to our expectations, the anti-Rieske-FeS leaves were not more susceptible to photoinhibition than were wild-type leaves. Further, when we regulated the irradiance so that the excitation pressure on photosystem II was equivalent in both the anti-Rieske-FeS and wild-type leaves, the anti-Rieske-FeS leaves experienced much less photoinhibition than wild-type. The evidence from the anti-Rieske-FeS tobacco suggests that rapid photoinactivation of Photosystem II in vivo only occurs when closure of Photosystem II coincides with lumen acidification. These results suggest that the model of photoinhibition in vivo occurring principally because of limitations to electron withdrawal from photosystem II does not explain photoinhibition in these transgenic tobacco leaves, and we need to re-evaluate the twinned concepts of photoinhibition and photoprotection.

Abbreviations: Chl – chlorophyll; DCMU – 3-(3',4'-dichlophenyl)-1,-dimethylurea; \( F_0 \) and \( F_0' \) – minimal fluorescence when all PS II reaction centers are open in dark- and light-acclimated leaves, respectively; \( F_m \) and \( F_m' \) – maximal fluorescence when all PS II reaction centers are closed in dark- and light-acclimated leaves, respectively; \( F_v \) – variable fluorescence \( (F_m - F_0) \) in dark acclimated leaves; \( F_v' \) – variable fluorescence \( (F_m' - F_0') \) in light-acclimated leaves; NPQ – non-photochemical quenching of fluorescence; PS I and PS II – Photosystem I and II; \( P_680 \) – primary electron donor of the reaction center of PS II; PFD – photosynthetic flux density; \( Q_A \) – primary acceptor quinone of PS II; \( q_P \) – photochemical quenching of fluorescence; \( V+A+Z \) – violaxanthin + antheraxanthin + zeaxanthin

Introduction

Photo inhibition is the loss of photosynthetic efficiency when the light-harvesting antennae absorb excitation energy in excess of that used for photosynthesis (Powles 1984; Osmond 1994). Of the four macromolecular complexes found in thylakoid membranes, Photosystem II (PS II) is the most vulnerable to dam-
age from excess irradiance, although damage to Photosystem I (PS I) has also been shown (Sonoike and Terashima 1994). In the field, the point where excitation capture exceeds the capacity for electron withdrawal from PS II varies, with effects of fluctuations in temperature, water and nutrient status superimposed on fluctuations in irradiance. Consequently, the responses of plants to variations in energy capture requirements are complex, and include both avoidance and tolerance mechanisms.

A range of avoidance mechanisms have been described for higher plants, including both long-term acclimative and fast dissipative mechanisms. Long-term responses include modulation of thylakoid composition (Anderson and Osmond 1987) and changes to leaf reflectance (Robinson et al. 1993). Fast avoidance mechanisms, estimated collectively by non-photochemical quenching of chlorophyll fluorescence, dissipate absorbed photons as heat and rapidly down-regulate PS II energy capture efficiency during excess irradiance. Both reaction center and antenna-based mechanisms have been implicated in non-photochemical quenching of chlorophyll fluorescence, and the principal driving force behind these dissipative reactions is the development of the thylakoid ΔpH (Briantais et al. 1979; Krause et al. 1982). Low lumenal pH has been shown to slow electron donation from water to P680+ and under these conditions excitation energy trapped by PS II may be dissipated as heat by fast internal charge recombination (Krieger and Weis 1990). The mechanistic basis for antennae-quenching, which is also driven by the ΔpH and is thought to be enhanced by the activity of the xanthophyll cycle (reviewed by Demmig-Adams and Adams 1992; Pfundel and Bilger 1994), remains unclear. Current models include both direct (Chow 1994; Frank et al. 1994; Owens 1994) and indirect (Ruban et al. 1992, 1993; Mohanty et al. 1995; Gilmore et al. 1996a, b) quenching roles for the xanthophyll zeaxanthin. However, the primary site(s) and mechanism(s) involved in antenna-quenching, and the degree to which such quenchers can divert energy from PS II, has not been established unequivocally. An important characteristic of these putative antennae-based photoprotective mechanisms is that they develop coincidently with the reduction of PS II electron acceptors, and as far as is known, they do not respond directly to the reduction state of the PS II electron acceptor pool.

The principal mechanism that enables higher plants to tolerate excess irradiance is the D1 protein repair cycle. Light-dependent turn-over of the D1 protein subunit of the PS II core was early identified with photoinhibition (Mattoo et al. 1981; Kyle et al. 1984) and net photoinhibition has been shown to occur when the rate of D1 protein degradation exceeds the capacity for D1 protein synthesis (Greer et al. 1986; Aro et al. 1993a,b). However, D1 protein synthesis has been shown to saturate at low irradiances (Aro et al. 1993b, 1994; Park et al. 1996a), and pea leaves are known to have the same intrinsic capacity for D1 protein synthesis regardless of growth irradiance (Park et al. 1995a,b, 1996a,b). Furthermore, the efficacy of this tolerance mechanism will vary depending on the particular environmental conditions (e.g. low temperature) that prevail, and when combined with high irradiance, results in photo-inhibition.

A model has begun to emerge from the many biochemical and biophysical studies that suggests photoinhibition of PS II can occur because of either limitations in electron delivery to oxidised PS II reaction centers (donor-side limitation) or because of a limited capacity for electron withdrawal from PS II (acceptor-side limitation). In vitro studies have shown that D1 degradation can occur because of cleavage on either the lumenal or stromal side of the thylakoid membrane (De Las Rivas et al. 1992; Aro et al. 1993b). However, the evidence for which of these limitations is primarily responsible for photo-inhibition in vivo in higher plant leaves remains equivocal. Some reports suggest primarily acceptor-side related damage (De Las Rivas et al. 1993; Shipton and Barber 1994) while others suggest significant donor-side related degradation of the D1 protein (Russell et al. 1995; Kettunen et al. 1996).

In normal higher plant leaves the coincident development of PS II reaction center closure, leading to acceptor-side limitations, and lumen acidification, which may give rise to donor-side limitations by slowing electron donation from water to P680+ (Krieger and Weis 1990), can only be broken by chemical means such as with the use of uncouplers (e.g. nigericin) or inhibitors of electron transport (e.g. DCMU). In this paper we describe experiments using tobacco transformed with an antisense mRNA construct against the transcript for the Rieske-FeS subunit of the cytochrome b8f complex (Price et al. 1995). Even at low irradiances, the electron carriers between P680 and the cytochrome b8f complex are highly reduced in these leaves, and under these conditions little non-photochemical quenching develops. The principal aim of these experiments was to test whether photoinhibition in vivo occurs primarily as the result of the over-reduction of electron carriers on the acceptor-side