Photoinhibition, 77K chlorophyll fluorescence quenching and phosphorylation of the light-harvesting chlorophyll-protein complex of photosystem II in soybean leaves*

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Abstract. When the capacity of leaves for orderly dissipation of excitation energy in photosynthesis is exceeded, one mechanism by which the excess energy appears to be dissipated is through a non-radiative decay process. This process is observed as a reversible quenching of chlorophyll fluorescence emission (77K) from both photosystem II and photosystem I which persists in darkness (Demmig and Björkman 1987, Planta 171, 171–184). Fluorescence quenching was induced in soybean (*Glycine max* (L.) Merr.) leaves by two methods: 1) changing the composition of the gas surrounding the leaf from normal air to 2% O₂, 0% CO₂ at a low, constant photon flux density (PFD = photon fluence rate), and 2) increasing the PFD in the presence of normal air. In either case the quenching was fully reversible after return to the original condition (low PFD, normal air). The half-time of the relaxation of the quenching was in the order of 30 min. Both treatments resulted in reversible dephosphorylation of the light-harvesting chlorophyll-protein complex of photosystem II (LHC-II). Treatment under photoinhibitory conditions (high PFD plus chloramphenicol) also caused dephosphorylation of LHC-II. Therefore, phosphorylation of LHC-II cannot account for the observed fluorescence quenching. In addition, our results indicate that in vivo a factor other than the redox state of the plastoquinone pool controls LHC-II phosphorylation. This factor may be ΔpH, the pH gradient across the thylakoid membranes.

Key words: Chlorophyll fluorescence quenching – Light-harvesting chlorophyll-protein complex – ΔpH – *Glycine* (photoinhibition) – Phosphorylation (LHC-II) – Photoinhibition (photosynthesis).

Introduction

Plants are subject to a constantly changing light environment. In order to maintain maximum photosynthesis at all light levels and to avoid damage by overexcitation the plant must be able to regulate the distribution of incoming excitation energy. On a physiological scale such regulation is sometimes achieved by leaf and chloroplast movement. At the molecular level it can be observed as changes in the photosystem II/photosystem I (PSII/PSI) stochiometries, the concentration of electron-transport components and antenna size. However, the latter changes essentially only allow the plant to adapt to long-term light conditions. The mechanisms available to the plant to cope with short-term changes in light levels are even less well understood. State transitions involving a change in the distribution of light energy between the photosystems have been invoked. Evidence for a process by which the plant harmlessly removes excess excitation energy by a non-radiative dissipation has recently been obtained (Demmig and Björkman 1987).
This increase in energy loss by non-radiative dissipation was manifested as a general and reversible quenching of chlorophyll (77K) fluorescence emission from both PSII and PSI. This type of fluorescence quenching could be induced by excess excitation at 1) high light levels where it was superimposed on effects caused by photoinhibitory damage (probably to the PSII reaction centers; Cleland et al. 1986), and 2) in weak light by removal of the terminal electron acceptor (a CO2-free gas stream containing 2% O2 and 98% N2) (Demmig and Björkman 1987). Both instantaneous (F0) and maximum (Fm) fluorescence at 692 nm and 734 nm were quenched and this was interpreted as reflecting an increased non-radiative energy dissipation in the pigment bed. Since 734-nm fluorescence was also quenched, increased distribution of excitation energy to PSI at the expense of PSII was unlikely to be the explanation for the quenching of PSI fluorescence.

Light is known to induce phosphorylation of thylakoid membrane proteins, including the light-harvesting chlorophyll-protein complex of PSII (LHC-II) (Bennett 1979). Phosphorylation of LHC-II has been suggested to be controlled by the redox state of the mobile plastoquinone (PQ) pool (Allen et al. 1981). It is also widely considered to decrease the rate of excitation of PSII while increasing the rate of excitation of PSI and to play an important role in light regulation of photosynthesis (for a review, see Horton 1985). It has also been suggested that LHC-II phosphorylation may have a protective function against photoinhibition (Haworth et al. 1982; Horton 1983; Horton and Lee 1985). Under conditions of high PFD or removal of the terminal electron acceptor (e.g. in an atmosphere of 2% O2, 0% CO2) the plastoquinone pool is likely to become more reduced. It has therefore of interest to explore the possibility that the fluorescence quenching observed in such treatments is related to an increased phosphorylation of LHC-II.

Material and methods

**Plant material.** Glycine max (L.) Merrill (soybean) cv. Corsoy was grown from seed (DeKalb-Pfizer Genetics, Beaman, Ia., USA) in an air-conditioned greenhouse (February–March 1985). The mean daily photon receipt by the plants was approx. 15 mol m⁻² d⁻¹. The plants were watered daily with a solution containing, in mM, 3 K, 1.3 Ca, 1 Mg, 2.5 N (as nitrate), 1 P, 1 S, 0.1 Fe-EDTA, and standard micronutrients. Plants had not yet flowered; the leaves had reached about 70-100% of full expansion.

**Experimental treatments of the leaves.** All exposures of leaves to changes in photon flux density (PFD) or gaseous composition were made on intact leaves. A single leaf was sealed into a gas-exchange cuvette and exposed to the desired PFD in a humidified gas stream of either air (21% O₂, 0.034% CO₂) or a CO₂-free mixture of 2% O₂ and 98% N₂. The leaf temperature was kept constant at 25°C. The leaf was held normal to the light beam so that the upper surface received direct radiation. Light was provided from a multivapor arc lamp, equipped with a diffusor and a water filter; for details see Demmig and Björkman (1987). Chloramphenicol (CAP) treatment: one leaflet of a trifoliate leaf was pretreated, prior to the 32P labelling, with CAP as described by Greer et al. (1986). The upper surface of the leaflet was gently abraded and, during a 30-min period, was repeatedly dipped into a 0.31 mM CAP solution containing Tween-20 (polyoxyethylene sorbitan monolaurate). During the CAP treatment the plant was kept in dim roomlight.

**Chlorophyll fluorescence determinations.** Measurements of 77K fluorescence emission were made as described by Björkman and Demmig (1987). Leaf samples were always kept in total darkness (air, room temperature) for 5 min before freezing. The redox state of Q (the acceptor for PSII) was estimated from room-temperature chlorophyll fluorescence using a pulse-modulation chlorophyll fluorometer (Model PAM; H. Walz, Effeltrich, FRG) as described by Schreiber et al. (1986). The ratio Qred/Qtotal was taken to equal (Fm-Fo)/(Fm-Fp), where Fm is the fluorescence emission in the light, Fo the emission during a 1-s saturating flash in the light, and Fp the emission in the presence of the weak, modulated measurement beam alone, determined within 2 min after the actinic light was extinguished.

**Protein-phosphorylation determinations.** A trifoliate leaf of Glycine max was allowed to take up [32P]-orthophosphate (Amer- sham, Arlington Heights, Ill., USA) from a carrier-free solution (18 MBq in 2 ml H₂O) through the cut common petiole for 30 min. The solution was then replaced with water and the treatment was begun. At different times during the treatments a leaf disc was removed for 77K-fluorescence analysis and the remaining leaflet detached and immediately immersed into liquid N₂. Thylakoids were prepared by homogenizing the frozen leaflet and leaf disc in ice-cold buffer containing 0.3 M sorbitol, 50 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid-(Hepes)-NaOH, pH 8.0, 15 mM NaCl, 5 mM MgCl₂, 0.1% bovine serum albumin and 10 mM NaF (to inhibit protein-phosphatase activity; Bennett 1980). After filtration through Miracloth (Behring Diagnostics, La Jolla, Cal., USA), thylakoids were centrifuged, washed and resuspended in the same buffer. Separation of thylakoid proteins was performed according to Ryrie (1983) using a resolving gel containing a gradient of 10 to 22.5% acrylamide/bisacrylamide (39:1) overlaid by a 7% polyacrylamide stacking gel (Biorad, Richmond, Cal., USA), 0.1% lithium dodecylsulphate (LDS; Sigma Chemical Co., St. Louis, Mo., USA) was present in the gel and in the upper reservoir buffer. Samples were dissociated in 0.1 M 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl, pH 6.8, 13% glycerol, at an LDS :dithiothreitol:chlorophyll ratio of 50:50:1 and heated for 90 s at 100°C. Ten micrograms chlorophyll were loaded per lane. Electrophoresis was carried out in a laboratory-built apparatus overnight at constant current. The temperature was maintained at 4°C. Molecular-weight standards obtained from Biorad were run concurrently with the thylakoid samples. The standards were lysozyme 14 kilodaltons (kDa) trypsin inhibitor 22 kDa, carbonic anhydrase 31 kDa, ovalbumin 45 kDa, bovine serum albumin 66 kDa and phosphorylase B 93 kDa. Gels were stained with Coomassie Brilliant Blue (Sigma) prior to drying. Autoradiograms were prepared by exposing the dried gel to X-ray-sensitive film (XAR-5; Eastman Kodak, Rochester, N.Y., USA).