Concepts in Photobiology: Photosynthesis and Photomorphogenesis
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16. How Higher Plants Respond to Excess Light: Energy dissipation in photosystem II

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Summary

In this chapter we present current views concerning the adaptation and acclimation of the higher plant photosynthetic apparatus to excess light levels. The primary focus is at the level of the chloroplast thylakoid membrane which is where the primary events of photosynthetic energy transduction occur. We first summarize our current understanding the molecular composition and macromolecular organization of Photosystem II (PS II) because these factors pertain directly to photosynthetic function during environmental stress. We then discuss the biochemical and biophysical interpretations obtained from the most commonly used tool for probing the photosynthetic function of PS II, namely PS II chlorophyll (Chl) *a* fluorescence. We explain how PS II Chl *a* fluorescence yield measurements have provided insights into the dynamic relationships between the primary photosynthetic light-energy transduction processes and the important light adaptation and acclimation strategies utilized by PS II. The basic biochemical and biophysical aspects of the light-energy dissipation, avoidance and damage-repair mechanisms that influence PS II function are discussed in relation to a physiological gradient of increasing environmental stress. The future areas of research interest and importance regarding the optimization and preservation of PS II function during environmental stress are also briefly highlighted.

1. Introduction

In many natural environments the photosynthetic apparatus of higher plants is exposed to light levels that significantly exceed the photosynthetic capacity (Long et al., 1994). As will be explained in this chapter, excess light levels are potentially harmful to the pigments and proteins of the photosynthetic apparatus. Therefore, higher plants must adapt and acclimate to the light levels in their environment to optimize and preserve photosynthetic productivity (see e.g., reviews by Demmig-Adams and Adams, 1996; Osmond and Grace, 1995; Srivastava and Strasser, 1997). Optimizing photosynthetic productivity in a given light environment requires balancing the use of absorbed light for photosynthesis with the safe, photoprotective dissipation of potentially harmful excess light energy (Björkman and Demmig-Adams, 1994; Demmig-Adams

et al., 1996; Horton et al., 1996). Further, in order to maintain photosynthetic productivity, a balance must be struck between light-induced damage and repair of light-induced damage to the component pigments and proteins of the photosynthetic apparatus (Chow, 1994; Andersson and Barber, 1996).

The goal of this chapter is to outline our current understanding of the adaptation and acclimation of the higher plant photosynthetic apparatus to excess absorbed light levels experienced by the plant during environmental stress. However, in this chapter we focus on the effects of the environmental stress factors in relation to the basic biochemical, bioenergetic and biophysical aspects of photosynthetic light-energy transduction, as well as the primary photoprotective and light-induced damage-repair mechanisms. The discussion is based on an integration of several recent findings with the established, classical biochemical and biophysical principles in this research area. The main intent is to explain how plants optimize and preserve the function of the photosynthetic apparatus during environmental extremes wherein the biochemical processes of photosynthesis may be attenuated by various factors, such as temperature, carbon-dioxide concentration and water availability, among others. To obtain a complete understanding, the chapters by A. Mattoo et al., B. Logan et al., and C. Critchley in this book must also be consulted.

Although the photosynthetic apparatus in higher plants essentially utilizes two separate Photosystems, PS I and PS II, that act in concert to transduce light energy into biochemical reducing potential and high-energy phosphate (Whitmarsh and Govindjee, 1995; also see their chapter in this book), we focus here on specific characteristics of PS II. This is both because the adaptation and acclimation of PS II is very highly characterized and also because the functioning of PS II serves as one of the most informative indicators of the integrated functioning of the entire photosynthetic apparatus (see Baker, 1996; Bricker and Ghanotakis, 1996; Diner and Babcock, 1996; Satoh, 1996 for various aspects of PS II). We emphasize here the utility and basic principles of one of the primary research tools used to probe the function of PS II, namely, Chl *a* fluorescence analysis (for reviews on Chl *a* fluorescence, see various chapters in Govindjee et al., 1986; Govindjee, 1995; Joshi and Mohanty, 1995). It is important to realize that measurements of fluorescence intensity (F = the number of emitted photons) alone do not necessarily reflect the quantum yield of fluorescence, (i.e., Φ_f = number of photons emitted/number of photons absorbed by the fluorescing system), which itself is the parameter related to the rate constants (i.e., the number of transitions per unit time) of the various deexcitation pathways (e.g., fluorescence, f; photochemistry, p; heat loss, h; and energy transfer, tr). As described below,

$$F = \Phi_f \cdot I_a = \Phi_f \cdot f_a \cdot I_i, \tag{1}$$

where I_a is the number of photons absorbed by the fluorescing system, I_i is the number of photons incident on the entire system, and f_a is the fraction of light absorbed by the fluorescing system. In addition to changes in Φ_f,

changes in, f_a, that are directly influenced by changes in the absorption cross section, σ, of the fluorescing system can also strongly influence the measured "F". On the other hand, the lifetime of fluorescence (τ_f) is related to Φ_f as follows:

$$\Phi_f = \tau_f/\tau_o, \tag{2}$$

where $\tau_o = 1/k_f$ is the intrinsic lifetime of fluorescence when the only deexcitation pathway is fluorescence; τ_o is assumed to be constant.

Based on the above arguments it is easy to see why combined measurements of the steady-state fluorescence intensity (F) and time-resolved components of Chl *a* fluorescence (i.e., fluorescence lifetime) can provide valuable insight into the complex interactions among the various light-energy-deexcitation processes that compete for absorbed excitation energy within the PS II unit. The PS II Chl *a* fluorescence yield, ϕf_{PSII}, is determined by the various competing deexcitation processes as described by the following expression:

$$\phi f_{PSII} = \frac{k_f}{k_f + k_p + k_h + k_{tr \rightarrow PSI}} \tag{3}$$

where k_f is the rate constant of PS II Chl *a* fluorescence, k_p is the rate constant of PS II photochemistry, k_h is the rate constant of heat dissipation from PS II and $k_{tr \rightarrow PSI}$ is the rate constant of energy transfer from PS II to PS I. It is important to mention here that although this basic expression can be used to describe the fundamental principles of PS II Chl *a* fluorescence, as will be described below, our current model of PS II excited state dynamics is considerably more complex. This is because our current model must take into consideration the molecular and macromolecular structural organization of the PS II unit, as these factors directly influence the deexcitation processes associated with PS II photochemistry, photoprotection and photodamage.

In view of the above, we begin the chapter with a discussion of the current views on the molecular and structural organization of PS II in relation to photosynthetic light-energy transduction and excited state dynamics. We then proceed to discuss the biochemical and biophysical properties of the most important and well characterized photoprotective and photodamage-associated energy dissipative mechanisms, respectively. Of primary importance in this chapter is the relationship between the biochemical and biophysical principles that govern PS II function and the widely variable physiological -ecological factors of the natural environment. Therefore, we also discuss the physiological response range of adaptation and acclimation of the PS II apparatus in relation to the increasing extent and or duration of environmental stress. We conclude with a brief discussion of future research interest and significance in the context of optimizing and preserving PS II function during environmental extremes.

2. Structural and Functional Organization of Photosystem II

PS II exists mainly in the granal stacks of the chloroplast thylakoid membranes and is best described as a holocomplex because it is composed of several specially organized sub-sets of pigment-proteins (Gantt, 1996; Whitmarsh and Govindjee, 1995). The enzymatic function of PS II in the photosynthetic apparatus is to catalyze the light-driven process of water-splitting to yield molecular oxygen and protons, and to transfer the functional reducing equivalents (electrons) extracted from the water to plastoquinone; hence PS II can otherwise be termed the water-plastoquinone oxidoreductase (see chapter by G. Renger). As outlined below the molecular structure and function of PS II in photosynthesis are integrally related and understanding these structural aspects is a prerequisite to further discussions concerning photosynthetic light energy transduction and energy dissipation.

2.1 Pigment-Protein Complexes

Recent experimental studies and reviews have brought to light many aspects concerning the molecular and integrated structural organization of the PS II unit (Bassi et al., 1993; Diner and Babcock, 1996; Debus, 1992; Hansson and Wydrzynski, 1990; Kühlbrandt et al., 1994; Whitmarsh and Govindjee, 1995). We are beginning to understand how structural factors relate to and determine the absorption and transfer of excitation energy from antennae complexes to the reaction center complexes, where primary photochemistry takes place. Figure 1 shows a scheme of the current view of the gross structural organization of a typical higher plant PS II unit as it is situated in the chloroplast granal thylakoid membranes. The pigment-protein complexes are organized into three main subsets, namely, the peripheral (distal) antenna (Light-harvesting complex IIb or LHCIIb), the inner antenna (CP24, CP26 and CP29, where CP stands for chlorophyll protein and the number for its molecular mass) and the PS II core (mainly the CP43, CP47, D1, D2 and cytochrome b_{559} protein complexes), see Vermaas et al. (1993). The PS II peripheral antenna is composed mainly of trimeric complexes of LHCIIb, abbreviated here as II b. A subset of LHCIIb is functionally mobile between the granal PS II-enriched membranes and the stromal PS I-enriched thylakoid membranes (Allen, 1992; Bennett, 1991). The mobilization process of LHCIIb is controlled by phosphorylation of specific stromal exposed residues. This is suggested to regulate the spectral light energy distribution and redistribution among the two photosystems to optimize non-cyclic electron transport. The minor CPs of the inner antenna are immobile relative to the PS II core and probably act as bridges in transferring excitation energy from the peripheral antenna to the PS II core. The minor CPs are also proposed to make up an integral part of an important photoprotective mechanism (see review by Gilmore, 1997), to be discussed below. They may also strongly affect the structural and conformational integrity/stability of the PS II core. The PS II

core is composed mainly of the CP43 and CP47 complexes, the cytochrome b_{559} complex and the heterodimer complex known as D1/D2, which houses the special P680 Chl *a* molecules responsible for the primary charge-separation reaction (for a three dimensional molecular model of PS II, see Xiong et al., 1996). Note that for simplicity we have ignored here in our discussion several low molecular weight proteins also associated with PS II (see, Funk et al., 1995; Gantt, et al., 1996).

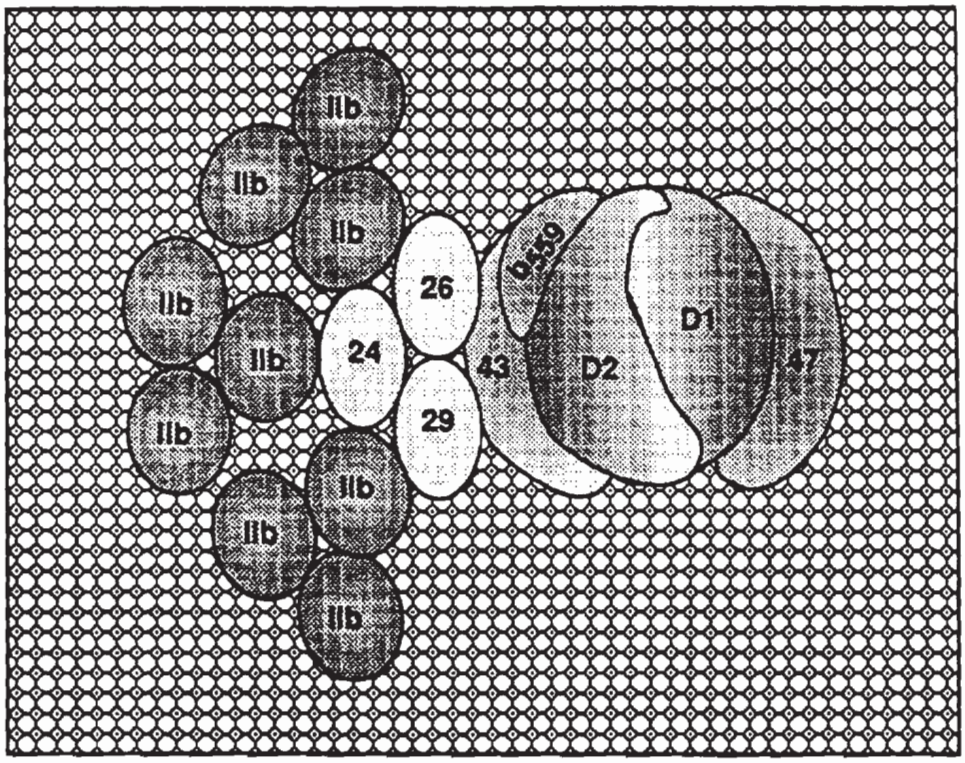


Fig. 1 A schematic top view of the organization of the major pigment-protein complexes of PS II in the chloroplast thylakoid membrane. The peripheral (or the distal) antenna is composed of trimeric complexes of the main light harvesting complex, LHCIIb (II b). The inner antenna is composed of the minor chlorophyll *a* + *b* binding proteins CP24, CP26 and CP29 (24, 26 and 29). In contrast to a mobile fraction of LHCIIb, the minor CPs always remain bound to or tightly associated with the PS II core. The PS II core includes the chlorophyll *a* binding proteins CP43 and CP47 (43 and 47), the core-heterodimer (D1 and D2) proteins as well as the cytochrome b_{559} complex (b_{559}); however, there are numerous other small protein components in the PS II core that are not detailed here.

2.2 Partitioning of Pigments with Respect to Pigment-Protein Organization

Figure 2 shows the chemical structures of the various carotenoids of PS II. Figure 2A shows the 'xanthophyll cycle' carotenoids, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z), Fig. 2B shows β, β-carotene and Fig. 2C shows lutein and neoxanthin. The structures of chlorophyll *a* and *b*

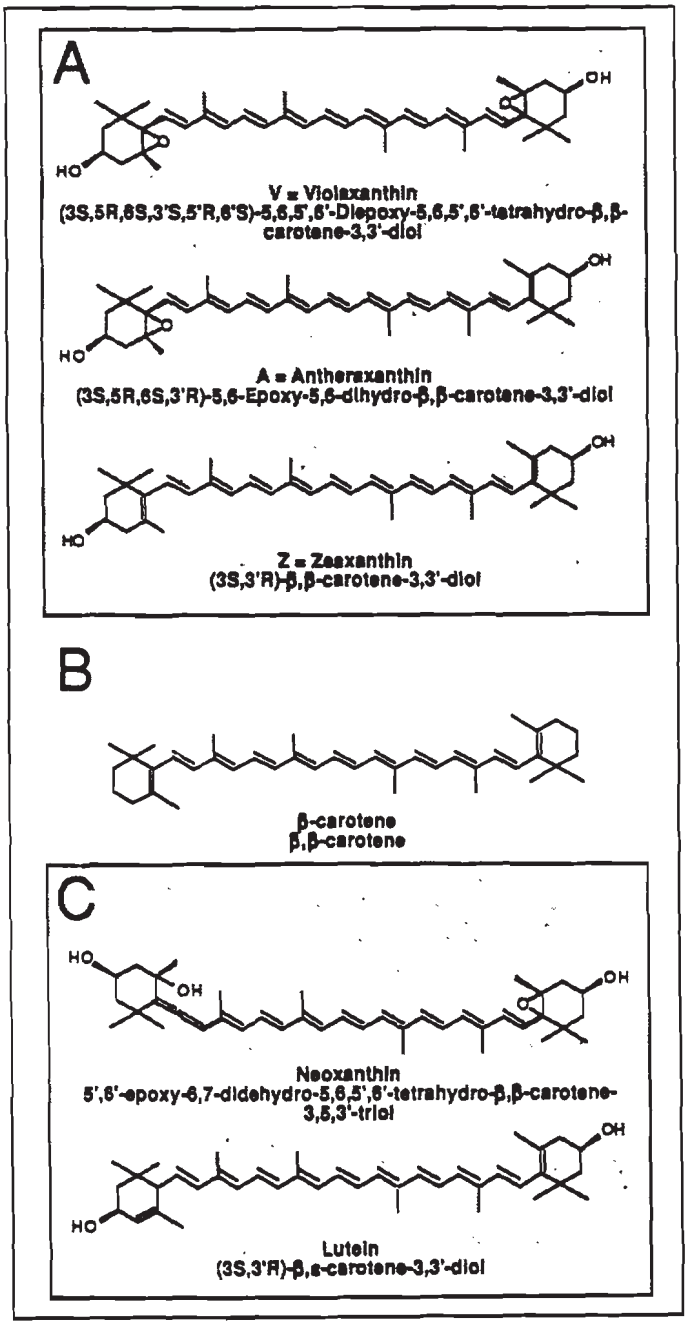


Fig. 2 Chemical structures of the xanthophylls and other carotenoids of the PS II unit. (A) Xanthophyll cycle carotenoids that include V = violaxanthin, A = antheraxanthin and Z = zeaxanthin (Yamamoto, 1979); their major role is in the dissipation, as heat, of excess absorbed light energy in the minor CPs of PS II. (B) β,β-carotene in the PS II core; their main proposed role is to act as photoprotective antioxidants. (C) Neoxanthin and lutein are proposed to maintain the structural integrity and stability of LHCIIb and the minor CPs (Bassi et al., 1993; Kühlbrandt et al., 1994) and/or act as accessory light-harvesting pigments (Demmig-Adams et al., 1996; Siefermann, 1987).

(Chl *a* and *b*) are shown in the chapter by Whitmarsh and Govindjee *in this volume*. Figure 3 illustrates the relative stoichiometric distribution and partitioning of the chlorophylls, xanthophylls and other carotenoid pigments among the three main protein subsets (LHCIIb, minor CPs and the core) comprising the PS II unit (Bassi et al., 1993; Lee and Thornber, 1995). The effective absorption cross section of the PS II unit, σ, determines the rate of photon absorption and is directly proportional to the total number of Chls $a + b = N_{pig}$ serving the PS II unit. The LHCIIb complexes of the peripheral antenna contain up to 50% (110–120 Chl $a + b$) of the total Chl (N_{pig} = 240–300) of the PS II unit with a minimum of 7 Chl *a* and 5 Chl *b* molecules per LHCIIb monomer (Bassi et al., 1993; Kühlbrandt et al., 1994). The main carotenoid constituent of LHCIIb is lutein (2 luteins per LHCIIb monomer), which is believed to play a central role in maintaining the structural integrity of the LHCIIb monomer (Kühlbrandt et al., 1994). Lutein may also act as an accessory light-harvesting pigment, transferring absorbed light-energy to Chl thus increasing σ of the PS II unit (Siefermann, 1987; Demmig-Adams et al., 1996). Interestingly, Pogson et al. (1996) recently showed in *Arabidopsis* mutants with altered carotenoid biosynthetic pathways that lutein, a derivative of β, ε-carotene (not shown), may be both structurally and functionally replaced with a β,β-carotene derivative such as violaxanthin (for chemical structures, see Fig. 2). LHCIIb also contains neoxanthin. Although, the structural association and its function in LHCIIb is presently unclear; possibly neoxanthin maintains LHCIIb structure and functions in the light-harvesting process. LHCIIb contains significant levels of the special xanthophyll cycle carotenoids;

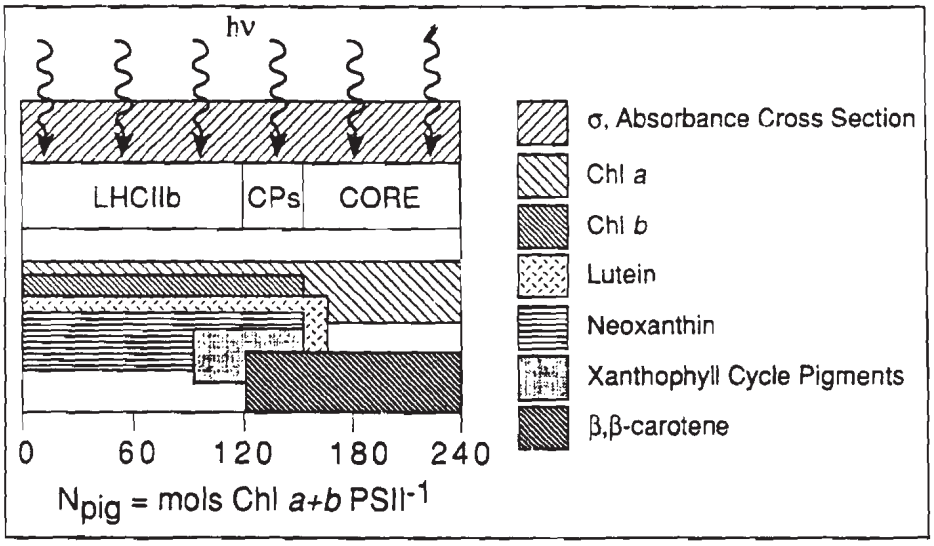


Fig. 3 Relative distribution of the Chls, xanthophylls and carotenoids among the three main subsets of PS II proteins, namely, the peripheral antenna (LHCIIb), the inner antenna (CP24, CP26 and CP29, labeled as CPs) and the PS II core (CORE) (based generally on the data from Bassi et al., 1993 and Lee and Thornber, 1995). The x-axis scale is N_{pig} = mols Chl $a + b$ PS II^{-1}.

however, as with neoxanthin, the nature and stoichiometry of their association with the LHCIIb protein subunits are presently unresolved in the three dimensional crystal structure (Kühlbrandt et al., 1994).

The minor CP proteins are believed to have a high degree of amino acid sequence and secondary and tertiary structural homology to the LHCIIb monomers (Crofts and Yerkes, 1994). In comparison to LHCIIb, the minor CPs contain a much smaller fraction of the total Chl *a* + *b* of the PS II unit; further, the CP26 and CP29 complexes have Chl *a*/Chl *b* ratios of around 2, while for CP24 the Chl *a*/*b* ratio is around 1. Also similar to LHCIIb, the minor CPs contain lutein and neoxanthin as significant constituents. In contrast to LHCIIb, the minor CPs and LHCIIb are enriched in the xanthophyll cycle pigments and associated with β,β-carotene; this is interesting because the biosynthetic pathways for all the xanthophyll cycle pigments (V, A and Z) proceed from β,β-carotene (Pogson et al., 1996). Funk et al., (1995) reported that the minor intrinsic 22 kD protein of PS II (termed the PS II-S protein) also binds significant levels of the xanthophyll cycle pigments. Bassi et al. (1993) reported that of the total pool of the xanthophyll cycle carotenoids violaxanthin, V, antheraxanthin, A, and zeaxanthin, Z, in PS II, approximately 80% are functionally associated with the CP complexes, compared to only 20% with LHCIIb. The distribution is disputed in the literature where there are reports that the xanthophyll cycle pigment distribution within the PS II unit is roughly 50% in LHCIIb and 50% in the minor CPs (Horton et al., 1994; Lee and Thornber, 1995). In all reported cases so far, however, the consensus is that on a per Chl basis there is a larger fraction of V + A + Z in the minor CPs than in LHCIIb. The xanthophyll cycle pigments are probably associated with the periphery or interface of the minor CPs (Bassi et al., 1993), i.e., as opposed to being bound in the protein backbone like lutein (Kühlbrandt et al., 1994). This explains the different allocation patterns for pools of lutein and the xanthophyll cycle pigments during sun-shade acclimation and when comparing barley wild-type and LHCIIb deficient *chlorina* mutants, as will be discussed later (see Gilmore, 1997). In sum, it is clear from studies of normal wild-type plants and Chl *b* and LHCIIb deficient mutants that the molecular nature of the association of xanthophyll cycle pigments in PS II is clearly different from that for the bulk of lutein (and neoxanthin), which is consistent with their playing different functional roles in the PS II unit.

In contrast to the peripheral and inner antennae complexes, the D1/D2 and CP43 and CP47 complexes do not contain Chl *b*, neoxanthin or the xanthophyll cycle pigments; however, the PS II core contains significant levels of β,β-carotene and traces of lutein, as well as, minor levels of pheophytin (not discussed here). van Dorssen et al. (1987) report that each PS II core contains two spectrally distinct β,β-carotenes with different orientations. Trebst and Depka (1997) reported that oxidation of the reaction center β,β-carotenes triggers PS II degradation under conditions of extreme excess light. As discussed below, the reaction center β,β-carotenes also may serve

an important photoprotective function by deactivating potentially damaging triplet excited states of Chl and harmful oxygen species in the PS II core (for a review of the *in vivo* functions of carotenoids in higher plants, see Demmig-Adams et al., 1996).

2.3 A View of Photosystem II Excited State Dynamics

Based on previously considered models of PS II excited state dynamics (Schatz et al., 1988; Dau, 1994; van Grondelle et al., 1994; Jennings et al., 1996), pairwise excitation energy transfer rates between antenna Chls are believed to be ultra-fast, i.e., hundreds of femtoseconds (10^{-15}s = fs) to picoseconds (10^{-12} s = ps). The mechanism of the energy transfer is believed to be of almost purely Förster resonance type and hence depends on the distance, orientation and Förster overlap integrals of the chromophores involved (see a minireview by Pearlstein, 1996). An exciton is believed to rapidly "hop" from one antenna Chl site to an adjoining antenna site in the lattice of Chls comprising PS II unit. By virtue of this rapid, 'incoherent' or diffusive motion, the exciton was proposed to rapidly equilibrate, within tens of picoseconds, throughout all the Chls $a + b = N_{pig}$ of the entire PS II unit. Essentially, the previous models assumed that PS II behaves as a single pigment pool (including all Chls *a* of the peripheral, inner and core/antenna). Located within the lattice framework of PS II Chls are special Chl *a* molecules, known as the *P680* Chls, that trap the exciton and are the sites of the photochemical charge separation reaction in the PS II core (see e.g., Greenfield et al., 1997). The probability *p680* of the exciton being trapped at the special *P680* Chls (which determines the measured charge separation time of PS II units) is supposed to be simply determined by the prevailing thermodynamics, namely the enthalpic and entropic factors, of the PS II unit. According to the expression $P680 = c_H N_{pig}^{-1}$ derived by Dau (1994), the enthalpy factor is the c_H term, a sum of the free-energy differences (and relative spectral heterogeneity) between all the antenna Chls relative to the special P680 trap Chls. The entropic factor is the N_{pig}^{-1} term, where N_{pig} sums the total number of antenna Chls in PS II that have comparable probability in harboring the exciton as do the special P680 Chls. As will be discussed below, we suggest here that this view is oversimplified; it needs to be modified and expanded since it does not adequately account for the distinctive heterogeneity of pigment distribution or the unique topological architecture/arrangement of the various component complexes of the PS II unit.

Our simple hypothetical views center on the importance of heterogeneous entropic factors for governing exciton migration and behavior in the PS II unit. Because exciton migration must be controlled by thermodynamic factors, an exciton should dwell, in a time-integrated manner, in the area of the PS II unit where it finds the lowest possible energy and the largest possible entropy. We believe that the structural organization of the PS II units, namely, the arrangement of the LHCIIb assemblies and the PS II core (Fig. 1), as

well as the distinctive partitioning of the individual pigments (Fig. 3), dictate that PS II can neither be viewed as isoenergetic nor as isoentropic. The latter point is especially critical in our judgment because of newer and recently revived views concerning exciton motion in a lattice system where the geometric arrangement of the pigments in the lattice is the major factor controlling the probability of exciton residence on the trap Chls and hence the exciton lifetime (Holcomb and Knox, 1996; Pearlstein, 1996). One important consideration is that the lattice sites in the PS II unit may not necessarily be individual Chl molecules but instead may be excitonically coupled (di-or even multimeric) clusters of pigments (Holcomb and Knox, 1996; Pearlstein, 1996). It is also important to consider, as will be explained below, that antenna Chls exhibit a high degree of kinetic homogeneity, with respect to energy transfer, that is largely independent of spectral heterogeneity (Pearlstein, 1996).

In light of the above concepts, Fig. 4 (Top) depicts that the PS II unit comprises two major and structurally distinct lattice systems, defined as the LHCIIb and PS II core. Because the minor CP complexes comprise only a minor fraction of the total Chl *a* and *b*, they are viewed here as an interface between the two main lattice networks and are believed to behave as a part of the core compartment with respect to exciton equilibration; this is because as mentioned previously they share a tight structural association with the core (Bassi et al., 1993). Both the main lattice compartments are defined with unique terms that summarize their absorbance energy (ΔE term), the number of lattice sites (N term) and the geometric arrangement of the lattice sites (geo term). The product of the N and geo terms represents the entropic contribution (ΔS) of the respective compartment to the overall PS II unit. The stylistic top view of the PS II unit (Fig. 4, top) conveys the proposed heterogeneity of pigment clustering and geometric lattice arrangements that determine the large proposed entropic differences, $\Delta\Delta$S, between LHCIIb and the PS II core. In contrast to the large $\Delta\Delta$S, it is well established the $\Delta\Delta$E is much less significant between LHCIIb and the core (Dau, 1994; Jennings et al., 1996). It is concluded that the isoenergetic properties and thermal equilibration of Chl *a* forms in PS II (Dau, 1994; Jennings et al., 1996; Renger et al., 1995) dictate that the entropic factors are probably the main factors determining *P680*. The crux of Fig. 4 (top) centers on the predominant importance of entropic considerations for calculating the rate constant of energy transfer between LHCIIb and the PS II core i.e., as exemplified by Holcomb and Knox (1996). Because the exciton migration is essentially a diffusion process, estimations of energy transfer rates between light-harvesting and core compartments in a photosynthetic unit must also consider the surface areas of contact, and distances, between the two compartments. The concepts outlined in Fig. 4 (top) explain several observations, discussed below, that are not consistent with isoenergetic/isoentropic models of PS II excited states dynamics (Schatz et al., 1988; Dau, 1994; Jennings et al., 1996). Primarily,

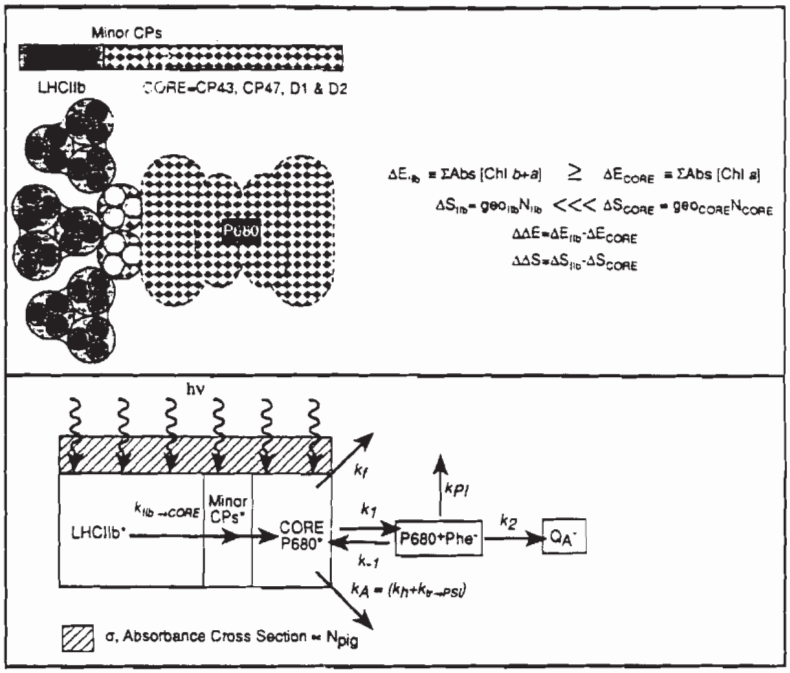


Fig. 4 Relationships between the gross structural organization and the enthalpic and entropic factors governing exciton migration in the PS II unit. *Top Panel* illustrates the energetic and entropic differences between the main pigment pools of PS II, namely the LHCIIb and the PS II core. The equations in the top *Panel* summarize the differences between the absorbance (free energy differences, ΔE) and entropic factors (ΔS) between LHCIIb and the PS II core. The geometric arrangement and possible excitonic coupling of pigment clusters in LHCIIb is shown to be symmetrical (small shaded circles) within and between LHCIIb trimers (groups of 3, larger shaded circles). The PS II core (CP43, CP47 and D1/D2) is proposed to comprise monomeric and dimeric clusters of Chl *a* (CORE, checkerboard pattern). The minor CPs (CP26, CP29 and CP24) are believed to be structurally homologous to LHCIIb monomers and are shown here as a transition zone for exciton equilibration between LHCIIb and the PS II core. In the *bottom Panel*, the absorption cross section, σ, determines the rate of photon absorption and is proportional to the total number of Chl $a + b$ molecules $= N_{pig}$ comprising the PS II unit. Absorption of light in LHCIIb leads to the excited state LHCIIb*; transfer of excitation from LHCIIb* through the minor CPs to the PS II core is proposed to be very rapid and is determined by the rate constant $k_{IIb \rightarrow CORE}$, see Table 1. Trapping and detrapping of the exciton by the reaction center Chls, i.e., exciton transfer between a nearest neighbor antenna Chl *a* and P680 ($Chl^* \leftrightarrow P680^*$) is not included in this figure for simplicity. The charge separation reaction to form $P680^+Phe^-$ and the reverse charge recombination reaction of the PS II reaction center are determined by the rate constants k_1 and k_{-1}, respectively. The stabilization of the charge separated state to form the reduced primary quinone electron acceptor Q_A^- is determined by the rate constant k_2. The rate constant of fluorescence emission from the PS II core is k_f. The sum of all other deexcitation processes in the PS II antenna including heat loss (k_h) and energy transfer to PS I ($k_{tr \rightarrow PSI}$) is defined by a combined rate constant k_A. Aerobic photoinhibitory energy dissipation from the charge separated state is determined by the rate constant k_{PI}.

the proposed entropic differences between LHCIIb and the PS II core help explain observations of similar behavior of PS II Chl *a* fluorescence lifetimes and lifetime distributions in both wild-type and LHCIIb-deficient barley (*Hordeum vulgare* L.) *chlorina* mutants (Briantais et al., 1996; Gilmore et al., 1995; 1996a, b). The general idea is that an exciton may move rapidly both out of LHCIIb into the PS II core, as well as rapidly between separate PS II cores through shared pools of LHCIIb. We note this idea parallels the idea that separate PS II cores can share excitation *via* restricted connections, as outlined in the model analyses of Trissl and Lavergne (1995).

Figure 4 (bottom) illustrates the interactions among the various competing deexcitation processes that influence measurement of the PS II Chl *a* excited state or fluorescence lifetimes, τ_xs. As mentioned in the Introduction, measurements of the PS II Chl *a* fluorescence lifetimes normally assume that the intrinsic rate constant of Chl fluorescence, k_f, remains constant. Accepting this assumption, the respective minimal and maximal effects of PS II photochemistry on the PS II Chl *a* fluorescence lifetimes can be defined according to the following expressions (see Fig. 4 (bottom) and Equation (3) for symbols of rate constants):

$$\tau_{F_m} \propto \frac{1}{k_f + k_{1(min)} + k_{A(min)}} \tag{4}$$

and

$$\tau_{F_o} \propto \frac{1}{k_f + k_{1(max)} + k_{A(min)}} \tag{5}$$

where τ_{F_m} and τ_{F_o} are the PS II Chl *a* fluorescence lifetimes under conditions of minimal and maximal rate constants of PS II photochemistry. The conditions for both the τ_{F_m} and τ_{F_o} in equations (4) and (5) are strictly defined such that both the nonradiative dissipation rate constant, k_A, is a constant and presumably of minimal value and the rate constant of charge recombination of the primary radical pair ($P680^+Pheo^-$), k_{-1}, is constant; here the rate constant k_A sums all energy dissipation not owing to fluorescence or photochemistry and includes heat losses (k_h) and transfer of excitation energy from PS II to PS I ($k_{tr \rightarrow PSI}$). As defined in equation (4), the condition for the maximal fluorescence lifetime, τ_{F_m}, is defined such that the rate constant of PS II photochemical charge separation, k_1, to form the primary radical pair (Greenfield et al., 1997) is at a minimum because the primary quinone electron acceptor of PS II, Q_A, is reduced (oxidized Q_A is a quencher of chlorophyll fluorescence, whereas reduced Q_A, Q_A^-, is not; see Duysens and Sweers, 1963). For measurements of τ_{F_m}, Q_A can be kept reduced by either high light-intensities that saturate the rate of Q_A^- oxidation or even at low light-intensities by the addition of a compound such as DCMU, that inhibits Q_A^- oxidation by Q_B, by displacing Q_B from its natural binding site (Velthuys,

1981). According to Schatz et al. (1988), electrostatic charge-repulsion between Q_A^- and the $Pheo^-$ of the primary radical pair slows k_1 to minimum. The rate constant of charge stabilization ($P680^+Pheo^-Q_A \rightarrow P680^+PheoQ_A^-$), k_2, determines the formation of the reduced primary quinone acceptor Q_A^-. The minimal PS II Chl *a* fluorescence lifetime condition, τ_{F_0}, is observed when k_1 is maximal since Q (top) is fully oxidized (at low light intensities used for its measurement). As explained in Fig. 4(top) above, the rate constant of excitation energy transfer from LHCIIb to the PS II core $k_{IIb \rightarrow CORE}$ is be proposed to be rapid because an overriding $\Delta\Delta S$ factor conducts the exciton into the core (Briantais et al., 1996; Gilmore et al., 1996a; also see Lin and Knox, 1991). The structural source of the variable PS II Chl *a* fluorescence is mainly the Chl *a* molecules in the PS II core, not LHCIIb. This concept is experimentally supported by the facts that both the fluorescence lifetimes (Briantais et al., 1996; Gilmore et al., 1996a) and emission spectra (Knoetzel and Simpson, 1991) are similar in both the wild type barley and in the LHCIIb-lacking *chlorina* mutants. It is also fully consistent with all the early literature where no fluorescence band originated from LHCIIb except at 4K (see reviews in Govindjee et al., 1986). Decreased levels of LHCIIb and N_{pig} in the *chlorina* mutants correlate with a decreased absorption cross section, σ, as indicated by the reduced rates of PS II photochemistry (Chow et al., 1989; Falk et al., 1994).

3. Energy Dissipation at the Antenna Level

In Fig. 4 it was assumed that the rate constant of energy dissipation in the PS II core compartment, $k_A = k_h + k_{tr} \rightarrow PSI$, remained constant. As will be explained below, the rate constant of the heat loss component of k_A, k_h, is under a feedback control and is important for regulating the *in vivo* function of PS II under environmental conditions where the absorbed light flux exceeds the photosynthetic capacity. Described below are our current views of the primary mechanism that functions in PS II to dissipate excess absorbed excitation energy as heat, namely, the xanthophyll cycle-dependent energy dissipation mechanism (Demming-Adams et al., 1996; Gilmore, 1997).

3.1 Biochemical and Bioenergetic Control of the Xanthophyll Cycle-dependent Energy Dissipation Mechanism

The PS II unit of higher plants is believed to be photoprotected by the xanthophyll cycle-dependent energy dissipation mechanism (Demmig-Adams et al., 1996; Gilmore, 1997). This feedback-controlled mechanism is precisely tuned to react under excess light levels and help dissipate excess absorbed light energy as heat. The primary controlling factor is the uncoupler-sensitive trans-thylakoid proton gradient, ΔpH, that is otherwise used to drive photophosphorylation according to the chemiosmotic principle (Mitchell, 1968). In relation to the impinging light intensity, the ΔpH constitutes a very efficient feedback-control point for photosynthesis because it is very sensitive

to limitations in the ATP-consuming CO_2-fixing reactions. This is important to consider because the latter rates are extremely sensitive to temperature as well as environmental factors such as water and salinity stresses, that affect movement of the terminal electron acceptors of photosynthesis (CO_2 and sometimes O_2 in the so-called Mehler reaction) into the leaf *via* the stomata (Björkman and Demmig-Adams, 1994).

Of particular interest concerning the sensitivity of the ΔpH as a feedback control point are the effects of temperature on the overall trans-thylakoid energy coupling system, including chloroplast ATP synthetase-hydrolase (ATPase) activity. The trans-thylakoid membrane ΔpH is determined by the relative fluxes of protons in and out of the thylakoid lumen (see Fig. 5). Compared to the higher physiological temperature conditions (solid arrows) shown in Fig. 5A, lower temperatures, as shown in Fig. 5B, decrease all proton fluxes (dashed arrows). The decreased proton fluxes are consistent with expected decreased rates of all chemical diffusion processes at lower temperatures. Thus it follows that the out-flux of protons through the ATPase is slowed because the diffusion-limited, enzyme-mediated processes of CO_2-fixation that consume ATP are inhibited. The low temperatures also retard the electron-transport mediated H^+ pumps as well as the rates of ATP-hydrolysis mediated proton-pumping. However, it is important to note that decreasing temperatures decrease the diffusion or leakage of protons across the thylakoid membrane to a significantly larger extent than they slow inward proton pumping (Avron and Schreiber, 1977; Gilmore and Björkman, 1995; Mills and Mitchell, 1982; Schreiber and Avron, 1979). This has the important consequence of increasing the lumen $[H^+]$ relative to a given rate of trans-thylakoid proton pumping, i.e., by either light driven electron transport or ATP hydrolysis (see e.g., Gilmore and Björkman, 1995). It is also important to understand that the chloroplast ATPase activity (inward proton pumping) is controlled both by pH-activation of its trans-thylakoid proton conducting channel (CF_o, the membrane-bound part of ATPase) as well as the reduction status of key cysteine residues (SH-HS) of the γ-subunit proteins of the CF_1 (the stromal exposed, extrinsic portion of the ATPase that has the enzymatic activity) (Mills and Mitchell, 1982). Therefore, it is easy to envision two main reasons why lowering the temperature favors reverse-proton pumping of the ATPase and, thus, enhances lumen acidification: (1) by decreasing the proton leakage from the lumen, the pH-activation state of the ATPase proton conducting channel is maintained and (2) since the enzyme-mediated processes that oxidize the γ-subunits of CF_1 are retarded at low temperatures, the active state of the enzyme can be maintained for longer times (Gilmore and Björkman, 1995; Mills and Mitchell, 1982).

Figure 6 outlines our current view of the biochemical mechanism of xanthophyll cycle-dependent energy dissipation constructed mostly from studies with isolated thylakoid membranes (see a review Gilmore, 1997). It is important to understand the advantages and perceive the significance of

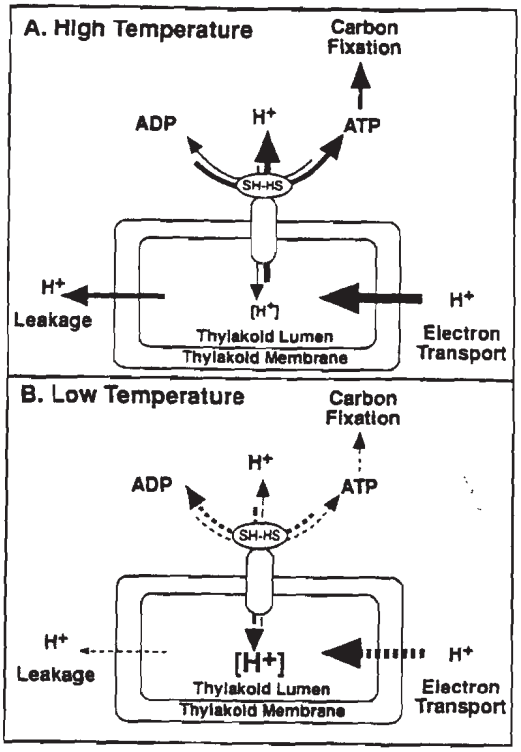


Fig. 5 A scheme to explain the difference in the concentration and translocation of protons at high (A) and low (B) physiological temperatures. The concentration of protons in the lumen [H^+] is determined by the relative fluxes of protons into and out of the lumen. The thickness of the arrows is relatively proportional to the proton flux. At high physiological temperatures (A) the rates of proton consumption for ATP synthesis and the use of ATP during carbon fixation as well as trans-thylakoid proton leakage are relatively fast in comparison to the rates at which protons are translocated into the lumen as a consequence of electron flow. Thus, the concentration of protons in the lumen is low. At low physiological temperatures (B) all proton fluxes are decreased (as depicted by broken arrows); however, the relative proton efflux due to both ATP synthesis and trans-thylakoid proton leakage are decreased relatively more than the proton influx, thus, allowing for a larger [H^+] for a given rate of proton influx by either ATP-hydrolysis or light-driven electron flow. See text for further explanation.

controlling the xanthophyll cycle and energy dissipation mechanism at the thylakoid membrane level. A primary consideration is of course the trans-thylakoid membrane pH gradient that is sensitive to the photosynthetic capacity as described above. It is clear in Fig. 6A that increasing the lumen proton concentration [H^+] plays two roles in the energy dissipation mechanism. First, with a pK_1 around 5.2 there is the activation of the violaxanthin deepoxidase that converts [V] to [Z + A] (Yamamoto, 1979). Secondly, with a pK_2 around 4.5 there is the suggested protonation of key glutamate carboxyl groups of the CP26 and CP29 proteins of the PS II unit (CP( )*-COOH) (Crofts and Yerkes, 1994; Walters et al., 1994). We suggest that the protonation

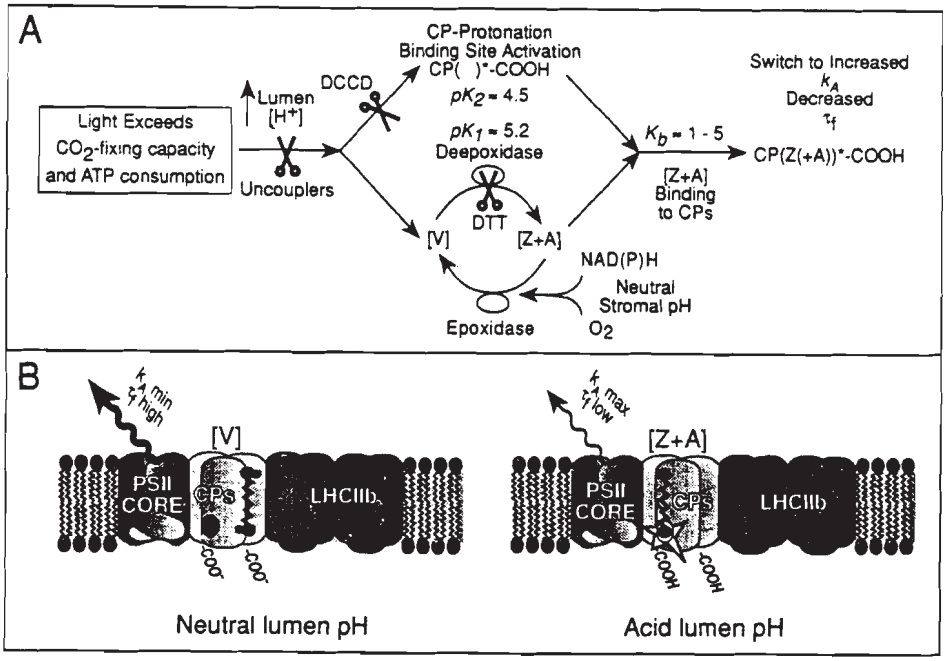


Fig. 6 A model for the xanthophyll cycle-dependent energy dissipation mechanism. *Panel A* shows that light levels in excess of the CO_2-fixing capacity increase the thylakoid lumen proton concentration. Activation of the violaxanthin deepoxidase enzyme occurs with a $pK_1 \approx 5.2$ (Yamamoto, 1979); this enzyme catalyzes the conversion of [V] into [Z + A]. The epoxidase enzyme can in the presence of an inactive deepoxidase, a neutral stromal pH, NAD(P)H and molecular oxygen, convert [Z + A] back to [V]. Protonation of lumen exposed carboxyl groups of glutamate residues of the CP26 and CP29 proteins (CP ( )*–COOH) occurs with a $pK_2 \approx 4.5$ and activates a binding site for the Z + A molecules. The binding association constant K_b (for the binding of Z + A to the CPs) is estimated to range between 1 to 5 mols [Z + A]/mol PS II. Binding of Z + A to the CPs causes the PS II unit to *switch* to have an increased rate constant of deexcitation, k_A (mostly the k_h component) and hence a decreased fluorescence lifetime, τ_f. Uncouplers of the lumen [H^+] inhibit pH-dependent deepoxidation and CP-binding site activation. The sulfhydryl reagent dithiothreitol, DTT, inhibits the deepoxidase without affecting the lumen [H^+]. The carboxyl labeling reagent dicyclohexylcarbodiimide, DCCD, inhibits the CP binding site activation. *Panel B* shows a simple model of the effects of the lumen pH and [Z + A] on the PS II unit in the thylakoid membrane. *Panel B (left)* shows that at neutral lumen pH, the CP carboxyl groups are unprotonated, the binding site is inactive (dark circle on lumen side of CPs) and the [V] is high; under these conditions the fluorescence lifetime of the PS II unit is high because the rate constant k_A is low. *Panel B (right)* shows that upon lumen acidification the [Z + A] is increased, the CP carboxyl groups are protonated and the Z + A can bind (spark) to the active binding site on the lumenal side of the CPs; under these conditions the fluorescence lifetime is low because the rate constant k_A is high.

of the CP carboxyl groups causes a conformational change in the inner antenna region that is associated with the activation of a binding site on the lumenal side of the thylakoid membrane. We propose that this has a high affinity for the β-cyclic endgroup structures of either Z or A; the binding step can be described with the binding association constant K_b. The binding of Z (or A) (Gilmore and Yamamoto, 1993) to this site causes the PS II unit to *switch* to an unit with an increased rate constant of heat dissipation, k_A (Wagner et al., 1996) and a decreased fluorescence lifetime, τ_f (Gilmore et al., 1995, 1996a).

Outlined schematically in Fig. 6A are the sites and scope of action of some of the more commonly used chemical agents for controlling and studying xanthophyll cycle-dependent energy dissipation in thylakoid membranes and leaves. Uncouplers that dissipate the proton gradient can thus inhibit and reverse both pH dependent steps; however, uncouplers will not directly alter the [Z + A] concentration in isolated thylakoid membranes unless exogenous NAD(P)H and bovine-serum albumin are added to activate the zeaxanthin epoxidase enzyme that converts Z to A and A to V (see Siefermann and Yamamoto, 1975; Gilmore et al., 1994). The epoxidation enzyme is located on the stromal side of the thylakoid membrane and has a neutral pH optimum. The maximal rate of epoxidation is normally one tenth that of deepoxidation; thus, when the deepoxidase is active, the faster rates of deepoxidation reverse and thereby prevent net epoxidation. The sulfhydryl reagent dithiothreitol, DTT, inhibits the energy dissipation by preventing violaxanthin deepoxidation (Yamamoto, 1979); however, DTT does not inhibit either the light-driven or ATPase mediated proton pumping and therefore does not directly change the lumen [H^+]. Further, DTT will not change the [Z + A] after addition, unless of course the zeaxanthin epoxidase is also activated (Siefermann and Yamamoto, 1975). The carboxyl-labeling reagent dicyclohexylcarbodiimide, DCCD, reportedly inhibits the energy dissipation mechanism by affecting the CP protonation steps (Crofts and Yerkes, 1994; Horton et al., 1994).

Figure 6B illustrates our model of the *concerted effects* of the lumen pH and deepoxidation on the structure and fluorescence yield of the PS II unit. We start our description on the left hand side in the diagram under conditions where a steady state has been reached in the presence of a neutral lumen pH. Here, we assume that most of the xanthophyll cycle pigments exist in the form of [V] and the CPs are not protonated; thus, because there is no Z or A bound to the special site (circle) on the lumenal side of the CPs in the inner antenna, the rate constant k_A is low and the fluorescence lifetime τ_f is high (see Equations 4 and 5). On the right hand side of the diagram, we assume conditions where a steady-state has been reached under conditions of an acid lumen pH. With an acid lumen pH, the [Z + A] is increased and the protonation of the CPs causes a conformational change in the inner antenna region that activates the binding site for Z and A. The binding of the Z and A to the CPs switches the rate constant k_A to an increased value and thus decreases τ_f according to Equations 4 and 5.

3.2 Biophysical Characterization of Xanthophyll Cycle-dependent Energy Dissipation Mechanism

We describe below a quantitative pH-dependent binding model and present supporting data for the xanthophyll cycle-dependent energy dissipation mechanism. This model was formulated in collaboration with Prof. V. Shinkarev (Gilmore et al., 1997); it takes into consideration the following four assumptions concerning the relationships between the lumen pH, the [Z + A] and the time-resolved PS II Chl *a* fluorescence parameters: (1) steady-state conditions for the lumen pH and [Z + A] (this assumption eliminates complications associated with complex kinetic changes in the intrathylakoid pH or xanthophyll cycle pigment concentrations); (2) the [Z + A] applies only to a PS II unit in the granal thylakoids membranes; (3) we assume that there exists approximately one pH-activated binding site per PS II unit that has a high (and probably stereospecific) binding affinity for one of the (3S or 3'R) β-cyclic endgroup structures of Z or A (see Fig. 2 for structures); and (4) the pH-activation of the binding site is accomplished by protonation of several carboxyl moieties on the lumenal side of the CP26 and or CP29 proteins (Crofts and Yerkes, 1994; Walters et al., 1994); the protonation is believed to change the charged properties of the amino acid side chains thus leading to a refolding/reorganization of the minor CP proteins and the ultimate exposure of a binding domain for the Z and A molecules within or between the CP complexes. The model is designed to simulate changes within the typical stoichiometric range of the [Z + A]/PS II unit (minimally around 1 and maximally less than 10 moles Z + A per mol PS II, see Gilmore, 1997). Filling of the binding site by a Z or A molecule switches the entire PS II unit, in one step, to a unit with an increased rate constant of heat dissipation k_A. Finally, the model is assumed to be applied under conditions when PS II Chl *a* fluorescence lifetime is maximized because the rate constant of primary charge separation (k_1) is minimized such as by the addition of DCMU or by a brief flash of intense light to minimize the rate of Q_A^- oxidation.

The reaction scheme in Fig. 6A and the equations (A. Gilmore, V. Shinkarev, T. Hazlett and Govindjee, personal communication, 1997, 1998) discussed below describe the mechanism with two main steps: Step (1) is binding site activation due to protonation of the minor CPs with $pK_2 = 4.5$, typical for a glutamate carboxyl group, followed by step (2) the binding interaction between CP complexes and the [Z + A] that can be described with the binding (association) constant K_b. Regarding step (1) the quantitative relationship between the pH and the fraction of PS II units that have all their necessary CP complexes fully protonated. Thus, an active binding site is given by the Henderson-Hasselbach equation,

$$\mathrm{pH} = pK_2 + \log \frac{[\mathrm{CP}(\,) - \mathrm{COO}^-]}{[\mathrm{CP}(\,)^* - \mathrm{COOH}]} \qquad (6)$$

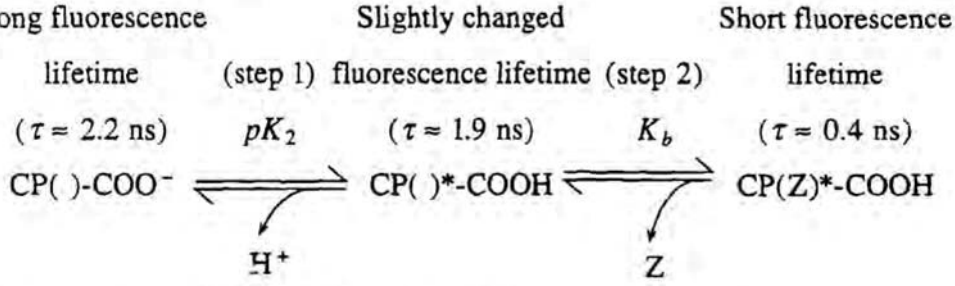
where CP( )–COO^- and CP( )*–COOH represent the unprotonated (inactive)

and protonated (fully active) binding sites. In step (1) we assume that several COOH moieties may be involved (perhaps more than 4 assuming two COOH groups for each CP26 and CP29 complex per PS II), (Crofts and Yerkes, 1994).

Regarding step (2) the quantitative relationship between the [Z] (for simplicity A is left out) and the fraction of binding sites filled by a Z molecule [CP(Z)*–COOH] is defined by the binding (association) constant equation,

$$K_b = \frac{[\mathrm{CP(Z)^*} - \mathrm{COOH}]}{[\mathrm{CP(\ )^*} - \mathrm{COOH}]\,[\mathrm{Z}]} \qquad (7).$$

Both step 1 and step 2 are characterized by their effects on the time-resolved PS II Chl *a* fluorescence parameters as described in the concerted reaction scheme,



The unprotonated PS II unit has a long fluorescence lifetime that shifts to a slightly shorter lifetime upon protonation and again to a much shorter fluorescence lifetime upon binding of Z. With the following equation (see Gilmore et al., 1997, 1998) we combine the information from the step (1) and step (2) equations and summarize the above reaction scheme concerning the relationships between the lumen pH the [Z + A] PS II^{-1} and the fractional concentration of PS II units with increased heat dissipation and hence shortened fluorescence lifetime, $\tau \approx 0.4$ ns:

$$[\mathrm{CP(Z)^*} - \mathrm{COOH}] = \frac{K_b[Z]10^{pK_2-\mathrm{pH}}}{1 + 10^{pK_2-\mathrm{pH}}(1 + K_b[Z])} \qquad (8),$$

where [CP(Z)*–COOH] = 1 – ([CP( )*–COOH] + [CP( )–COO–]).

Now we explain how these ideas are confirmed experimentally by studies of the PS II Chl *a* fluorescence lifetimes. We have accepted that PS II Chl *a* fluorescence lifetimes are best modeled assuming a distribution of lifetimes (Govindjee et al., 1993; Gilmore et al., 1995, 1996a, b). This is because the excited state characteristics of the fluorescent PS II Chl *a* molecules should be sensitive to their molecular environment that consists of a heterogeneous protein matrix. The possible heterogeneity of the Chl molecular environment should also be considered because the pigment-proteins probably exist in a distribution of conformational (sub)states, any of which may represent

a significantly different molecular environment for the Chls. The above ideas are derived from the concept of the existence of "conformational substates in proteins" as enunciated by Frauenfelder et al. (1988). Fluorescence lifetime distribution analyses yield data on the fluorescence lifetime center of a component distribution x (τ_x), and in the cases where there are more than one distribution, they also provide information regarding the relative intensity fractions (f_x) of the various component distributions. The average fluorescence lifetime is thus the integrated sum of the products of the respective fractional intensity and fluorescence lifetime values, $\langle \tau \rangle = \sum f_x \tau_x$. Fluorescence lifetime distribution analyses also yield data on the width of the distribution. The distribution width is believed to reflect changes in the fluorophore molecular-environment heterogeneity and therefore provide information concerning protein conformational changes around the fluorophore (Alcala et al., 1987). In the following paragraph, we explain how the fluorescence lifetime distribution parameters can be used to interpret both the Chl-protein conformational changes as well as changes in the deexcitation rate constants within the PS II unit.

We have compared the changes in the PS II Chl *a* fluorescence lifetime distributions and intensity with the changes in the [Z + A]/PS II and the pH in spinach thylakoids. For this purpose lifetime distributions were measured in the presence of DCMU to maximize Q_A^- formation and to minimize the rate constant of charge separation, k_1 and the [Z + A] was varied but the chloroplast ATPase-mediated lumen pH was kept constant (Gilmore et al., 1995). Fig. 7A shows a 3-dimensional plot of the bimodal distribution of PS II Chl *a* fluorescence lifetimes (τ), thus obtained, as a function of both the fractional intensity (f_x) and the maximal steady-state fluorescence intensity, F'_m, measured at different concentrations of Z + A per PS II. This plot provides a look at the effects of the xanthophyll cycle-dependent energy dissipation on the quantum yield of fluorescence, as measured by the lifetime of fluorescence (see Equation 3), and relates it to the maximal fluorescence intensity (F'_m) that has been measured in many photosynthesis laboratories throughout the world with commercially available Chl fluorometers. It is clear from Fig. 7A that the decreases in the maximal steady-state fluorescence intensity, F'_m, are caused by, and linearly correlated with, an increase in the fractional intensity, f_2, of a fluorescence lifetime distribution centered around $\tau_2 \approx 0.4$ ns. the increase in the f_2 of the lifetime distribution for τ_2 is accompanied by decreases in the f_1 of the lifetime distribution centered around $\tau_1 \approx 2$ ns; this according to Eq. (8) can be attributed to the increase in [CP(Z)*–COOH]. It is also clear that the widths of the lifetime $\tau_1 \approx 1.9$ ns and $\tau_2 \approx 0.4$ ns distributions are significantly different suggesting that they may originate from PS II units that have different overall protein conformations and hence excited state characteristics.

We now explain the significance of the time resolved fluorescence data and model as they pertain to most conventional studies involving the

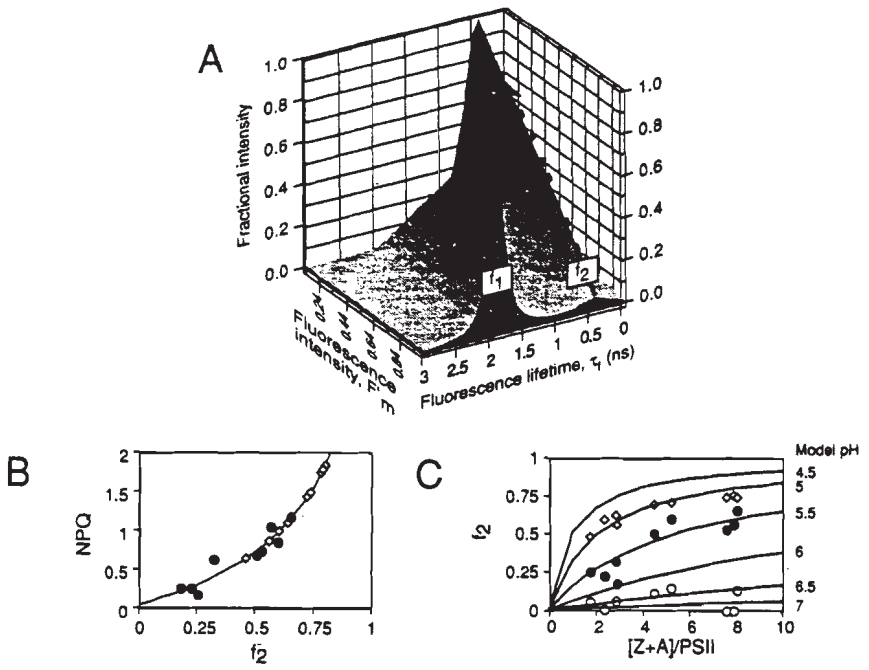


Fig. 7 Relationship among the PS II Chl *a* fluorescence lifetime distributions, the maximal fluorescence intensity and the stoichiometry of xanthophyll cycle pigment concentrations on a per PS II unit basis in spinach thylakoids. In *Panel A*, the three dimensional plot shows the relationship between the bimodal distribution of fluorescence lifetimes (τ in ns), fractional intensities (f_1 and f_2) and the maximal fluorescence intensity (F_m'). The surface of the three dimensional plot was drawn using the average fluorescence lifetime centers and widths as well as the best fitting linear relationship between the fractional intensities f_1 and f_2 and F_m'. The dark solid symbols on the black arrow denote the actual data points for the relationship between the fractional intensity f_2 of the $\tau_2 \approx 0.4$ ns component and the maximal fluorescence intensity F_m'; the relationship is linear where $r^2 = 0.90$ and $F_m' = -0.76\ f_2 + 0.96$. *Panel B* shows the relationship between the fractional intensity f_2 of the $\tau \approx 0.4$ ns component and the decrease in the maximal fluorescence intensity calculated as nonphotochemical quenching, NPQ (= $F_m/F_m' - 1$); the drawn line was calculated from the linear relationship between f_2 and F_m' in *Panel A*. The closed circles represent the correspondence between the f_2 data from *Panel A* and the NPQ measured under conditions of an ATP hydrolysis mediated pH changes (data adapted from Gilmore et al., 1995). The open diamonds represent the NPQ measured in the same thylakoid samples under conditions of a light saturated ΔpH; the f_2 values were calculated based on the linear relationship from *Panel A*. *Panel C* shows the relationship between the [Z + A]/PS II and the experimentally derived f_2 values from *Panel B* (same symbols); the data for the open circle symbols were collected from the same thylakoid samples after uncoupling all proton pumping, see Gilmore et al. (1995, 1996a). The drawn lines were calculated using the [Z +A]/PS II (x-axis) and model pH values (at the right) from Equation (8) assuming $pK_2 = 4.5$ and $K_b = 2$ for spinach thylakoids, see Gilmore et al. (1995). Collaboration with Prof. V. Shinkarev for Fig. 7C is gratefully acknowledged.

xanthophyll cycle-dependent energy dissipation mechanism where only the steady-state fluorescence intensities are measured. We first explain the relationship between the time-resolved fluorescence data and the steady-state fluorescence intensity data measured in parallel in these experiments. We define

$$F'_m/F_m \equiv \langle \tau \rangle'/\langle \tau \rangle \tag{9}$$

where we further define the normalized values,

$$F_m \equiv \langle \tau \rangle \equiv 1 \tag{10}.$$

Here F_m corresponds to the maximal fluorescence intensity corresponding to the conditions of τ_{F_m} defined in Equation (4) above where both the rate constants of charge separation (k_1) and energy dissipation in the antenna (k_A) are minimal. Next, by convention, we express the measured decreases ($F_m - F'_m$) in the steady-state PS II Chl *a* fluorescence intensity using NPQ, the nonphotochemical quenching coefficient:

$$\frac{F_m - F'_m}{F'_m} = \frac{F_m}{F'_m} - 1 = NPQ \tag{11}.$$

It follows that because F_m is normalized to unity and since the f_2 parameter (fractional intensity of the 0.4 ns lifetime component) is linearly proportional to the F'_m intensity (Fig. 7A), increases in f_2 are curvilinearly related to increasing values of NPQ ($=F_m/F'_m - 1$), as shown in Fig. 7B. The closed circles in Fig. 7B represent the measured NPQ values plotted against the f_2 values given in Fig. 7A (under conditions of an ATPase mediated ΔpH). The open diamonds represent NPQ data collected under conditions of a higher ΔpH (more acid lumen pH) during light-saturated electron transport in the same thylakoid samples (see Gilmore et al., 1995); the corresponding f_2 data were calculated based on the linear relationship between f_2 and F'_m shown in Fig. 7A. It is clear that the maximum NPQ value is predicted to occur when $f_2 = 1$ and that the relationship between f_2 and NPQ becomes increasingly steep as f_2 approaches unity.

In Fig. 7C we compare the experimentally derived f_2 values from Figs. 7A and 7B to those predicted from the model Equation (8) with the assumed and calculated values for the pH, pK_2 and K_b described in the Fig. 7 legend (A. Gilmore, V.P., Shinkarev and Govindjee, unpublished, 1997; Gilmore et al., 1998). The drawn lines depict the predicted values of f_2 for the corresponding lumen pH values (shown on the right ordinate of Fig. 7B) and [Z +A]/PS II values (shown on the abscissa). It is clear that the experimental values follow a curvilinear pattern similar to that predicted by equation (8). The data as analyzed with Equation (8) suggest the lumen pH differed by about 0.5 pH units when comparing the light-saturated pH conditions (diamonds; pH $\approx$ 5) to those under conditions of ATP-hydrolysis (filled circles; pH $\approx$ 5.5). Further, the pH value $\geq$ 7 for the f_2 values denoted by the open circles would be

consistent with the expected neutral lumen pH after the addition of an uncoupler of trans-thylakoid proton pumping.

It is clear from the data in Fig. 7 that the model parameters can be extrapolated, within the bounds of the physiological and biochemical constraints of a typical leaf or chloroplast system (i.e., lumen pH and [Z + A]). If the lumen pH were to reach a value around 5 (as might be expected under light-saturating conditions in leaves or chloroplasts, see Heber and Krause, 1971), very low levels of [Z + A] (< 1 mol per PS II) may cause significant levels of NPQ (> 1); indeed, such levels of [Z +A] and NPQ are well documented in many studies of leaf and chloroplast systems wherein deepoxidation is inhibited with DTT (see a review by Gilmore, 1997). Further, if f_2 was 1, and thus $f_1 = 0$, then based on the relationship shown in Fig. 7B, the maximal level of NPQ might reach, but probably not greatly exceed 6 in most higher plants. Indeed these maximal levels of NPQ are well documented and only observed in plants with high levels of [Z + A] and under conditions where one would expect a light-saturated lumen [H^+] (see Demmig-Adams et al., 1996).

As mentioned earlier, the xanthophyll cycle- and lumen pH-dependent changes in the PS II Chl *a* fluorescence lifetimes for spinach thylakoids, shown in above Fig. 7A, are strikingly similar to those obtained both in wild-type and in the LHCIIb deficient barley *chlorina* mutants (Gilmore et al., 1996a). Thus, another conclusion is that the observed NPQ values are independent of structural or organizational changes in LHCIIb, as thought before (Horton et al., 1994, 1996); instead, NPQ probably involves *only* changes in the minor CPs and PS II core (Gilmore et al., 1996a). We propose here that the Z + A binding site is structurally associated with the PS II core compartment, such that removing LHCIIb does not affect the intrinsic rate constant of the [Z + A]-dependent heat dissipating trap. In sum, the binding of the Z and A molecules creates a heat dissipating trap, with a very rapid 'intrinsic' rate constant of heat dissipation, that essentially drains the entire PS II unit. We propose that the Z and A molecules either directly accept energy from Chl *a* molecules in the inner antenna, as proposed by Frank et al., (1994) or otherwise directly influence the environment of Chls in the vicinity of the binding site causing them to have much higher rate constants of heat dissipation (see a review by Demmig-Adams et al., 1996).

4. Energy Dissipation Associated with Photosystem II Reaction Center Damage and Inactivation

4.1 Biochemical Changes Associated with Reaction Center Damage and Inactivation

As outlined in Figs. 6 and 7, each PS II unit has a finite capacity to dissipate excess absorbed light energy via the xanthophyll cycle-dependent mechanism, and, thus to prevent molecular damage to the PS II core; this limit is defined

on a PS II unit basis by the rate constant, k_A, with k_h playing the major role as described above. Figure 8 outlines the consequences of exceeding the photoprotective capacity. Excess light promotes overexcitation of the PS II reaction center Chls leading to various deleterious side-reactions that ultimately degrade and or inactivate the reaction centers (Andersson and Barber, 1996). In the presence of air (with oxygen being normally in the triplet state, $^3O_2$), these reactions are believed to involve the formation of long-lived triplet excited states of the Chl (^{3}Chl) which themselves, through photosensitization, can stimulate the formation of damaging singlet oxygen ($^1O_2$) and oxygen radical species as well as superoxide anions (O_2^-)that can attack, oxidize and decompose the component pigments, lipids and proteins of PS II (Fig. 8). The steps can be written for example, as: $^1Chl^* + {}^3O_2 \rightarrow {}^3Chl + {}^1O_2$. The complex events associated with PS II inactivation in the presence of oxygen are collectively termed 'aerobic photoinhibition'. There are a host of various

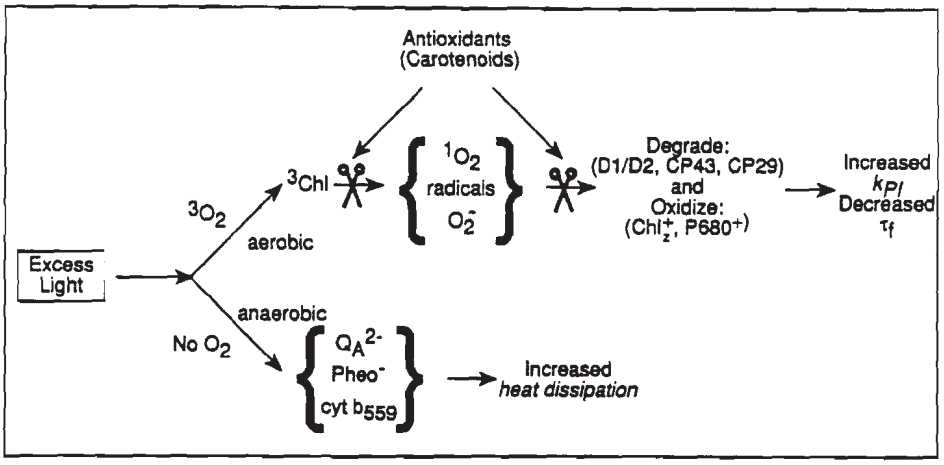


Fig. 8 A scheme relating the effects of excess absorbed light in the presence and absence of air on the function and composition of the PS II unit. Under aerobic conditions high excess light leads to overexcitation of PS II and increased yields of triplet excited state Chl (^{3}Chl). Triplet Chl can be deactivated by carotenoids (^{1}Car); however, the triplet excited Chl can, through photosensitization, cause the formation of singlet oxygen ($^1O_2$), oxygen radicals and superoxide (O_2^-) anions that can attack, decompose and oxidize the pigments, proteins and lipids of PS II. Singlet oxygen and radical oxygen species can be also deactivated by carotenoids thus alleviating oxidative damage; other antioxidant systems can also function along with ^{3}Car to alleviate oxidative degradation of PS II (see B. Logan et al., this volume for references and further discussion). Oxidation of PS II reaction center components causes an increased rate constant of nonradiative (heat) dissipation from the charge separated state, i.e., k_{PI} as shown in Fig. 4. Under anaerobic conditions excess light leads to overreduction and redox state changes in the electron-transport components of PS II including Q_A^{2-}, cytochrome b_{559} and pheophytin; these redox components can be associated with energy dissipation processes in the PS II reaction center under anaerobic conditions.

photoprotective antioxidants and radical scavenging mechanisms, including β,β-carotene as mentioned above, that help to alleviate accumulation of damaging radical species thus preventing oxidative PS II damage. Some of these reactions are: $^3Chl + Car \rightarrow {}^1Chl + {}^3Car$; $^3Car \rightarrow {}^1Car$ + heat; $^3Car + {}^1O_2 \rightarrow {}^3O_2 + {}^1Car$, etc.... For a summary of several of these protective processes and how they relate to environmental adaptation and acclimation, see Grace and Logan (1996) and B. Logan et al. in this volume.

In the absence of oxygen, excess light levels also cause various deleterious excited state interactions and redox-state changes within the electron transport components of the PS II reaction center, that are associated with overreduction of PSII electron transport components and photochemical inactivation, collectively referred to as 'anaerobic photoinhibition' (Vass et al., 1993; Kirilovsky et al., 1994; also see below).

4.2 Biophysical Characterization of Aerobic and Anaerobic Photoinhibition

Both the aerobic and anaerobic photoinhibition effects are associated with increased nonradiative (i.e., heat) dissipation of absorbed light energy by the PS II unit. The biophysical characterization of the respective nonradiative dissipation pathways suggests that the aerobic and anaerobic photoinhibition effects are mechanistically different. An example of the effects of aerobic photoinhibition on the PS II Chl *a* fluorescence lifetimes and maximal fluorescence intensity are shown in Fig. 9; the data shown here were obtained with barley thylakoids where the ΔpH was uncoupled, the [Z + A] was low and the rate constant k_1 was minimized by the addition of the herbicide, DCMU. Figure 9A shows that increased time of exposure to high excessive light levels leads to a progressive decrease in the center of the main fluorescence lifetime distribution, i.e., the lifetime of fluorescence decreases gradually from 2 ns to as low as 0.5 ns. Figure 9B shows that NPQ (= $F_m/F'_m - 1$), is correlated with the decrease in the fluorescence lifetime. Figure 9C shows a two-dimensional view of how the lifetime distribution center decreases while the fractional intensity and distribution width parameters remain relatively constant in response to increasing times of exposure to high excessive light levels in air. Since changes in the fluorescence lifetime center were observed, it is clear that the changes in steady-state fluorescence intensity are attributable to changes in the quantum yield of fluorescence under these conditions. Because it is believed that the 2 ns lifetime is inversely proportional to the rate constant of PS II charge separation k_1 (Dau, 1994), it is proposed that the decreased fluorescence lifetime is caused by an increased rate constant of heat dissipation that is in direct competition with charge separation (see Renger et al., 1995). The rate constant describing this process in the PS II unit is represented by k_{PI} in Fig. 4B. Kirilovsky et al. (1994) have suggested that aerobic photoinhibition is complex and involves combined PS II acceptor side inactivation and overoxidation of the PS II donor side components. One

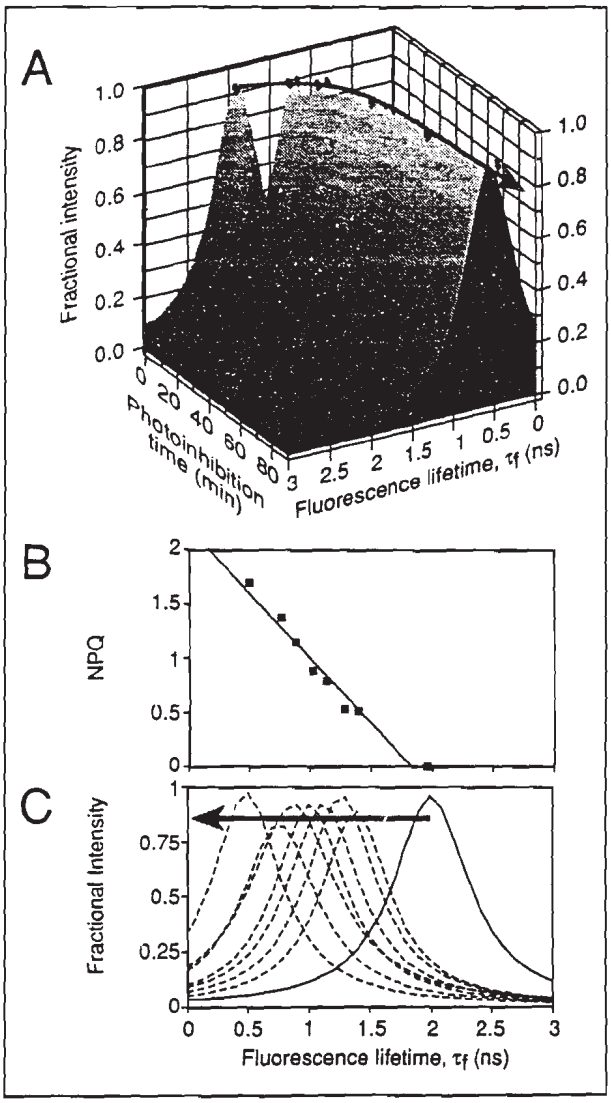


Fig. 9 Relationship among the PS II Chl *a* fluorescence lifetime distributions, the time of exposure to high excess light in air (photoinhibition time) and the decrease in maximal fluorescence intensity in barley thylakoids. In *Panel A*, the surface of the three dimensional plot was drawn using the average fluorescence lifetime distribution fractional intensity and width parameters and the best fitting relationship between the photoinhibition time and the fluorescence lifetime. The solid circle symbols show the actual data relating the fluorescence lifetime and the photoinhibition time. *Panel B* shows the linear relationship between the fluorescence lifetime and the decrease in the maximal fluorescence intensity calculated as NPQ = $F_m/F'_m - 1$. *Panel C* shows a two-dimensional view of the effects of increasing times of exposure to high excess light in air on the main PS II Chl *a* fluorescence lifetime distribution; the solid line represents the distribution at time = 0 min exposure, the dashed lines are the lifetime distributions after a given period of exposure and the gray arrow indicates increasing time. The data were taken from Gilmore et al. (1996b).

of the pathways for the loss of the energy may involve the formation of a special Chl_Z^+, e.g., from $P680^+$: $Chl_Z + P680^+ \rightarrow Chl_Z^+ + P680$. We consider it likely that Chl_Z^+ may be as efficient in quenching Chl fluorescence as $P680^+$. The aerobic photoinhibition effects are not associated with the accumulation of doubly-reduced forms of the primary quinone electron acceptor, Q_A^{2-}; it had been predicted that Q_A^{2-} should be accompanied by a long-lived ($\tau_f \approx 10$ ns) fluorescence lifetime component, that itself is believed to be attributed to the slow spin dephasing and recombination of the triplet radical pair to $^3P680$ (see Vass et al., 1993). Clearly, no such component was observed. Further, anaerobic photoinhibition effects are reportedly associated with other energy dissipation pathways and with redox state changes in cytochrome b_{559} involving electron transfer in a cyclic manner, from pheophytin ($Pheo^-$) to cytochrome b_{559} and back to P680 (for a complete discussion, see Whitmarsh and Pakrasi, 1996).

5. Physiological-Ecological Response Range of Photosystem II to Excess Light

Table 1 summarizes a current view of the gradient of responses for PS II unit by relating the degree and duration of environmental stress to the biophysical response of the PS II units as well as the biochemical changes that mediate and correlate with the response.

5.1 Short-term Response

Table 1 shows that the first order of defense for a typical PS II unit, for example of a plant growing in a shady environment when exposed to rapid intermittent doses of excess light, is the xanthophyll cycle-dependent energy dissipation. Typically, otherwise nonstressed, healthy plants show a diurnal time course where the level of xanthophyll cycle-dependent energy dissipation tracks the solar light intensity, peaking at midday when it becomes most excessive. Indeed, it is now clear that xanthophyll cycle-dependent energy dissipation is part of the daily and day-to-day life of almost all terrestrial higher plants and many algae and diatoms (Demmig-Adams et al., 1996).

5.2 Longer-term Responses

As an extension to the highly chloreographed short-term (secs to hours) photoprotective responses of the PS II unit to excess light levels described above, there is also a well defined long-term (hours to seasons) pattern of events involving changes in the overall pigment-protein content and structural composition of the PS II unit. As shown in Table 1, when the duration and or extent of excess light exposure increases, for example, in a fully sun exposed environment, or during suboptimal temperatures, it is possible that the molecular content of the PS II unit is altered such that there is a decrease in the size of the peripheral PS II antenna (Anderson, 1986; Tyystjärvi et al., 1994). This is mostly attributed to a decrease in the LHCIIb and subsequent

Table 1

Extremity and Duration of Excess Light Stress	Typical Environmental Situation	Biophysical Response of PS II Unit	Biochemical Symptom	Reference
	Shade Acclimated; Optimal Temperature	Dissipate Excess Light; Increase k_A	Lumen acidification [V] → [Z + A]	Demmig-Adams et al. (1996) Gilmore (1997)
	Sun Acclimated; Sub-optimal Temperatures	Avoid and Dissipate Excess Light; Decrease σ; Increase k_A	Decrease N_{pig} mostly LHCIIb (Chl *a* + *b*, lutein, neoxanthin)	Demmig-Adams et al. (1996) Falk et al. (1995) Gilmore (1997)
	Extreme Excess Light; Freezing Temperatures (Overwinter)	Degrade and Increase Heat Loss from PS II Reaction Center *Increase k_{PI}	Loss of Core and Inner Antenna Proteins (D1, D2, CP43, CP29)	Ottander et al. (1996) Russel et al. (1995)

decrease in its component pigments, namely, Chls $a + b$, lutein and neoxanthin. In most cases, long term acclimation of PS II is probably accompanied by no change or a significant increase in the number of xanthophyll cycle pigments, V, A and Z; thus the ratio of V + A + Z to total Chl $a + b$ increases (Demmig-Adams et al., 1996; Gilmore, 1997). According to our current model of the exciton dynamics of PS II (Fig. 4A and B), the purpose of the decreased σ and N_{pig} may be to avoid absorption of excess light, thereby optimizing and preserving the photochemical function of the PS II unit. It has been reported that N_{pig} and σ change by up to a factor of 2 in sun versus shade-adapted plants for example, and by a considerably larger factor in several varieties of the Chl *b* deficient *chlorina* mutants (Anderson, 1986; Tyystjärvi et al., 1994; see review Gilmore, 1997). Another interesting consideration regarding changes in the LHCIIb levels is that decreasing LHCIIb could lead to a larger population of PS II units that have their fluorescence quenched by the xanthophyll cycle-dependent mechanism. This is expected from model predictions because decreasing the concentration of LHCIIb usually correlates with a higher proportion of [V] converted to [Z + A] on a per PS II unit basis (Demmig-Adams et al., 1996; Gilmore, 1997). Therefore, as outlined in Equation (8) the higher [Z + A] should increase the total concentration of PS II units that have their fluorescence quenched (see Fig. 5). In conclusion, the decreased σ and/or the increased [Z + A] are probably the most important primary factors in shade to sun or low temperature acclimation on a per PS II unit basis.

5.3 Extreme Responses-damage and Repair of Photosystem II Reaction Centers

A natural follow up of the above concepts concerning the limitations of the photoprotective avoidance and dissipation mechanisms is to understand what happens when these photoprotective strategies fail under prolonged and or extreme stresses. It is important to consider that the degree of stress required to inflict failure of PS II function is usually associated with an environmental condition so severe that the primary biochemical functions are almost completely inhibited. For instance, the diffusion-limited processes of carbon-fixation and respiration are extremely slowed down at temperatures less than 5°C or when the atmospheric carbon-dioxide or oxygen concentrations are greatly reduced (Berry and Björkman, 1980; Björkman and Demmig-Adams, 1994). Thus, the observed degradation of the PS II core (D1, D2, CP43, CP29, etc.) proteins under extremely high-light exposure, at freezing-low temperatures or with limiting electron acceptors (CO_2, O_2) can be viewed (see Table 1) as symptomatic, as opposed to a causal effect, of the inhibited photosynthesis (Ottander et al., 1995; Russell et al., 1995). However, the sustained inhibition of photosynthetic productivity upon return to favorable environmental conditions is a significant and genuine concern and is the primary reason for the intense interest in the photoprotection and photodamage/

repair phenomena. It has been demonstrated in many studies that when the rate of reaction center damage exceeds that of repair (reaction center protein synthesis and assembly) accumulation of inactivated PS II units ensues (Chow, 1994). Thus, optimizing the balance of PS II damage and damage-repair mechanisms is clearly an important photoprotective strategy in light of extreme stress (see chapters by A. Mattoo et al., B. Logan et al., and C. Critchley, this volume). Further, because inactive or photoinhibited PS II centers have increased rate constants of heat dissipation, it has been proposed that inactive centers may serve a photoprotective purpose by dissipating excess light that may otherwise cause further damage to intact-functional PS II centers that share excitation energy (see Chow, 1994).

6. Future Research Regarding Adaptation and Acclimation of Photosystem II

As indicated in the previous sections, a great deal has been learned about how higher plants can optimize and preserve the function of the photosynthetic apparatus under environmental stress. Obviously, future research in this area will focus on understanding the balance between productive light-energy consumption by PS II photochemistry and energy dissipation by photoprotective and photoinhibitory mechanisms as well as avoidance and damage-repair strategies. We expect that there will be the enhanced utilization of molecular biological, biochemical and biophysical research tools that will enable us to probe the complex molecular and structural function of PS II and thus the photosynthetic apparatus as a whole.

The future use of molecular biotechnology will facilitate the ongoing efforts to understand the crucial details of not only the pigment-protein content and composition of PS II, but the way these details are regulated during the life of a plant under variable environmental conditions. For example, molecular manipulations, including generation of site-directed mutations, anti-sense constructs and deletions targeting the xanthophyll cycle processes and the xanthophyll cycle-chlorophyll-protein interactions may further elucidate the biophysical mechanism by which PS II fluorescence and photochemistry are reduced. Indeed, several recent advances have been made concerning cloning and sequencing of both the violaxanthin deepoxidase (Bugos and Yamamoto, 1996) and zeaxanthin epoxidase (Marin et al., 1996) genes, as well as identification of single nuclear gene mutations affecting key steps in carotenoid biosynthesis (Pogson et al., 1996). Likewise, molecular manipulation of the complex enzymatic processes controlling reaction-center protein synthesis and assembly may unlock the potential to engineer plants with improved regenerative properties and photosynthetic yields.

Regarding the future utility of biophysical techniques, improved X-ray and electron diffraction studies of the LHCIIb (Kühlbrandt et al., 1994) and of the inner antenna CP complexes, as well as the overall PS II reaction center unit, will hopefully provide us with a more quantitative framework

for modeling and understanding the complex energy transfer and energy dissipation process. Moreover, the continued use and improvement of time-resolved PS II Chl *a* fluorescence and absorption technology will be crucial in relating the structure to the function of PS II. In conclusion, it is hopefully anticipated that future research will proceed along the combined fronts of molecular and structural biology and biophysics and computational biology and through the innovative and integrative application of new and established research tools.

Acknowledgments

A.G. was initially supported by a training grant (DBI 96-02240) from the National Science Foundation Program for Collaborative Research in Plant Biology at UIUC and presently by a Research Fellowship at the RSBS at the Australian National University. We thank Drs. B. Demmig-Adams, M. Ball, W.W. Adams, W.S. Chow and T.J. Wydrzynski for making suggestions towards the improvement of the text presented here. Special thanks are offered to visiting. Prof. V. Shinkarev at UIUC Center of Biophysics and Computational Biology for help and stimulating discussions regarding the formation and analysis of pH-dependent binding models. We also thank Dr. T.H. Hazlett and Prof. P. Debrunner, whose collaborative research is discussed in this chapter.

Abbreviations

A—antheraxanthin; Car—carotenoid species; Chl *a, b*—chlorophyll *a, b*; c_H—enthalpy factor summing the spectral free energy differences between the antenna and P680 trap Chls of PS II; CPX—chlorophyll binding protein of PS II inner antenna of molecular mass X; DCCD—dicyclohexylcarbodiimide; DCMU—3-(3, 4-dichlorophenyl)-1,1-dimethylurea; DTT—dithiothreitol; D1/D2—PS II core heterodimer composed of D1 and D2 proteins; ΔE_{IIb},ΔE_{CORE}—absorption energy (kT) of LHCIIb, PS II core; F_m, F'_m—maximal PS II Chl *a* fluorescence intensity with all Q_A reduced in the absence, presence of thylakoid membrane energization; F_O—minimal PS II Chl *a* fluorescence intensity with all Q_A oxidized; $F_v = F_m - F_O$—variable level of PS II Chl *a* fluorescence; f_x—fractional intensity of fluorescence lifetime component x; k_A—rate constant summing all antenna energy dissipation processes in PS II antenna; K_b—equilibrium binding (association) constant between Z + A and minor CP complexes; k_f—rate constant of PS II Chl *a* fluorescence; k_h—rate constant of heat dissipation in SP II; $k_{IIb \rightarrow CORE}$—rate constant of excitation transfer from LHCIIb to PS II core; k_p—rate constant of photochemistry in PS II; $k_{tr \rightarrow PSI}$—rate constant of energy transfer from PS II to PSI; k_1—rate constant of PS II charge separation; k_{-1}—rate constant of PS II charge recombination; k_2—rate constant of PS II charge stabilization; LHCIIb—main light harvesting pigment-protein complex (of PS II); PFD—photon flux density (μmols photons $m^{-2}s^{-1}$); Pheo—reaction center pheophytin

(active); NPQ=F_m/F'_m – 1, —nonphotochemical quenching of PS II Chl *a* fluorescence; N_{pig}—molecules of Chl *a* or *b* per PS II; N_{IIb}, N_{CORE}—number of lattice sites (excitonically coupled Chl clusters) in LHCIIb, PS II core; PS I—PS II Photosystem I, II; P680—special pair of Chl *a* molecules of PS II reaction center; *p680*—probability of an exciton residing on the special pair Chls; Q_A—primary quinone electron acceptor of PS II; V—violaxanthin; w_x—width at half maximum of Lorentzian fluorescence lifetime distribution x; Z—zeaxanthin; ΔS—entropy difference; ΔpH—trans-thylakoid proton gradient; σ—absorbance cross section of PS II; $\langle \tau \rangle$, $\langle \tau \rangle' = \Sigma \tau_x f_x$—average lifetime of Chl *a* fluorescence in the absence, presence of nonphotochemical fluorescence quenching; τ_f—fluorescence lifetime; τ_x—lifetime (in ns) of fluorescence component x.

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