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On the relationship between non-photochemical quenching and photoprotection of Photosystem II

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ABSTRACT

Non-photochemical quenching (NPQ) of chlorophyll fluorescence is thought to be an indicator of an essential regulation and photoprotection mechanism against high-light stress in photosynthetic organisms. NPQ is typically characterized by modulated pulse fluorometry and it is often assumed implicitly to be a good proxy for the actual physiological photoprotection capacity of the organism. Using the results of previously published ultrafast fluorescence measurements on intact leaves of w.t. and mutants of Arabidopsis (Holzwarth et al. 2009) we have developed exact relationships for the fluorescence quenching and the corresponding Photosystem II acceptor side photoprotection effects under NPQ conditions. The approach based on the exciton–radical pair equilibrium model assumes that photodamage results from triplet states generated in the reaction center. The derived relationships allow one to distinguish and determine the individual and combined quenching as well as photoprotection contributions of each of the multiple NPQ mechanisms. Our analysis shows inter alia that quenching and photoprotection are not linearly related and that antenna detachment, which can be identified with the so-called qE mechanism, contributes largely to the measured fluorescence quenching but does not correspond to the most efficient photoprotective response. Conditions are formulated which allow simultaneously the maximal photosynthetic electron flow as well as maximal acceptor side photoprotection. It is shown that maximal photoprotection can be achieved if NPQ is regulated in such a way that PSII reaction centers are open under given light conditions. The results are of fundamental importance for a proper interpretation of the physiological relevance of fluorescence-based NPQ data.

1. Introduction

Photoprotection in plants and algae incorporates a variety of signaling mechanisms and regulatory responses to excess light conditions ranging from immediate local changes in the light-harvesting antennae or the photosynthetic reaction centers (RC), pH regulation of the cytochrome b_6/f complex [1–3], to changes in chloroplast organization to system-level acclimation responses affecting gene expression (see [4,5] and refs. therein). Despite these elaborate regulation and defence systems, excess light conditions can cause photodamage and, when persisting over extended time or combined with other stress factors, can lead to photoinhibition [6–9] – i.e. persistent reduction of the overall photosynthetic yield – and eventually to large scale destruction of the photosynthetic apparatus and plant death. Here we must clarify that photoinhibition, in the sense of sustained reduction (or downregulation) of photosynthetic capacity and decreased number of functional RCs, is in itself a photoprotection process that is actively regulated via signaling and genetic programs [10] and is crucial for the plant's acclimation and survival, e.g. in overwintering evergreens [11,12]. In this paper, however, we restrain the meaning of the term "photoprotection" only to processes that potentially prevent or decrease photoinhibition. Similarly, the term "photodamage" should be read "primary photodamage", which may cause photoinhibition but not necessarily irreversible physiological damage.

The main sites of photodamage are the photosynthetic RCs, primarily of Photosystem II (PSII), the antennae of PSII and likely also PSI. Photodamage steps are mediated by reactive oxygen species, the most important of them being singlet oxygen and H_2O_2 [13–15]. While the overall reaction chains leading to photoinhibition are very complex [16,17], the large majority of them start with the creation of chlorophyll triplet (^{3}Chl) states sensitizing the production of singlet oxygen by interaction with ground state molecular oxygen ($^3O_2$) in the thylakoid membrane [18,19]. ^{3}Chl is produced by intersystem crossing from singlet excited Chl in the light-harvesting antenna complexes or even more efficiently by charge recombination processes in the RCs. Up to now there exists no conclusive evidence that major photodamage effects to the photosystems occur from

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^{3}Chl produced initially in the antennae. This is explained by the fact that antenna Chls are in close contact with carotenoids, which efficiently and rapidly quench the ^{3}Chl states [20–26]. The PSII RC Chls on the other hand do form triplet states by a special radical pair mechanism and subsequent triplet charge recombination at a rate much faster than normal intersystem crossing from the Chl singlet excited states, in particular if PSII RCs are closed [27–32]. These RC triplets live long enough to interact efficiently with ground state O_2 [33]. Thus the primary route for photodamage in PSII is acceptor-side photodamage due to the formation of ^{3}Chl in the RC [34,35]. Other photodamage pathways exist, e.g. direct light-induced damage to PSII [8], but we maintain the notion that PSII acceptor-side damage is the dominant photodamage mechanism under physiologically relevant light intensities. Following this notion *photoprotection* of PSII (acceptor side) can be defined as *any process that reduces the yield of triplet states in the PSII RC*. With this generalization a wide range of photodamage and photoprotection reactions to PSII can be treated in a quantitative manner without going into the very details of these reactions. Fundamentally, since ^{3}Chl formation is in competition with the forward electron transfer processes, it cannot be completely avoided in any photosynthetic system and needs to be taken care of by various protection mechanisms.

The most important short-term reversible photoprotective process in higher plants, eukaryotic algae, and essentially all other oxygenic photosynthetic organisms, is the so-called non-photochemical quenching (NPQ) of chlorophyll fluorescence [36–38,38–42]. It is also often referred to as "feedback de-excitation" [43] since it is triggered primarily by the high pH gradient across the thylakoid membrane induced by high light (HL) irradiation. NPQ is clearly a photoprotective process in the sense of the above definition since enhanced thermal (i.e. non-photochemical) de-excitation of singlet excited states in the PSII antenna will reduce the excitation pressure on the RC and hence the ^{3}Chl yield. It has been shown that NPQ is indeed essential both for the optimal growth and survival of plants under natural conditions [44]. Typically the NPQ response and maximal NPQ capacity of photosynthetic organisms is determined by the PAM fluorimeter method which compares the PSII fluorescence intensity of the system under HL stress (F_m') to the maximal fluorescence intensity emitted from the system after dark adaptation (F_m), each measured after a short strong light pulse applied to close all PSII centers [40]. This type of measurement yields the so-called "NPQ parameter", which is termed here as NPQ_{SV} and is defined as $NPQ_{SV} = F_m/F_m' - 1$, denoted in the literature also as SV_N or qN_{SV} according to its derivation based on the assumption of Stern–Volmer quenching [45]. The maximal NPQ_{SV} measured in higher plans at saturating light is up to 5–6 [46,47] but much higher values can sometimes be observed, e.g. in diatom algae [48].

So far, the quantitative relationship between NPQ and the actual photoprotection of PSII has never been derived and is essentially unknown because the latter is not easily measurable. In order to judge and differentiate the physiological importance of the different NPQ processes it is essential to understand the impact of NPQ on real photoprotection. The goal of the present paper is to derive a quantitative relationship between the NPQ_{SV} parameter and the degree of acceptor-side photoprotection to PSII based on a kinetic model that allows a detailed consideration of various quenching effects as well as different possible quenching sites. NPQ cannot be described by a single mechanism but comprises several biochemically and biophysically distinct mechanisms and likely also different quenching sites in the photosynthetic apparatus [41,49–56]. In addition to enhanced heat dissipation in the PSII antenna NPQ can be realized e.g. by detaching parts of the antenna from the RC, with or without enhanced dissipation in the detached antenna [50,52,53]. Yet another site of NPQ may be the RC itself [57,58]. A detailed study of the fluorescence emission spectra associated with the different temporal components of NPQ induction in Arabidopsis (*Arabidopsis thaliana*) leaves revealed specific differences confirming the presence of at least two and possibly more independent NPQ mechanisms, one dependent primarily on PsbS and the other dependent on zeaxanthin (Zx) [59]. It is thus important to ask which of these mechanisms, or sites, plays the dominant role in protecting PSII from acceptor side photodamage, and under which conditions and how the combination of the different mechanisms will contribute to overall photoprotection and regulation.

To answer these questions, one must take into consideration the details of charge separation, charge recombination, and triplet formation processes in PSII. Fortunately ultrafast spectroscopy on isolated photosynthetic complexes as well as intact systems has provided the necessary information on the energy and electron transfer kinetics leading to precise kinetic descriptions of the primary processes in the photosystems [60,61] and in particular in PSII [62–66]. This now allows us to derive quantitative relationships between the fluorescence yield (or integrated intensity) and all photochemical and non-photochemical rate constants obtainable from time-resolved measurements. The analysis in this paper is based on ultrafast fluorescence lifetime data collected from wild type (w.t.) and various NPQ-affected mutants of Arabidopsis [50]. For the description of the PSII excited state kinetics we use the basic exciton–radical pair equilibrium model [50,64]. This allows us to directly relate fluorescence quenching and the derived NPQ_{SV} based on the fluorescence kinetics parameters on the one hand to changes in the RC ^{3}Chl yield of PSII in response to the different NPQ mechanisms on the other hand. We also compare quantitatively the different possible NPQ mechanisms for their actual acceptor-side photoprotective efficiency on PSII. Taking all these points together it follows that the quantitative relationship between the simple phenomenological parameter NPQ_{SV} and the actual photoprotection effect should be expected to be rather complex. As will be shown in this work it is best to consider NPQ_{SV} simply as an easily measurable technical parameter and the aim is to find quantitative descriptions for the actual photoprotection effect.

2. Materials and methods

The theoretical modeling of the kinetics of PSII was based on the exciton–radical pair equilibrium model [50,64]. The model system, containing compartments corresponding to singlet excited (fluorescing) states, charge-separated states (radical pairs) and triplet states, was described by the transfer matrix in which diagonal elements represent the total decay rate constants of each compartment and off-diagonal elements are the rate constants of exciton/electron transfer between components [67]. All rate constants used in the modeling were obtained from analysis of the picosecond time-resolved fluorescence data of intact Arabidopsis leaves. For details on the measurement and analysis procedure see Ref. [50]. For the initial conditions the population of the excited state compartment was set to unity and the concentration of all other compartments to zero. The system of homogeneous linear differential equations was solved by numerically determining the eigenvalues of the transfer matrix using matrix inversion and calculating the weighted eigenvector (amplitude) matrix. This allowed calculation of the lifetimes of the system (reciprocal eigenvalues) and the time-dependence of the population (concentration) of each compartment. All calculations were performed in MATLAB (The MathWorks, MA, USA).

3. Results and discussions

3.1. Kinetic model of Photosystem II

A kinetic model of the energy and electron transfer reactions in PSII, based on the so-called exciton–radical pair equilibrium (ERPE) model [64], was used to draw quantitative information about the relationship between NPQ and photoprotection of PSII. The kinetic

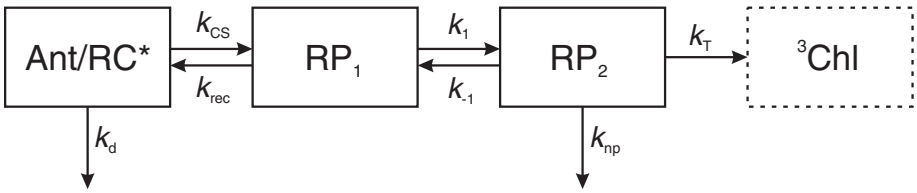

Fig. 1. ERPE compartment model used for simulating the effects of different photoprotective mechanisms in PSII. The rate constants in the scheme are determined from time-resolved fluorescence kinetics data obtained in the dark-adapted (unquenched) and light-adapted (quenched) states of intact Arabidopsis leaves [50]. k_d is the effective antenna deactivation rate constant, k_{CS} is the charge separation and k_{rec} the charge recombination rate constant from the first radical pair RP_1. k_1 and k_{-1} are the secondary electron transfer forward and backward rate constants to/from radical pair RP_2. k_T is the rate constant for triplet (^{3}Chl) formation in the RC and k_{np} is the total rate of other non-photochemical losses from RP_2 (non-radiative recombination, etc.).

model is shown in Fig. 1. We chose this particular scheme in our simulations because it has been demonstrated in the past as the minimal kinetic scheme that can reliably describe the experimental fluorescence kinetics as well as the transient absorption kinetics of early PSII reactions in a large variety of PSII complexes from different organisms and intact systems, thus correctly explaining both the excited state dynamics as well as the radical pair dynamics [61–64,68–71]. It was for these reasons also applied successfully to analyze the fluorescence kinetics of intact leaves under quenched and unquenched conditions [50]. The approach used here is valid regardless of the exact detail of the antenna description and regardless of the exact energy transfer parameters. Extension to more elaborate antenna descriptions would be straightforward if required at a later stage.

The ERPE reaction scheme (Fig. 1) consists of the following compartments: Ant/RC* represents all excited states in the system (antenna and RC); RP_1 and RP_2 represent the primary and secondary radical pairs [71], respectively, and ^{3}Chl represents the RC Chl triplet state. Note that the last compartment in the reaction sequence (^{3}Chl) does not influence the overall kinetics but serves as an accumulator for loss processes from the radical pairs. It allows in a convenient way to describe quantitatively the Chl triplet formation kinetics and yield, i.e. the crucial quantities in the present model. We use the model in the form appropriate for closed PSII (Q_A reduced) because NPQ by definition is the decrease in fluorescence yield in the system with closed RCs (where photochemical quenching is zero) [40]. Thus the forward electron transfer rate from RP_2 is assumed to be zero. In reality, even when Q_A reduction is completely blocked, there will be some additional processes deactivating RP_2 via alternative pathways other than triplet formation alone. Such processes, represented by the rate constant k_{np}, are for example non-radiative recombination directly to the RC ground state or transfer to external electron acceptors. The latter processes are omitted here for simplicity without any loss of generality.

The rate constants k_i used in the basic ERPE model, determined by fitting the picosecond fluorescence decay kinetics of Arabidopsis leaves with closed PSII RC [50], are summarized in Table 1. Measurements were performed in the dark-adapted state (F_m) and light-adapted state (F_{NPQ}, 600 μmol photons $m^{-2}\,s^{-1}$ red light). In addition to the w.t. leaves, results for the PsbS-deficient and PsbS overexpressor mutants, *npq4* and *L17*, are shown. The F_m state was used as the reference unquenched state for all comparisons. The dynamics of the system intermediates in the unquenched state is illustrated in Fig. 2A in the form of population kinetics of the different compartments — the decay of excited states, the transient population of the two radical pairs, and the accumulation of ^{3}Chl. The resulting PSII fluorescence lifetimes are 76 ps, 628 ps and 1.9 ns. In a simplified interpretation these lifetime components correspond to the lifetimes of primary and secondary charge separation, and radical pair deactivation (loss processes from the RPs). The average excited-state (fluorescence) lifetime is given as

$$\tau_f = \tau^*_{av} = \frac{\sum a_{i,[\mathrm{Ant/RC^*}]}\tau_i}{\sum a_{i,[\mathrm{Ant/RC^*}]}}$$

where $a_{i,Ant/RC^*}$ and τ_i are the relative amplitudes and corresponding lifetimes [50]. The triplet yield is calculated as the relative population of the ^{3}Chl compartment at time t_∞ (in practice $t_\infty = 20$ ns):

$$\varphi_T = \sum a_{i,[^3\mathrm{Chl}]} e^{-t_\infty/\tau_i}.$$

The absolute value of φ_T depends on the branching between k_{np} and k_T (Fig. 1), which is not derived from the experimental data. We have scaled the ratio of k_{np} and k_T such that the resulting φ_T of the unquenched PSII with closed RC is 20% (for reasons explained below). It is important to note that our goal is not to precisely determine the absolute triplet yield but to calculate the photoprotection effect on the basis of *relative triplet yield change*. The latter quantity is independent from the absolute yield or the scaling of the k_{np} and k_T.

Table 1
Rate constants for the PSII kinetics in Arabidopsis leaves determined from time-resolved fluorescence data [50].

Genotype	w.t. Arabidopsis		*npq4*	*L17*
State	Dark-adapted (F_m)	Light-adapted (F_{NPQ})	Light-adapted (F_{NPQ})	Light-adapted (F_{NPQ})
k_d	0.4	1.7	1.3	1.6
k_{CS}	3.0	2.1	1.0	0.9
k_{rec}	8.5	22.4	22.4	15.0
k_1	2.0	3.7	5.5	2.5
k_{-1}	0.5	2.0	1.4	0.8
k_p	–	–	–	–
k_{np}	0.54	0.54	0.54	0.54
k_T	0.36	0.36	0.36	0.36
% detached LHCII	–	30%	–	52%
k_d (det. LHCII)	–	2.3	–	3.4

3.2. Modeling the photoprotection effect

To study the effect of different photoprotection mechanisms we simulate their consequences on the triplet yield by altering the appropriate model parameters and then comparing the new state's solution to that of the reference unquenched state (F_m). Two parameters play a key role — the photoprotection factor and the fluorescence quenching factor. They provide the relevant direct link between the time-resolved fluorescence data on the one hand and the typically used steady state (time-integrated) fluorescence data (e.g. from a PAM fluorimeter) commonly used to determine NPQ_{SV}.

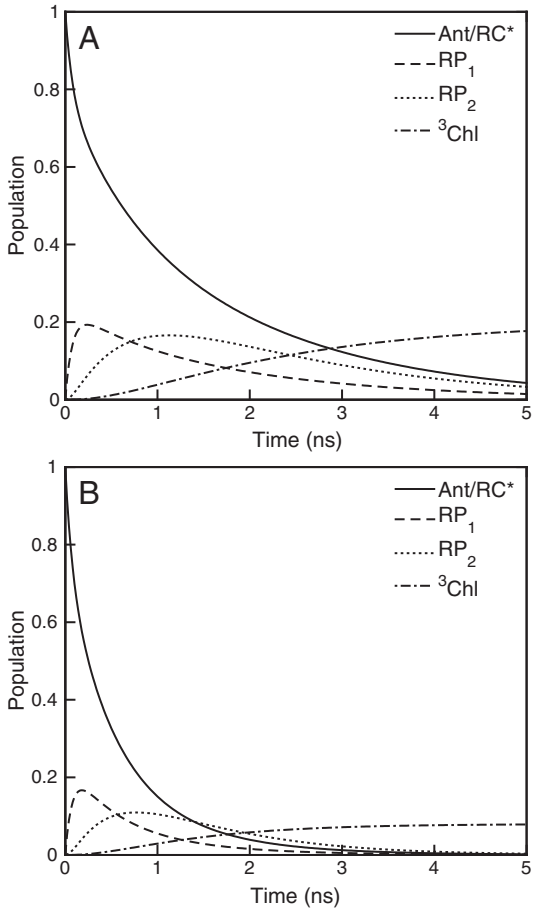

Fig. 2. Time-dependence of the populations of the four ERPE model compartments (Fig. 1). A. Solution of the model for the dark-adapted, unquenched state with closed PSII RCs. B. Solution for a state with increased rate constant of antenna deactivation, $k_d = 1.6\ ns^{-1}$.

We define the *photoprotection factor*, *P*, as the factor by which the triplet yield φ_{T1} of the new (quenched) state is reduced compared to the reference (unquenched) state's triplet yield φ_{T0}:

$$P = \frac{\varphi_{T0}}{\varphi_{T1}}.$$

A *P* value greater than one means increased photoprotection and a value less than one — decreased photoprotection as compared to the original "unquenched" state.

The fluorescence quenching factor *Q* is defined analogously as the factor by which the fluorescence yield (or fluorescence intensity) is decreased:

$$Q = \frac{\varphi_{f0}}{\varphi_{f1}}.$$

A value of $Q<1$ would mean that the fluorescence is increased rather than quenched. The fluorescence quenching factor *Q* is related to the commonly determined NPQ_{SV} by

$$NPQ_{SV} = Q - 1.$$

In the following we describe separately, and then in combination, the effects of three different potential mechanisms of photoprotection (i.e. antenna deactivation, partial antenna detachment, and photoprotective RC charge recombination). Note that all derivations only take into account PSII fluorescence and photoprotection effects. This is reasonable since PSI fluorescence contributes very little to the overall fluorescence, in particular in the states with closed PSII RCs. It is also important to point out here that all modeling results presented and discussed in this paper do not rely on or assume any particular photophysical or photochemical quenching mechanism, which is still a matter of intense debate. Rather only the different sites or locations of quenching in the photosynthetic apparatus are relevant for our results and conclusions.

3.3. Photoprotection by enhanced de-excitation in the antenna

NPQ is most commonly understood as increased thermal deactivation of Chl excited states in the PSII antenna. Simulation of this mechanism of quenching and photoprotection in our model is straightforward. It is achieved by increasing the rate constant of antenna deactivation k_d beyond the level of normal non-radiative antenna decay ($k_d = 0.4\ ns^{-1}$). Note that the deactivation occurs in the PSII antenna that is functionally attached to the RC, i.e. this type of quenching corresponds to the zeaxanthin-dependent Q2 type of quenching center [50]. The solution of the ERPE model where k_d was increased to $1.6\ ns^{-1}$ – a value that is typically reached in w.t. leaves at moderate light intensities [50] is shown as an example in Table 2 and Fig. 2*B* – as population kinetics. The change in k_d resulted in shortening of the average excited-state lifetime from 1.26 ns to 0.50 ns (also compare the excited state decays in Fig. 2A and B). This translates to a fluorescence quenching factor $Q = 2.5$. The ^{3}Chl yield is reduced from 0.20 to 0.08 (note the maximum level of ^{3}Chl in Fig. 2), i.e. the photoprotection factor *P* is also 2.5 — in this case there is a one-to-one relationship between quenching and photoprotection. This means that the traditionally determined NPQ_{SV} for this type of quenching directly reflects the photoprotection factor (note however that this is valid only when PSII RCs are closed). The photoprotection *P* and the quenching factor *Q* increase linearly with k_d as is illustrated in Fig. 3A. The results show that under physiologically relevant conditions

Table 2
PSII fluorescence lifetimes τ_f, triplet yields φ_T, quenching factors *Q* and photoprotection factors *P* calculated for selected simulated and experimental conditions.

Genotype	w.t. Arabidopsis					*npq4*	*L17*
State	Dark-adapted	Dark-adapted	Dark-adapted	Dark-adapted	Light-adapted	Light-adapted	Light-adapted
Simulation	–	Antenna 50%	k_d 1.6 ns^{-1}	k_{rec} 17 ns^{-1}	–	–	–
τ_f (ns)	1.26	0.84	0.50	1.64	0.56	0.75	0.60
φ_T	0.200	0.133	0.080	0.139	0.016	0.065	0.008
P	1.0	1.5	2.5	1.4	12.1	7.6	24
Q	1.0	2.0[a]	2.5	0.8	2.4	1.7	4.0

[a] Assuming k_d in the detached LHCII 2.5 ns^{-1}.

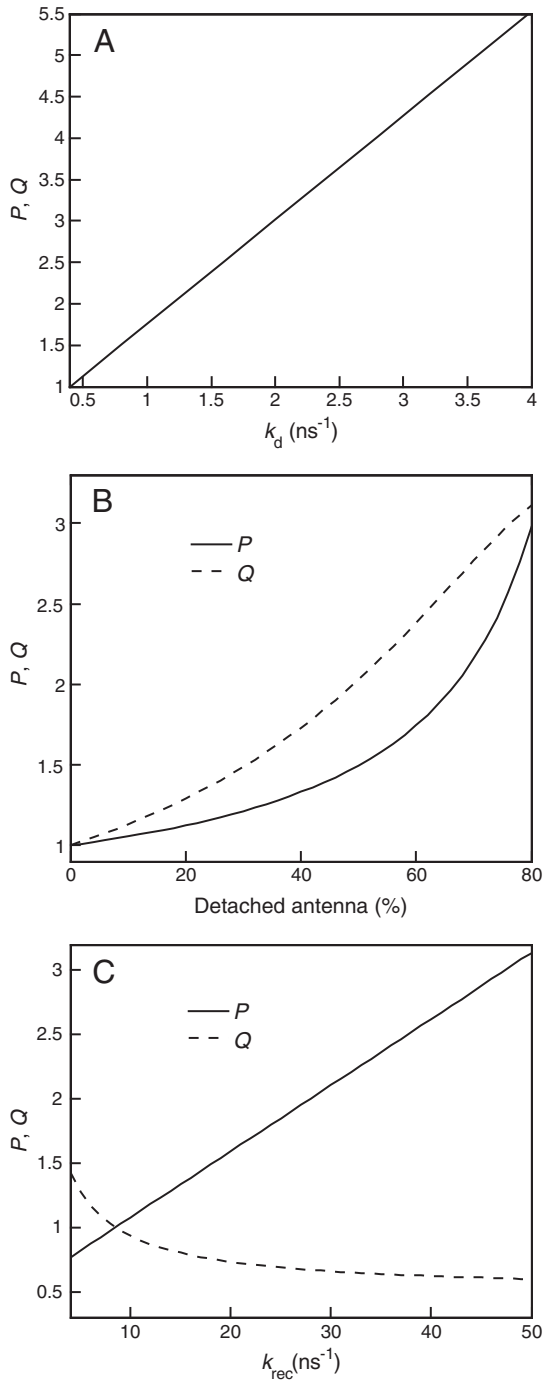

Fig. 3. Photoprotection (*P*, *solid lines*) and fluorescence quenching (*Q*, *dashed lines*) factors calculated from the ERPE model for different values of the antenna deactivation rate constant k_d (A), antenna detachment (B), or RC recombination rate constant k_{rec} (C). All other rate constants in the model are as in Table 1, state F_m. The factors are calculated as $P = \varphi_{T0}/\varphi_T$ and $Q = \tau_{f0}/[\tau_f(1-A)+0.4A]$, where $\varphi_{T0} = 0.2$ and $\tau_{f0} = 1.26$ ns are the triplet yield and fluorescence lifetime of the unquenched F_m state, φ_T and τ_f are the simulated triplet yield and fluorescence lifetime, *A* is the fraction of detached antenna, and 0.4 ns is the fluorescence lifetime of the detached antenna.

the experimentally observed change in k_d provides efficient photoprotection to PSII.

3.4. PSII antenna detachment

A second possible quenching and photoprotection type is the functional (and likely also spatial) detachment of parts of the antenna from the PSII supercomplex. Experimental evidence for this mechanism was derived from time-resolved fluorescence measurements in Arabidopsis leaves [50] and diatoms [72] as well as from biochemical and electron microscopy studies [53]. The antenna detachment, corresponding to the Q1 site of quenching [50], was shown to be strictly dependent on the action of the PsbS protein [50] and thus corresponds to the well-known qE type of quenching [73]. Reducing the effective size of the PSII antenna (Q1) is expected to have a photoprotective effect on the PSII RC, similarly to the quenching of excitation energy in the antenna (Q2), because both processes reduce the effective light capture in the antenna and thus reduce the excitation pressure on the RC.

To simulate the reduction in antenna size, the initial population of the antenna compartment [Ant/RC*] is set to the fractional size of the remaining PSII-attached antenna. Note that a change in the antenna size also requires a corresponding change in the rate constant k_{CS}, because k_{CS} represents not the *intrinsic* but the *effective* charge separation rate, which is proportional to the intrinsic rate divided by the antenna size *N* [64,74]. The lifetimes and yields resulting from solving the ERPE model with 50% reduced PSII antenna are shown in Table 2. Experimentally the PSII antenna cross-section was found to decrease by 30% in w.t. Arabidopsis under NPQ conditions and by 50% in the PsbS overexpressing mutant *L17* (Table 1). The decrease in PSII antenna size resulted in shortening of all PSII decay lifetimes even though the antenna deactivation rate constant k_d was unchanged. Consequently the average fluorescence lifetime, hence PSII fluorescence yield and the triplet yield are lower (Table 2). Clearly, the PsbS-dependent antenna detachment mechanism (Q1, qE type of quenching) has a significant benefit for photoprotection. It is however not as effective in providing photoprotection by triplet reduction as the direct antenna quenching (Q2 type).

The total fluorescence yield and quenching factor also depend on the fluorescence yield of the detached antenna. The decay rate constant of the detached antenna of w.t. leaves in NPQ conditions was $k_{d(\text{detached})} = 2.5\ \text{ns}^{-1}$ [50]. Using this value to simulate the observed fluorescence yield changes we calculated a quenching of 2.0 for 50% antenna detachment, as compared to a photoprotection factor of 1.5. Thus, when PsbS-dependent qE quenching is significant, the experimentally determined NPQ_{SV} can severely overestimate the actual photoprotection, as is illustrated in Fig. 3B.

3.5. Photoprotection by charge recombination

A third potential mechanism of photoprotection involves changes in the RC of PSII such that the balance of the various energetic pathways and back reactions is altered. The best way to achieve this condition consists in modifying the rate of charge recombination [9]. We consider the scenario in which the rate of radical pair (RP_1) charge recombination is increased. Recombination of the RP_1 state back to the singlet excited RC* is competitive with the triplet formation and therefore a photoprotection mechanism. It is described in the ERPE model (Fig. 1) by increasing the rate constant k_{rec}. The results relating photoprotection and quenching factors are plotted in Fig. 3C for a range of k_{rec} values. Lifetimes and yields are reported in Table 2 for $k_{rec} = 17\ \text{ns}^{-1}$. Enhancing the radiative recombination rate has a photoprotective effect (as judged by the reduction in triplet yield) similar in magnitude to the direct antenna quenching mechanism. There is however a drastic difference with the other mechanisms since this RC photoprotection mechanism actually enhances the fluorescence yield ($Q<1$).

This simulation demonstrates the possibility that photoprotection of PSII can be achieved without any decrease or in extreme cases even with an increase of the fluorescence yield. Whether and to what extent this particular mechanism actually occurs in reality is less well studied than the other two mechanisms and is still a matter of debate. There exists experimental evidence from several groups that the recombination rate in PSII can be adjusted under long-term HL stress [9,75]. Our time-resolved fluorescence data for dark-adapted and HL-adapted Arabidopsis leaves of various genotypes [50] indicate

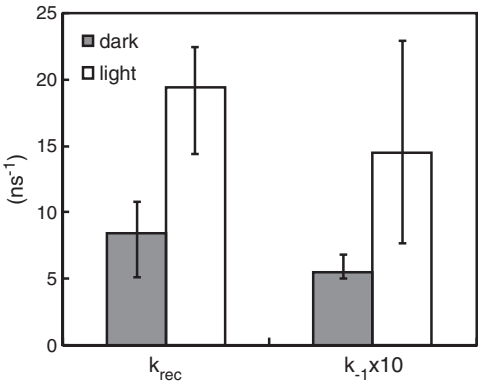

Fig. 4. Median values for the rate constants of charge recombination k_{rec} and k_{-1} determined from analysis of 14 measured fluorescence kinetics of Arabidopsis leaves (w.t. and various NPQ mutants — *npq1*, *npq2*, *npq4*, *L17* and other antenna mutants) in dark-adapted (shaded bars) and HL-adapted states (600 μmol m^{-2} s^{-1} red-orange light, white bars). The error bars represent lower and upper quartiles.

changes in the rate constant k_{rec} and the next backward electron transport rate constant k_{-1} (Fig. 4). Stimulation of the charge recombination rates is actually an expected and in some cases well-known effect of the light-induced electrical potential across the thylakoid membrane [76,77], thus it would be not surprising if it is a general consequence of the photosynthetic reactions but further detailed experiments will be needed to confirm the operation of such a mechanism in vivo.

3.6. Combined effects of two photoprotection mechanisms

Since in plants the different photoprotection mechanisms are usually triggered simultaneously, it is important to know what their cumulative effects will be. Fig. 5 shows the simulation results where two mechanisms are combined — antenna quenching with antenna detachment (panels *A*, *B*) and antenna quenching with charge recombination (panels *C*, *D*). For the first situation there exists now good experimental evidence from fluorescence kinetics studies on intact leaves from w.t. as well as various NPQ-impaired mutants of Arabidopsis and from microalgae. The RC recombination mechanism (panels *C*, *D*) is not well documented. It may become particularly important in cases when the other two quenching types are not activated, e.g. in NPQ-impaired mutants or under special physiological conditions.

As the surface plot in Fig. 5A shows, the photoprotection effects of antenna detachment and antenna quenching are additive, i.e. the total effect is the sum that the two mechanisms would exert separately. For a parameter range that is quite realistic to expect under natural conditions, the antenna quenching rate (k_d) has the dominant effect on the photoprotection whereas the role of the antenna detachment mechanism plays a smaller role for photoprotection. The fluorescence yield changes induced by antenna detachment and antenna quenching are not additive at all (Fig. 5*B*). When significant antenna detachment is present and the PSII antenna is not quenched, the fluorescence quenching

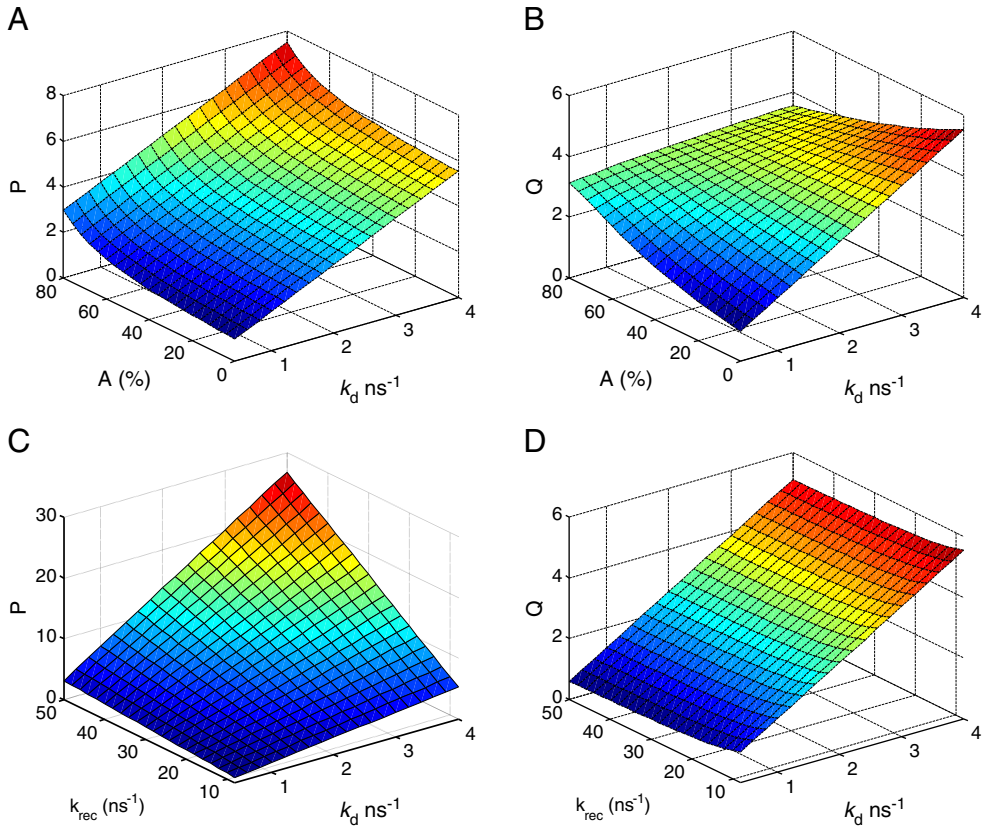

Fig. 5. Photoprotection (*P*, panels A, C) and quenching (Q, panels B, D) factors calculated from the ERPE model solved for different values of the antenna deactivation rate constant k_d (A), antenna detachment (*B*), or RC recombination rate constant k_{rec} (C). All other rate constants in the model are as in Table 1, state F_m. The factors are calculated as $P = \varphi_{T0}/\varphi_T$ and $Q = \tau_{f0}/[\tau_f(1-A)+0.4A]$, where $\varphi_{T0} = 0.2$ and $\tau_{f0} = 1.26$ ns are the triplet yield and fluorescence lifetime of the unquenched F_m state, φ_T and τ_f are the simulated triplet yield and fluorescence lifetime, A is the fraction of detached antenna, and 0.4 ns is the fluorescence lifetime of the detached antenna.

parameter Q is higher than the photoprotection P (compare panels A and B). In contrast, when strong antenna quenching is also present, the magnitude of fluorescence quenching would underestimate the photoprotection gain. This finding questions the assumption that qE quenching should also represent the dominant and most important photoprotection mechanism. Our results indicate that the most effective photoprotection mechanism is the Zx-dependent Q2 type quenching [50], which increases the PSII antenna deactivation rate.

A remarkable situation arises when antenna quenching and enhanced (singlet) charge recombination are activated simultaneously (Fig. 5C). In this case the total photoprotection factor is drastically higher than if the two separate effects would be added up. More importantly, this photoprotection gain would not be revealed by NPQ_{SV} at all or indeed by any parameter based on the fluorescence yield (Fig. 5D).

3.7. Photoprotection relationships for real-life cases

We now calculate the photoprotection factors for different actual experimental data sets obtained from Arabidopsis leaves under NPQ conditions. The ERPE model was solved using the rates from Table 1 and the results are summarized in Table 2. The most notable result of the calculation is that moderately high intensity irradiation (600 μE m^{-2} s^{-1} as compared to a growth light intensity of 150 μE m^{-2} s^{-1}) induces changes in the energetic pathways in PSII that drastically lower the triplet yield — resulting in photoprotection factors of more than 10 by the acceptor-side triplet mechanism. The fluorescence quenching factors Q (and likewise the equivalent experimental NPQ_{SV}) severely underestimate and are thus entirely inadequate measures of the degree of photoprotection. In w.t. Arabidopsis the measured fluorescence yield is only threefold reduced under these experimental conditions (600 μE m^{-2} s^{-1} actinic light) whereas the calculated photoprotection factor has a value of 12. An interesting case is the PsbS-deficient *npq4* mutant. Even though it shows a rather small NPQ_{SV}, the modeling results reveal that it is actually quite well photoprotected with a P factor of 8. This finding is in excellent agreement with the observation that the PsbS deficient *npq4* mutant grows very well under HL – without showing any photodamage effects – in the laboratory under steady state HL conditions. It is only impaired in growth under a rapidly changing light intensity regime as it occurs under natural environmental conditions [44,78]. Taking these findings together allows us to draw important conclusions with regard to the relevance and actual function of the PsbS-dependent qE quenching.

3.8. Modeling results with open and partially open reaction centers

Up to now all calculations were performed under the assumption that all RCs are closed as NPQ is commonly measured in the laboratory. A more physiologically relevant situation is the steady-state under given environmental conditions, in which the RCs are not necessarily fully closed. Therefore we estimated the photoprotection level also for situations with open and partially closed PSII RCs.

To estimate the photoprotection factors for open and partially closed PSII RCs, we use the ERPE kinetic model with rates obtained by fitting the fluorescence kinetics of dark-adapted leaves with open RCs [50], summarized in Table 3. In the case of partially open RCs the results depend on the absolute triplet yields of closed and open RCs. Unfortunately precise data on the PSII triplet yield in vivo are not available. We assume a value of 20% for closed RCs as a reasonable approximation, based on measurements with isolated PSII RCs [22,33,79]. In open RCs the ^{3}Chl yield must be drastically lower to allow for a sufficiently high quantum yield of Q_A reduction. Assuming a conservative value for the photochemical yield of 85% [80], the ^{3}Chl yield in open RCs is limited to 2%. Using these values, the photoprotection factors P were calculated for different ratios of open/closed RCs under steady state quenched conditions. The results are shown in Table 4 for three different cases (antenna quenching, detachment, and enhanced singlet recombination) and in Fig. 6 as dependences of P on the k_d rate constant (Q2 type quenching) and on the percentage of the detached antenna (Q1 type quenching). The curves corresponding to 100% closed RCs are identical with the respective curves in Fig. 3. Evidently, reopening of the RCs drastically amplifies the photoprotective efficiency of the quenching processes.

This analysis stresses the important role of the NPQ processes in maintaining the PSII RCs in an open state by reducing the excitation

Table 3
Rate constants for the PSII kinetics in Arabidopsis leaves with open reaction centers (F_o).

k_d	k_{CS}	k_{rec}	k_1	k_{-1}	k_p	k_T
0.3	3.3	4.0	30	1.7	3.5	0.1

Table 4
Modeling results for the PSII kinetics in dark-adapted state with open RCs.

	Unquenched	$k_d = 1.6$ ns^{-1}	50% det. ant.	$k_{rec} = 17$ ns^{-1}
τ_f, ps	326	229	171	463
φ_T	0.020	0.014	0.011	0.019
Q	3.9	5.5	7.4	2.7
P	10.0	14.2	19.0	10.4

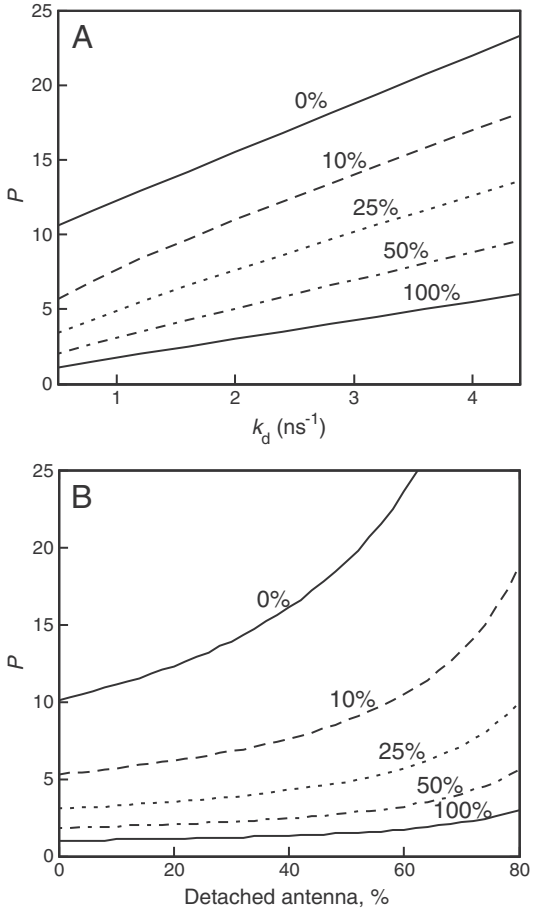

Fig. 6. Photoprotection factors calculated as a function of the antenna deactivation rate constant k_d (panel A) and the detached antenna (panel B) for different fractions of closed PSII RCs (indicated in %). Photoprotection is calculated as $P = 0.2/(B\varphi_T^{closed} + (1-B)\varphi_T^{open})$, where 0.2 is the triplet yield of closed unquenched RCs, B is the fraction of closed RCs, φ_T^{closed} and φ_T^{open} are the simulated triplet yields of closed and open RCs in the quenched state.

pressure. If NPQ is effective enough to reopen at least part of the PSII RCs the photoprotective function of NPQ is largely enhanced. It is not possible from the present data to predict the exact efficiency of NPQ in reopening the RCs since the percentage of open RCs under real-life steady state conditions depends on a variety of other factors like e.g. the ratio of excitation pressure to the downstream electron utilization [42], i.e. on the environmental conditions and the physiological state of the plant. For this reason it is of utmost importance to develop experimental procedures that are able to determine precisely the fraction of open PSII RCs under steady state illumination conditions. Since a direct method to probe the redox state of Q_A in vivo has not been developed so far to our knowledge, the best approximation is perhaps the qL parameter (but not qP) obtained from PAM fluorimetry-type measurements [42,81,82]. It follows as an important conclusion that taking into account the combined effects of the different photoprotective processes (antenna quenching, detachment, etc.) and RC reopening, the photoprotection efficiency of PSII in vivo due to NPQ under steady state conditions will typically far exceed the value that would be expected considering only the value of the experimentally measured NPQ_{SV}.

4. Conclusions

The kinetic considerations presented in this paper demonstrate clearly that NPQ and the resulting photoprotection due to NPQ-induced reduction of PSII triplet yields are neither linearly nor otherwise proportionally connected in general. In certain situations the discrepancy between the observable NPQ_{SV} and the actual photoprotection factor of PSII can be huge. This may be one reason for the apparently weak correlation between NPQ and photoinhibition, observed in a number of studies, e.g. by Sarvikas et al. [83]. Of course in many cases NPQ could still be a good indicator of the actual photoprotection such as in the case when NPQ is solely due to antenna quenching, but that would depend on species and specific conditions. For example comparable changes between NPQ and Fv′/Fm′ were found under natural conditions in evergreens [84]. The kinetic modeling approach allowed us to evaluate the two independent quenching mechanisms, identified recently [50] – antenna quenching vs. antenna detachment – for their relative contributions to the total photoprotection. The Zx-dependent antenna quenching provides the dominant photoprotective factor of NPQ quenching. The PsbS-dependent antenna detachment contributes strongly to the total NPQ but to a significantly smaller extent to the actual PSII photoprotection. Despite the lack of this mechanism and the reduced NPQ in PsbS-deficient plants, they are substantially protected by means of Zx-induced antenna quenching. This result is well in accord with the findings that *npq4* plants were not prone to photodamage even in HL as long as constant light conditions prevailed, but were only sensitive to reduction in photosynthetic yield and photodamage under largely and rapidly varying light intensities [44,78,83]. The PsbS-dependent antenna detachment mechanism is important in allowing the photosynthetic apparatus to respond rapidly to changing light conditions. The zeaxanthin-dependent antenna quenching on the other hand is a more slowly inducible process and therefore most effective during prolonged periods of HL stress. The considerations presented here just spread out the possible extreme ranges of photoprotection responses by plants due to non-photochemical quenching. In this multi-dimensional parameter space plants have several possibilities to choose their optimal photoprotection responses, which will vary with species and growth conditions. It will be the task of future experiments to determine the strategy chosen by different plant species under particular conditions. Such tremendous experimental work would by far exceed the scope of the present paper, which limits itself to point out the various possibilities and give the theoretical framework for studying “real life” situations of quenching and photoprotection for different species and external conditions.

All photodamage reactions in the scope of our calculations start with the ^{3}Chl generated in the RC. Antenna-derived ^{3}Chl states are not included explicitly in this first model but can be incorporated in our quantitative reaction model description once clear supporting evidence for such effects becomes available. There exist several other types of photodamage which do not originate from ^{3}Chl states produced in PSII RCs but which are directly connected to the production of primarily reactive oxygen or damaging radical species related to electron flow downstream of PSII. While it is clearly expected that NPQ will also have a reducing influence on such photodamage effects by reducing or better balancing electron flow they are not explicitly included in our model. Thus such photodamage (and protection) effects would occur in addition to the ones described here.

The prime purpose of the present work is to quantitatively describe photoprotection and NPQ parameters obtained from ultrafast time-resolved fluorescence data [50,59,85] and at the same time relate them to quenching parameters commonly obtained by steady-state fluorescence measurements. For a full description of the actual photoprotection effects in vivo under moderate intensity radiation and in steady-state conditions the model description must be extended to include the dependence of NPQ and photoprotection mechanisms on ΔpH and possibly other parameters like CO_2 supply, etc. [1,86]. This will be possible when detailed ultrafast time-resolved fluorescence data recorded at intermediate quenching levels and in dependence of such parameters become available. Such an approach can ultimately help to gain deeper insights into the actual roles of the different particular quenching mechanisms and their contribution to photoprotection. In the meantime, caution should be exercised when relying on NPQ_{SV} as an estimate for photoprotection and additional physiological parameters (photosynthetic rate, RC turnover, peroxidation, etc.) should be taken into account whenever possible.

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