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Light-induced degradation of PS II reaction centres analyzed by measurements of steady-state fluorescence spectra in D1/D2/cytochrome *b*-559 preparations from spinach

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The degradation of isolated D1/D2/cytochrome *b*-559 preparations from spinach induced by monochromatic blue-green light (476.5 nm) under aerobic conditions was investigated by measurements of the steady state fluorescence emission spectra. The overall fluorescence spectrum can be described by the superposition of four Gaussian bands. The dominating band peaking at 682.0 ± 0.4 nm in control samples at room temperature was found to be most susceptible to deleterious illumination of the sample. Therefore, this band, referred to as F682, was used as an indicator of sample integrity. It was found that (a) F682 gradually shifts to the blue and broadens with progressing degradation; (b) position and full width at half-maximum (fwhm) of F682 exhibit a linear correlation, which is independent of degradation temperature in the range of 275 K < T < 293 K; (c) the fwhm value Γ_{F682} of F682 exhibits a phenomenological temperature dependence of $\Gamma_{F682}(T) = \Gamma(0) \cdot (1 + C \cdot T^B)$ between 77 K and 273 K with a decrease of exponent B due to progressing sample degradation. Based on the data of this study the aerobic degradation of D1/D1/cytochrome *b*-559 preparations induced by monochromatic blue-green light is inferred to be a multistep conversion from the undamaged control sample into fully damaged comlexes, which comprises a multitude of intermediate states. These intermediate states are assigned to different conformational states of the protein matrix, which determine the electronic and vibrational interactions of the pigments consequently affecting their energy levels and the exciton dynamics within the complex.

Introduction

The primary steps of photosynthetic water cleavage in Photosystem II (PS II) comprise the following reactions (for a recent review see Ref. 1): (a) generation of excitons in the antenna and efficient transfer to a special chlorophyll *a* (Chl *a*)-containing pigment complex referred to as P680; (b) photochemical trapping by ejection of an electron from the excited singlet state ^{1}P680* and transfer to a pheophytin (Pheo) molecule with $\tau \approx 3$ ps (Ref. 2, but see Ref. 3) and; (c) stabilisation of the primary radical pair P680$^+$ Pheo$^-$ by Pheo$^-$ reoxidation with a special plastoquinone molecule Q_A as acceptor, taking place with $\tau \approx 300$ ps [4]. Although the detailed structural arrangement of the redox components P680, Pheo and Q_A within the protein matrix is not resolved, the striking functional similarities and sequence homologies led to the conclusion that these prosthetic groups are arranged within a heterodimer of polypeptides D1 and D2 in an analogous way to the corresponding reactants (special pair, BPheo, $Q_A \cdot Fe^{2+}$) in the L- and M-subunits of purple bacteria reaction centres [5,6]. This idea has gained strong support from the isolation of a D1/D2 cytochrome *b*-559 complex, which is able to perform the primary charge separation from ^{1}P680* into the primary radical pair P680$^+$ Pheo$^-$ [7,8]. Latest studies revealed that even an isolated D1/D2 complex without cytochrome *b*-559 exhibits this activity [9]. Although the loss of Q_A prevents the normal stabilization of the primary radical pair, exogenous silicomolybdate as acceptor was shown to support a light-driven linear electron transport with diphenylcarbazide acting as donor [10,11]. Despite of limitations due to lacking Q_A, the

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Abbreviations: Chl, chlorophyll; Cyt, cytochrome; F682, the main fluorescence band whose peak is located at 682 nm in control and shifts down to 676 nm in fully degraded samples; fwhm, full width at half-maximum of a Gaussian band; P680, primary electron donor of reaction centre II; Pheo, pheophytin; PS II, Photosystem II; Q, plastoquinone.

isolated D1/D2/cytochrome *b*-559 complexes provide a suitable tool to analyze the primary processes of exciton trapping and transformation into free energy within the PS II reaction centre.

The isolated D1/D2/cytochrome *b*-559 preparations contain one P680 and two pheophytin molecules, but the content of additional Chl *a* and of carotenoid molecules reported in the literature varies between 2 to 8 and 1 to 2, respectively [7,8,12–15]. Latest data reveal that the highly purified samples contain four chlorophylls and one β-carotene per two pheophytins [16]. The strucure of P680 is still a matter of debate, although latest studies [3] favour a dimeric structure analogous to the special pair in the reaction centres of purple bacteria (but see Ref. 17).

Freshly prepared samples, with high photochemical activity, exhibit absorption and emission spectra which are characterized by red peaks at 675.5 ± 0.5 nm and 681.5 ± 0.5 nm, respectively [18,19]. Illumination of these samples under aerobic conditions causes a rapid loss of the photochemical activity [21–24], together with a blue shift of the absorption [18–20,23,24] and emission peaks [18,20,24] in the red. This latter phenomenon can be used as an indicator for the degradation of the functional integrity [18–20].

In this communication we report on the modifications of the steady state fluorescence spectra due to degradation of isolated D1/D2/cytochrome *b*-559 complexes from spinach induced by monochromatic blue-green illumination (476.5 nm) under aerobic conditions. In addition to the well-known blue shift of the fluorescence peak, a significant broadening of the spectra was observed. A deconvolution into Gaussian bands reveals that the dominant component, referred to as F682, is most susceptible to sample damage. Accordingly, the marker band F682 has been used to analyze its change in peak position and FWHM value during progressing aerobic degradation in the range of $275\ \mathrm{K} < T < 293\ \mathrm{K}$.

Materials and Methods

D1/D2/cytochrome *b*-559 complexes were prepared from market spinach, as desribed by Barber et al. [8], and subsequently precipitated by polyethyleneglycol (PEG) according to McTavish et al. [11]. The final chlorophyll concentration was 0.054 mM Chl. The samples were stored at 77 K. Samples used for the studies in the temperature-range between 77 K and 273 K contained 50% glycerol.

Steady state fluorescence measurements were carried out with equipment described in detail by Stahl et al. [25]. The samples were illuminated at 476.5 nm by a continuous-wave argon ion laser, which excited the fluorescence emission and simultaneously caused the light-induced degradation of the sample. During the illumination the spectra were recorded at definite time intervals in the range of 640 nm to 750 nm, with a spectral resolution of 0.5 nm. The recording intervals were short enough (5 s) to avoid serious sample modification during this period. An optical multichannel analyzer (OMA-2, PAR) was used to monitor the spectra and to control the whole process of measuring and processing the data. The irradiation at laser intensities of either 300 W/m^2 (data of Fig. 2) or 400 W/m^2 (data of Fig. 3) was performed at different temperatures between 275 K and 293 K, which were kept constant by a temperature-controlling unit (TSK 200, ZWG). Low-temperature measurements, down to 77 K, were carried out with a directly cooled nitrogen cryostat. All fluorescence spectra reported in this communication are normalized to $F_{max} = 1$.

The spectra were deconvoluted into Gaussian bands on a wavelength scale on the basis of the formula

$$I(\lambda) = A \cdot e^{-\left(\frac{(\lambda - \lambda_0)^2}{(\Gamma/2)^2} \cdot \ln 2\right)} \qquad (1)$$

where $I(\lambda)$ is the intensity of the component at wavelength λ, A is the relative amplitude, Γ the fwhm value and λ_0 the peak position of the band. Within the narrow emission range (640–750 nm) the conversion of wavelength into an energy scale does not significantly change the spectral shape. The best fit of the experimental spectra by superposition of Gaussian bands with different positions, bandwidths and relative amplitudes was achieved with a computer program based on the down-hill simplex method of Nelder and Mead [26]. The program minimizes the least square difference between the measured fluorescence spectrum and a superposition of Gaussian bands. The significance of the deconvolution procedure was improved by: (a) calculation of second-derivative spectra of the emission spectra after a smoothing procedure; (b) use of the temperature-dependence of fluorescence in order to find a set of fluorescence bands describing the steady state fluorescence spectra at all temperatures; and (c) description of the spectra with a minimal number of free parameters.

Results

The effects of degradation on the steady state fluorescence spectrum of isolated PS II reaction centres were analyzed by comparing the data with the corresponding parameters extracted from measurements performed in particles which were not exposed to harmful illumination (referred to as 'undamaged control').

In order to check the integrity of our undamaged control samples, the red absorption band was measured. A peak value of 675.4 ± 0.4 nm was found di-

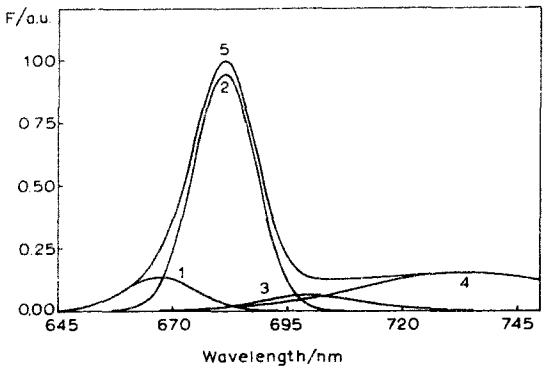


Fig. 1. Experimental steady state fluorescence spectrum of undamaged D1/D2/cytochrome *b*-559 complexes at 277 K and its numerical deconvolution into Gaussian bands. For detailed results of the deconvolution see Table I. The wavelength of the exciting laser beam was $\lambda_E = 476.5$ nm, with intensities of $I_E = (400 \pm 100)$ W/m^2. 1–4, spectral bands 1–4 (see Table I, spectrum A); 5, experimental spectrum.

rectly after thawing the samples stored at 77 K (data not shown). This finding indicates that our undamaged control is functionally highly active because the peak position of the red absorption band is a parameter that directly reflects the photochemical activity of D1/D2/cytochrome *b*-559 preparations [20,27]. The fluorescence emission spectrum of our undamaged control at 277 K exhibits a main band peak at 682.0 ± 0.4 nm and a broad satellite with a peak at 734 ± 3 nm (see Fig. 1). These features closely resemble those reported in literature (see Refs. 18, 19). A numerical deconvolution into Gaussian bands (see Materials and Methods) reveals that the overall emission spectrum can be satisfactorily described by four components, as shown in Fig. 1. The two main Gaussian bands, with peaks at 682 nm and 734 nm, are assumed to originate from the 0,0 and 0,1 transitions, respectively, of the dominant emitting Chl *a* species. It has to be emphasized that the deconvolution into Gaussian bands does not necessarily permit the drawing of unambiguous conclusions on the detailed optical properties of the individual Chl *a* species within the D1/D2 protein matrix (see Discussion). However, this procedure provides a suitable phenomenological tool to analyze the spectral changes of the fluorescence emission, which arise due to light-induced degradation of the samples under aerobic conditions. This is illustrated by a comparison of the parameters obtained by fitting the emission spectra of the control and heavily damaged samples, characterized by an emission maximum at around 676 nm. The compilation in Table I readily shows that the most prominent Gaussian band (which has its peak position at around 682 nm in the control) is most susceptible to sample degradation in both the peak position and the fwhm value. Therefore, it appears reasonable to use the properties of this Gaussian band, referred to as F682, as an indicator of the structural changes in the D1/D2/cytochrome *b*-559 complex, which are caused by light-induced degradation under aerobic conditions.

The degradative effect was measured as a function of the exposure time to continuous monochromatic ($\lambda = 476.5$ nm) light at different temperatures of the sample suspension in the range of 275 K to 293 K. Fig. 2A depicts typical spectra of the fluorescence emitted at room temperature from samples which were illuminated for different times at an intensity of 300 W/m^2. Phenomenologically, the data reveal a blue shift, together with a broadening of the spectra. Interestingly, the blue shift levels off at 676 nm, even after exposure to prolonged illumination (data not shown). Therefore, in terms of structural changes reflected by modification of the fluorescence emission spectra, these samples are referred to as 'fully damaged'. Sample illumination at room temperature under aerobic conditions also shifted the peak position of the red absorption band hypochromically down to 669.2 nm (data not shown). The extent of the blue shift depends on the exposure time. These findings correspond with data reported in the literature [18,23,28,29].

A numerical fit reveals that either a two-state model or a multistate model (see Discussion) can satisfactorily describe the experimental spectra of samples at different overall degradation states (data not shown). In an attempt to obtain additional information, the second derivative spectra were calculated (see Fig. 2B). In the undamaged control sample the spectrum exhibits a maximum at about 682 nm and a shoulder at 676 nm.

TABLE I

Numerical deconvolution into Gaussian bands of the steady state fluorescence spectra of undamaged (A) and fully damaged (B) D1/D2/cytochrome b-559 complexes at 277 K

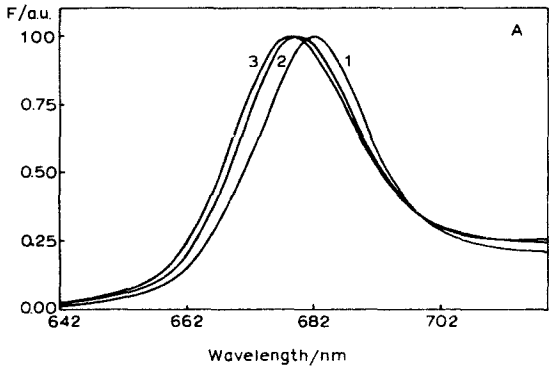
Spectrum A was recorded directly after thawing the sample. Spectrum B was obtained after a 30 min illumination with $\lambda_E = 476.5$ nm, $I_E = 400 \pm 100$ W/m^2 at 293 K. Experimental conditions as in Fig. 1. F is the relative amplitude of a fluorescence band when $F_{max} = 1$. The integral fluorescence IF represents the fraction of overall fluorescence emitted by each band.

Band No.	Position λ_0 (nm)	fwhm Γ (nm)	F	IF
1				
A	667 ±2	18 ±2	0.14±0.02	0.09
B	665 ±1	20 ±2	0.13±0.03	0.08
2				
A	681.8±0.5	15.9±0.4	0.95±0.06	0.55
B	676.6±0.6	21.6±0.3	0.92±0.07	0.59
3				
A	700 ±2	26 ±2	0.06±0.04	0.06
B	698 ±2	27 ±2	0.08±0.03	0.06
4				
A	734 ±2	55 ±2	0.15±0.02	0.30
B	733 ±2	56 ±1	0.16±0.01	0.27

With progressing degradation of the sample, both the maximum and the shoulder shift to the blue, without other significant changes in the shape. These features, however, do not permit an unambiguous distinction between a two- or multistate model.

Additional information is expected to be gathered from an analysis of the transformation kinetics. For this purpose, the experimental data were analyzed by using the F682 Gaussian band as an indicator of the structural degradative modifications induced by the exposure to monochromatic blue-green light under aerobic conditions.

Fig. 3 depicts the peak maximum (part A) and the FWHM values (part B) of the F682 band, as a function of exposure time (light intensity 400 W/m^2 at 476.5 nm) at different temperatures of the sample suspension. The data reveal that, in a comparatively narrow range of less than 20 K (275 K to 293 K) the degradation processes, as reflected by the F682 properties, are



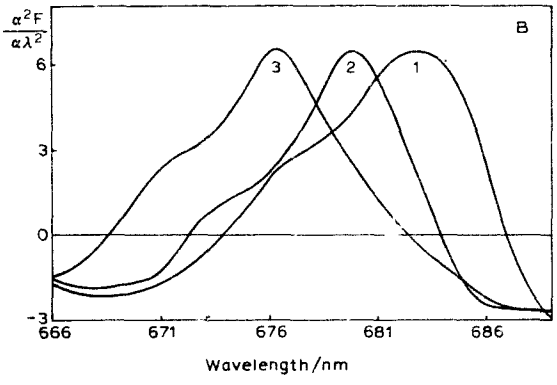
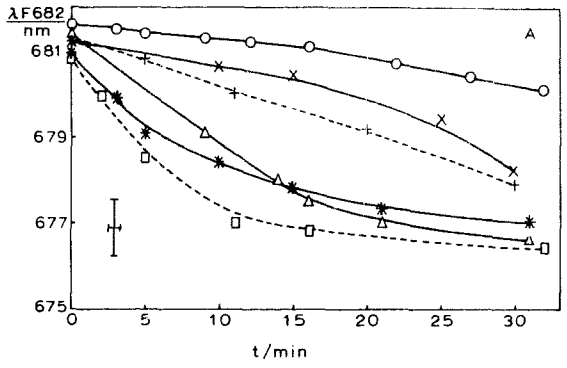


Fig. 2. Steady state fluorescence spectra of isolated D1/D2/cyt *b*559 complexes exposed to different intervals of deleterious illumination with monochromatic (476.5 nm) light at intensities of $I_E = 300 \pm 100$ W/m^2 at $T = 290$ K. Spectra are normalized to $F_{max} = 1$. 1, 0.5 min; 2, 3 min; 3, 5 min of illumination. A, Fluorescence spectrum; B, negative second-derivative spectrum.



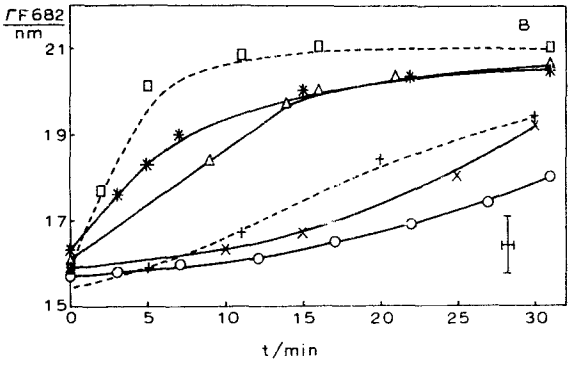


Fig. 3. Peak position (A) and fwhm (B) of the F682 Gaussian fluorescence band of isolated D1/D2/cytochrome *b*-559 complexes as a function of exposure time to deleterious illumination with monochromatic (476.5 nm) light at intensities of $I_E = 400 \pm 100$ W/m^2 at different temperatures. The parameters result from a deconvolution procedure described in the text. ○, 275 K; x, 277 K; +, 283 K; △, 288 K; *, 291 K; □, 293 K.

markedly temperature-dependent. Apart from the expected increase of the degradation rate at higher temperatures, the type of the kinetics also changes significantly. As will be outlined in the Discussion, the latter finding cannot be described by a simple degradation mechanism of the type

$$A \xrightarrow{k(T,I)} D \qquad (2)$$

where A represents the undamaged control, D the fully damaged sample and $k(T, I)$ the rate constant of degradation, which depends on temperature and light intensity.

A more complex mechanism has to be considered. Regardless of the complexity, the rate constant of the reaction, which triggers the overall reaction sequence by a modification of state A, can be obtained by determining the initial rate. It was found that at all temperatures the initial modification rate r_M (0) of

TABLE II

Initial rates of the blue shift and band broadening of F682

Initial rates of the blue shift and band broadening of F682 of isolated D1/D2/cytochrome *b*-559 complexes ($\delta\lambda_0/\delta t$ and $\delta\Gamma/\delta t$) and periods of time where a linear approximation provides a satisfying description of the data in Fig. 3A and B (t_{lin}). The values were estimated from Fig. 3.

T/K	Initial modification rates of F682 band shift		Initial modification rates of F682 band broadening	
	$r_M(0) = \delta\lambda_0/\delta t$	t_{lin}/min	$r_M(0) = \delta\Gamma/\delta t$	t_{lin}/min
	nm/min		nm/min	
275	$-(0.04 \pm 0.02)$	0–20	0.03 ± 0.02	0–15
277	$-(0.05 \pm 0.02)$	0–20	0.04 ± 0.02	0–15
283	$-(0.11 \pm 0.03)$	0–20	0.16 ± 0.06	0–12
288	$-(0.24 \pm 0.10)$	0–16	0.20 ± 0.10	0–12
291	$-(0.30 \pm 0.12)$	0– 5	0.39 ± 0.16	0– 7
293	$-(0.48 \pm 0.17)$	0– 5	0.84 ± 0.30	0– 5

both parameters, the blue shift of the peak maximum and the increase of the fwhm value of F682, remains almost constant in a certain time-domain. The $r_M(0)$ values and the corresponding time-domains are compiled in Table II. Fig. 4 shows that the initial modification rate $r_M(0)$ of both parameters can be described by an Arrhenius-type temperature dependence

$$r_M(0) = \text{const} \cdot e^{-(E_A/RT)} \quad (3)$$

where E_A represents an activation energy. Both parameters exhibit similar dependencies, characterized by E_A values of 1.0 ± 0.5 eV and 1.2 ± 0.6 eV for the blue shift and the fwhm increase, respectively, caused by light-induced sample degradation.

This finding suggests that λ_{F682} and Γ_{F682} are closely interrelated. Therefore, in Fig. 5 the fwhm values are depicted as a function of the peak maximum λ_{F682}.

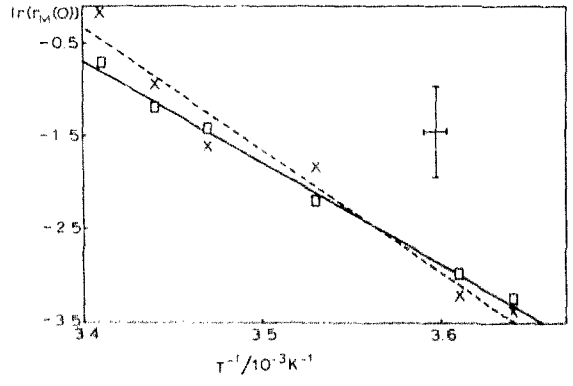


Fig. 4. Semilogarithmic plot of the initial modification rate $r_M(0)$ as a function of reciprocal temperature of isolated D1/D2/cytochrome *b*-559 complexes. The parameters are taken from Table II. □: rate of the blue shift of F682. ×: rate of the spectral broadening of F682.

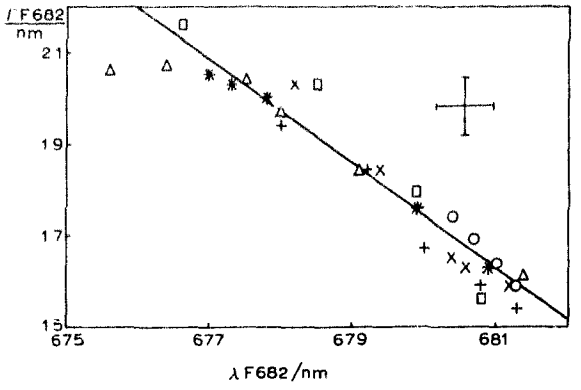


Fig. 5. Relation between fwhm and peak position of the F682 Gaussian band in isolated D1/D2/cytochrome *b*-559 complexes. The parameters are taken from Fig. 3. ○, 275 K; x, 277 K; +, 283 K; △, 288 K; *, 291 K; □, 293 K.

The data show that the fwhm increase, $\Delta\Gamma_{F682}$, linearly correlates with the extent of the blue shift, $\Delta\lambda_{F682}$, in the range from 681.5 nm down to 676 nm for the peak position. Although the degradation kinetics markedly differ between 275 K and 293 K, the linear dependence of $\Delta\Gamma_{F682}$ on $\Delta\lambda_{F682}$ is practically temperature-independent. This finding indicates that the underlying mechanism of the F682 modification, due to light-induced thermal degradation, is the same in the temperature range from 275 K to 293 K.

The integral fluorescence emission spectrum of a structurally highly organized pigment protein complex depends on different parameters. Apart from a direct chemical modification of the pigments (e.g., pheophytinization of the chlorophylls), the intermolecular distances, the mutual orientations of the pigments and the pigment - protein interactions are of special interest because the latter parameters are susceptible to protein degradation. In principle, information on the coupling between electronic and vibrational states can be obtained by measuring the linewidth of an optical transition as a function of temperature. Experiments were performed in the range of 77 K < T < 270 K with samples exhibiting different emission peak values of F682 due to varying exposure to degradative illumination W/m^2, λ = 476.5 nm) at room temperature. treatment the samples were frozen to 77 K. he experimental conditions of this study, the dification rate is negligibly slow in frozen samples, the temperature dependence of F682 could be determined over the whole range from 77 K to 270 K by successive measurements on the same sample. The temperature-dependence of fluorescence emission down to 77 K can be described with the same set of four Gaussian bands, which was used at room temperature (see Table I and Fig. 1). Cooling down to 77 K does not affect the positions of the Gaussian compo-

nents, but diminishes the fwhm value of F682, Γ_{F682}, and increases the overall fluorescence intensity of the sample by a factor of 4 to 5. The relations between the relative fluorescence intensities of the four Gaussian bands of a fluorescence emission spectrum at a certain temperature do not depend significantly on temperature (data not shown).

Fig. 6 depicts, in a double logarithmic plot, the dependence of Γ_{F682} on the temperature in samples which were adjusted to different modification states (as reflected by the peak maximum of the fluorescence marker F682). The data obtained reveal that the temperature-dependence of the bandwidth can be described by the phenomenological relation

$$\Gamma_{F682}(T) = \Gamma(0) \cdot (1 + C \cdot T^B) \quad (4)$$

where $\Gamma(0)$, C and B are fit parameters.

It has to be emphasized that these fit parameters cannot be assigned in a straightforward way to physical parameters of the system. The theoretical description of the fluorescence emission as a function of temperature is very complex for pigment protein systems like D1/D2/cytochrome *b*-559 preparations. In particular, the contribution of different broadening processes (homogeneous, inhomogeneous, dynamic inhomogeneous broadening [30]) to the spectral linewidth at different temperatures cannot be easily calculated [30,31]. To address this problem, thorough theoretical calculations, based on experimental data at sufficiently low temperatures (liquid helium) are required, which are far beyond the scope of the present study. Therefore, we will consider only the general feature of the temperature-dependence in the range of 77 K to 270 K and refrain from any detailed discussion. The key point

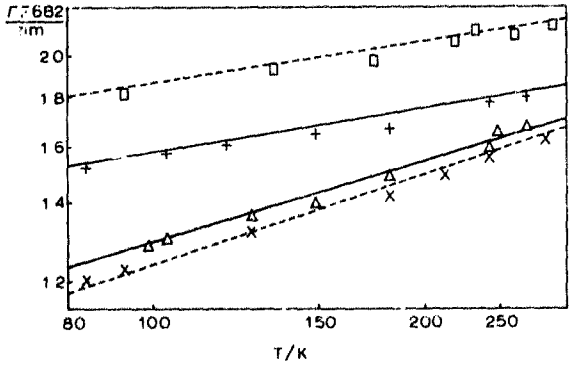


Fig. 6. Double-logarithmic plot of the spectral fwhm of F682 as a function of temperature for D1/D2/cytochrome *b*-559 complexes at different stages of degradation. The wavelength of the exciting laser beam was $\lambda_E = 476.5$ nm, with intensities of $I_E = 400 \pm 100$ W/m^2. The peak maxima of the F682 band are 680.9 nm (x), 680.6 nm (△), 677.3 nm (+) and 677.0 mm (□).

TABLE III

Temperature dependence of the spectral halfwidth of the F682 band in D1/D2/cytochrome b-559 complexes at different degradation stages.

λ_0 is the position of F682, B is a fitting parameter according to formula 4. The values of B were calculated from data of Fig. 6.

Sample nr.	λ_0/nm	B
1	681.6 ± 0.3	0.33 ± 0.12
2	680.9 ± 0.2	0.26 ± 0.12
3	680.6 ± 0.4	0.27 ± 0.10
4	677.3 ± 0.6	0.14 ± 0.06
5	677.0 ± 0.6	0.14 ± 0.05

of Fig. 6 is the finding that exponent B decreases if sample degradation progresses (see Table III), i.e., in damaged systems the fwhm values are less dependent on temperature than in the undamaged control. Implications of this observation will be briefly considered in the Discussion.

Discussion

In the present study the degradation of isolated D1/D2/cytochrome *b*-559 preparations induced by monochromatic blue-green light ($\lambda = 476.5$ nm) under aerobic conditions was investigated by analyzing changes of the fluorescence emission spectra. The deconvolution of the experimentally obtained spectra into Gaussians revealed that the dominant band, which exhibits a peak maximum at 682 nm in the undamaged control, is the most sensitive component. Therefore, this band, referred to as F682, provides a useful marker to investigate the aerobic photodegradation of the D1/D2/cytochrome *b*-559 complex. Progressing degradation of the sample was shown to be accompanied by: (a) gradual blue shift of F682; (b) increase of the fwhm value, which is linearly related to the blue shift of the peak position of F682, and; (c) less pronounced narrowing of the F682 band at decreasing temperatures in the range of 77 K < T < 273 K.

In general, two different types of models can be considered to interpret the experimental data: (i) two-state model, which assumes the conversion of a long-wavelength (characteristic for the undamaged sample) into a short-wavelength emitter, formed in the fully degraded sample. The superposition of the emissions from these two states will result in an overall blue shift and increasing halfwidth of the fluorescence spectrum, due to the increasing population of the fully damaged at the expense of the undamaged state, during progressive degradation; (ii) multistate model, where the different conformational states of the protein, which certainly are formed as intermediates during the overall degradation process, are assumed to give rise to differ-

ent fluorescence emission properties of each state. All these individual states contribute to the integral fluorescence spectrum of the sample (vide infra, Eqn. (6)).

The degradation of a structurally highly organized pigment protein system like the D1/D2/cytochrome *b*-559 complex certainly comprises a number of intermediate states. In general, the degradation process of each individual complex can be described by:

$$A \rightarrow I_1 \rightarrow I_2 \rightarrow \ldots \rightarrow I_n \rightarrow D \qquad (5)$$

where the undamaged complex is symbolized by A and the fully damaged complex by D, and $I_1 \ldots I_n$ represent the intermediate states. This raises questions about the nature of these states. In order to address this point, two questions have to be answered: (a) what is the underlying mechanism of the light-induced aerobic degradation? and; (b) what kind of modifications are monitored by the fluorescence technique applied in this study? In respect of the first question, recent studies revealed that illumination of isolated D1/D2/cytochrome *b*-559 complexes under aerobic conditions comprises at least three different types of reactions [32,33]: (i) generation of reactive singlet oxygen $^1\Delta_g$ O_2, sensitized by ^{3}P680 formed due to radical-pair recombination: (ii) cleavage of polypeptide D1, via autoproteolytic activities; and (iii) subsequent slow degradation of other polypeptides, especially of D2. A recent FTIR analysis indicates that, under exposure to similar photon flux densities as in the present study (2000 $\mu E/m^2$ per s of white light vs, 1600 $\mu E/m^2$ per s of blue-green light in this study), the high α-helix content of the D1/D2/cytochrome *b*-559 complex significantly decreases [34].

Based on these findings it appears reasonable to assign the intermediates I_n to different conformational states of the D1/D2 heterodimer. This idea also relates to the second question. If one assumes that the intermediates I_i ($i = 1 \ldots n$) of each individual complex in the ensemble give rise to fluorescence bands that are characteristic for the macroscopic state of the sample, then the integral F682 emission is given by

$$F_{F682}(\lambda) = \sum_{i=1}^{n} \rho_i \cdot F_{F682}(\lambda)_i + \rho_A \cdot F_{F682}(\lambda)_A + \rho_D \cdot F_{F682}(\lambda)_D \qquad (6)$$

where ρ_i, ρ_A and ρ_D represent the population probabilities of I_i, A and D, respectively, and $F_{F682}(\lambda)_i$, $F_{F682}(\lambda)_A$ and $F_{F682}(\lambda)_D$ the corresponding marker emission bands F682 (see Results) weighted with their fluorescence quantum yield.

Eqn. 6 readily shows that a two-state description provides a suitable fit of the fluorescence spectra if either the quantum yields $(\Phi)_i$ are negligibly small compared with those of states A and D or if the $F_{F682}(\lambda)_i$ values do not markedly differ from those of A and/or D. Therefore, additional information is required to address the problem of the degradation mechanism. Measurements of the overall fluorescence quantum yield revealed a 30–50% decrease during sample degradation by exposure to monochromatic (476.5 nm) ligth. Interestingly, a slight increase was observed if white light of comparable intensities (400 W/m^2) is used. The latter result is in qualitative agreement with a recent report of Booth et al. [27]. These findings indicate that, under monochromatic blue-green illumination, quenching state(s) are (is) created. The nature of this (these) state(s) remain(s) to be clarified.

Interesting information about the complexity of the degradation mechanism can be obtained by considering the time course of λ_{F682} and Γ_{F682} as a function of light exposure at different temperatures.

Direct evidence for the complexity of the light-induced aerobic degradation process is provided by the marked temperature-dependence observed for the time course of the blue shift $\Delta\lambda_{F682}$ and the line broadening $\Delta\Gamma_{F682}$ (see Fig. 3). Regardless of the detailed relationship between $\Delta\lambda_{F682}$ (also $\Delta\Gamma_{F682}$, see Fig. 5) and the population probability ρ_i of the different structural states according to Eqn. (6), the data of Fig. 3 exclude a simple, phenomenological kinetics of the type $A \xrightarrow{k(T,I)} D$, which would arise if either all ρ_i values of intermediates I_i are negligibly small or if all states I_i are indistinguishable from A in their fluorescence marker band $F_{F682}(\lambda)_i$. A more complex reaction pattern is expected to arise for the light-induced aerobic degradation of the D1/D2/cytochrome *b*-559 complex if one takes into account recent data from the literature ([32,33], vide supra). As the above mentioned reaction types of aerobic photo-degradation certainly exhibit greatly different temperature dependencies (e.g., the proteolytic cleavage of D1 becomes highly retarded at 0 °C compared with 20 °C, see Refs. 35, 36, while the light-induced impairment of photochemical activities in different PS II preparations still occurs, see Ref. 37), the strikingly different time courses of the marker band F682 (peak wavelength and fwhm value) depicted in Fig. 3 (A and B) are easily understandable. In this respect it is interesting to note that degradation processes in the dark can also exhibit markedly different kinetic patterns. It was found that the decline of the oxygen evolution capacity in PS II membrane fragments significantly deviates from an exponential decay if the samples are suspended in a Cl^--containing medium [38]. In contrast, incubation in Cl^--free medium [38] or thermal inactivation at higher temperatures [39] give rise to degradation curves that can be approximately described by an exponential decay. The above-mentioned considerations lead to the conclusion that a multistep model is much more realistic than a two-step model.

This idea also provides a simple explanation for the dependence of Γ_{F682} on the temperature in the range of 77 K to 270 K, as reflected by the fit parameter B in Eqn. 4. It appears reasonable to assume that, in the undamaged sample, the distribution of different structural states in the ensemble of individual D1/D2/cytochrome *b*-559 complexes, is very narrow. Therefore, homogeneous or dynamical homogeneous line-broadening effects can contribute significantly to the temperature-dependence of Γ_{F682}. In the damaged sample, however, the distribution of different structural states is markedly wider, thereby leading to dominance of inhomogeneous line-broadening, which is much less temperature-dependent. This phenomenological consideration rationalizes the decrease of fit parameter B with progressing degradation state of the sample (see Table III).

The idea of using parameter B as a qualitative indicator of the structural state distribution within a sample is supported by the finding that a value close to 0.5 has been observed for all Gaussian bands of absorption and emission in primary leaves of wheat seedlings [30] and the emission bands of thylakoid membranes, PS II membrane fragments and PS II core complexes (Maciy, K., unpublished results). A similar value was observed for Γ_{F682} in our undamaged control sample. Therefore, B-values approaching 0.5 could be characteristic for native pigment protein complexes with a very narrow distribution of different structural states. In line with these considerations, it is interesting to note that a band narrowing is observed during the assembly of native pigment protein complexes (Ref. 40 and U. Stahl, unpublished results). However, it has to be emphasized that the above-mentioned ideas have to be substantiated by more detailed quantitative analysis. Exponent B itself does not provide any further mechanistic information because the fwhm value of the Gaussian marker band F682 does not directly reflect the pigment-pigment and pigment-protein interaction. A much more thorough theoretical analysis is required to address this problem (studies in this direction are under progress).

Regardless of these problems, the present study shows that steady state fluorescence spectra can be used as a tool for analyzing the degradation of pigment protein complexes like the D1/D2/cytochrome *b*-559 complex of PS II.

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