Delayed Emission from Green Algae "Chlorella pyrenoidosa" at Various Temperatures

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Comparative studies of prompt and delayed emission from whole cells of Chlorella pyrenoidosa are presented for various temperatures between 4.2 and 300 K. Prompt emission was excited with continuous light (wavelengths between 420 and 670 nm). Light pulses (duration 6.9 msec) have been used to excite delayed emission. Their wavelength had to be shorter than 600 nm for investigations at 77 K.

A linear dependence of the delayed emission intensity on the excitation intensity at low temperatures (4.2 and 77 K) favours a one-quantum-mechanism to be responsible for delayed light production.

The response of the total intensities on temperature shows a similar behaviour for both types of emission up to 200 K. Above 200 K they behave quite differently.

1. Introduction

At room temperature living cells of Chlorella pyrenoidosa show prompt [1] and delayed [1, 2] emission of light in the spectral range from 640 to 800 nm if irradiated with visible light. Whereas the lifetime of the former is of the order of nanoseconds [1, 4], the latter shows a non-exponential decay in the time range of microseconds to hours [1, 5]. The total intensity of the prompt emission is about 10^3 to 10^5 times higher than that of the delayed emission [3]. Both kinds of radiation are attributed primarily to singlet transitions ($S_1 \rightarrow S_0$) of chlorophyll a, which is present in the pigment systems of the algae [1]. In order to explain the delayed fluorescence various models have been suggested, some of them involving triplet states and some based on charge-carrier recombination mechanisms [6–12, 13].

Low temperature measurements of prompt fluorescence down to 4.2 K have been reported previously [13–17]. In 1957, Tollin and coworkers observed also a long-lived emission after flash-excitation of 1.5 msec pulse duration at 103 K and temperatures up to 294 K [18]. These measurements were confirmed by Arnold [5], but no long-lived fluorescence was detectable upon excitation with continuous light. In 1974, Ruby observed a delayed emission at temperatures above 120 K after excitation with 2-μsec-flashes and additional background illumination [19].

Our investigations are a further attempt to clarify the properties of states which are involved in the emission of prompt and delayed fluorescence. The present paper reports some new experiments concerning the spectral structure and total intensity of the delayed emission at various temperatures down to 4.2 K as well as comparative studies of the prompt emission.

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2. Materials and Methods

The starting culture used to produce the Chlorella cells was obtained from the algae collection at the University of Göttingen. This material was held in a nutrient solution as given by Lorenzen [20], exposed to a carbondioxide-air mixture (1.5% CO_2) and illuminated with fluorescent lights.

For room temperature measurements on living cells a flow-arrangement as sketched in Fig. 1 was used in order to keep the photosynthetic activity of the algae efficient.

For low temperature investigations, the cells were separated from the nutrient solution by repeated washing and centrifuging. They could then be at-

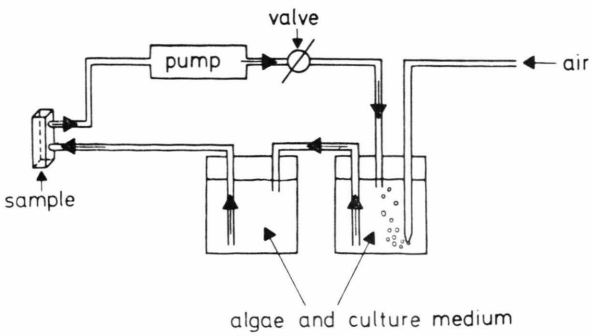

Fig. 1. Flow arrangement for measurements with cell suspensions at room temperature (flow rate $\leq$2 ml/sec).

tached as a paste to an optically inactive substrate and frozen in the dark. This preparation technique was necessary to prevent cuvette emission from falsifying the investigations of delayed fluorescence, since all available cuvette materials showed a comparatively strong interfering emission upon excitation in the blue region of the visible spectrum. As a consequence of using such highly concentrated specimen, the shapes of the observed spectra are distorted by reabsorption processes. This must be taken into account in a conclusive discussion of the experimental results.

Figure 2 illustrates schematically the experimental set-up. The cryostat allowed investigations between

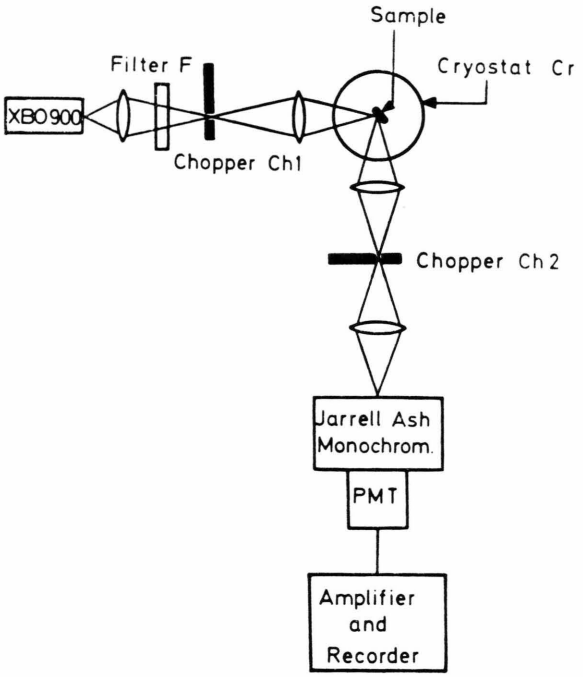

Fig. 2. Schematic sketch of the experimental setup.

4.2 and 310 K. Temperatures below 77 K were measured using a carbon resistor; an iron-constantan thermo-couple was used between 77 K and 310 K. In this way the sample temperature could be determined with an accuracy of ± 2 K. The experiments at intermediate temperatures were performed while the sample was warming up. To ensure a constant temperature over the entire specimen, the warm-up rate was very slow (0.3 K/min). The optical windows of the cryostat exhibited a slight and temperature dependent emission which was corrected for in the experimental results.

A xenon high-pressure lamp XBO 900 W was used for excitation in combination with a filter-set F. Unless otherwise specified, F consisted of a heat reflector, a Schott GG 420 cut-off filter and a $CuSO_4$-liquid filter, yielding an excitation bandwidth from 420 to 560 nm.

The emission of the sample was analyzed with a 0.25 m monochromator (Jarrell-Ash) and monitored using a cooled EMI 9658 photomultiplier tube. The spectra reported in the present paper were corrected for the spectral sensitivity of this detection scheme.

The set-up described permitted investigations of prompt fluorescence spectra. The delayed emission, which is always superimposed, is at room temperature roughly $10^3 - 10^5$ times weaker [3] and is therefore negligible. At low temperatures this factor is even greater. In order to investigate the spectra of the delayed emission separately, two choppers Ch 1 and Ch 2 (Fig. 2) operated synchronously at 3000 rpm in such a way that either the excitation or the observation path was clear (Figure 3). Using this sequence, only decays lasting longer than 0.3 msec could appear in the spectra of delayed fluorescence. Thus, this arrangement eliminates not only the prompt fluorescence but also the faster components of delayed emission.

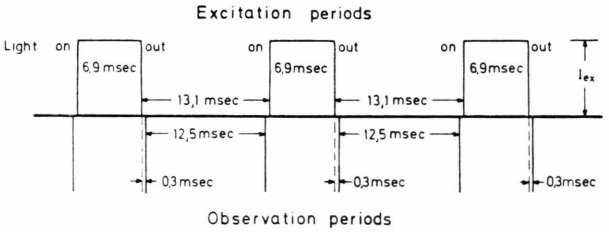

Fig. 3. Sequence of the excitation and observation periods used for measurements of delayed fluorescence spectra (I_{ex}: intensity of excitation). One cycle includes: excitation (6.9 msec) — dark period (0.3 msec) — observation (12.5 msec) — dark period (0.3 msec).

3. Experimental Results

Some emission spectra are presented in Figures 4 – 8. At room temperature a suspension of living, photosynthesizing cells exhibits an emission as sketched in Figure 4. Figures 5, 6 and 7 show the prompt and delayed emission spectra, respectively, for a paste of algae cooled to 4.2 K. Both shapes are replotted in Fig. 8 for a detailed comparison. The sample spectra observed during warming up are shown also in Fig. 8 for various temperatures.

The spectra of prompt and delayed emissions of living cells in nutrient solution at *room temperature*

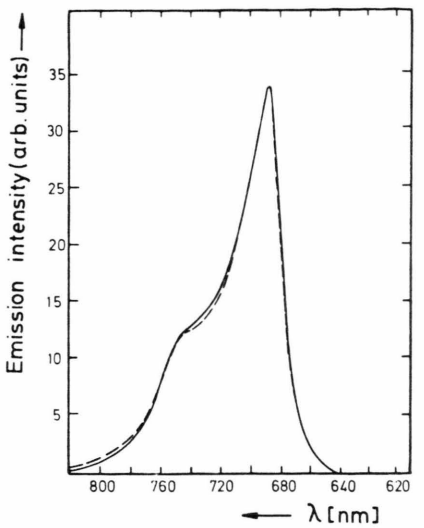

Fig. 4. Prompt (———) and delayed (– – –) emission spectra from a suspension of living cells at 298 K under saturating conditions for photosynthesis (concentration of algae: 10^7 cells/ml; excitation wavelengths: 420–560 nm). The dominant peaks of both spectra are situated at 687 ± 2 nm.

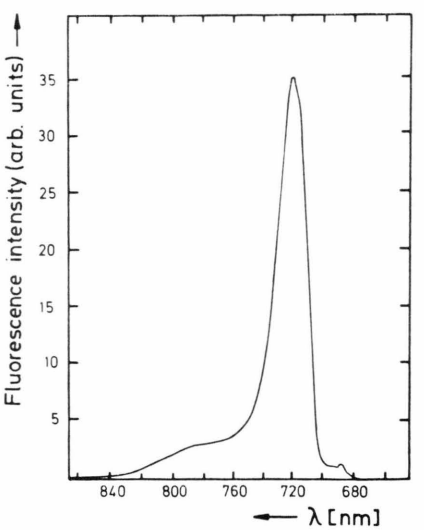

Fig. 5. Prompt fluorescence spectrum of a paste of cells at 4.2 K (excitation wavelengths: 420–560 nm).

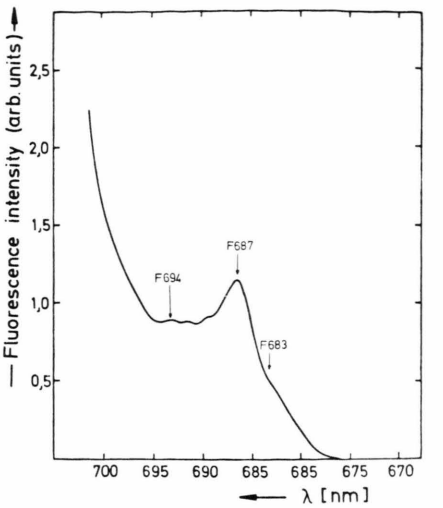

Fig. 6. Short-wavelength part of the prompt fluorescence spectrum as sketched in Fig. 5 on an expanded scale. The positions of some known emission bands are marked (limits of error: ± 2 nm).

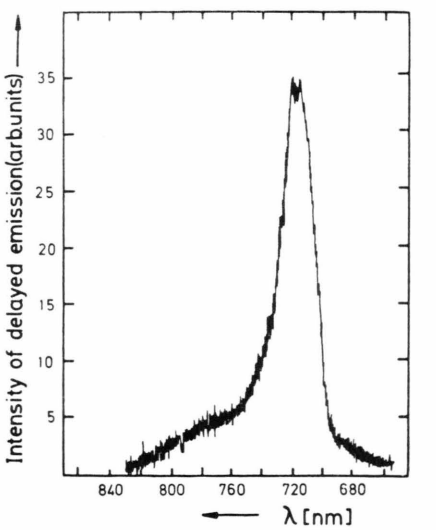

Fig. 7. Delayed fluorescence spectrum of a paste of cells at 4.2 K including components whose decay is longer than 0.3 msec (excitation wavelengths: 420–560 nm).

(Fig. 4) are identical, within the limits of error, and exhibit a pronounced maximum at 687 ± 2 nm. These results are in agreement with previous reports [1, 2, 21].

If a paste of algae is *cooled to 4.2 K* the shapes of the prompt and delayed emission spectra are distinctly changed (Figs. 5 and 7). The short-wavelength parts between 670 and 700 nm are rather weak, while intense peaks occur between 700 and 750 nm. Long-wavelength tails, that can be observed up to 820 nm, are attached to these peaks. This spectral structure is at least partially due to reabsorption processes which occur in highly concentrated samples [30].

The spectrum of prompt fluorescence (Figs. 5 and 6), which is more structured than that at room temperature, indicates the superposition of a series of emission bands. This is evident especially in

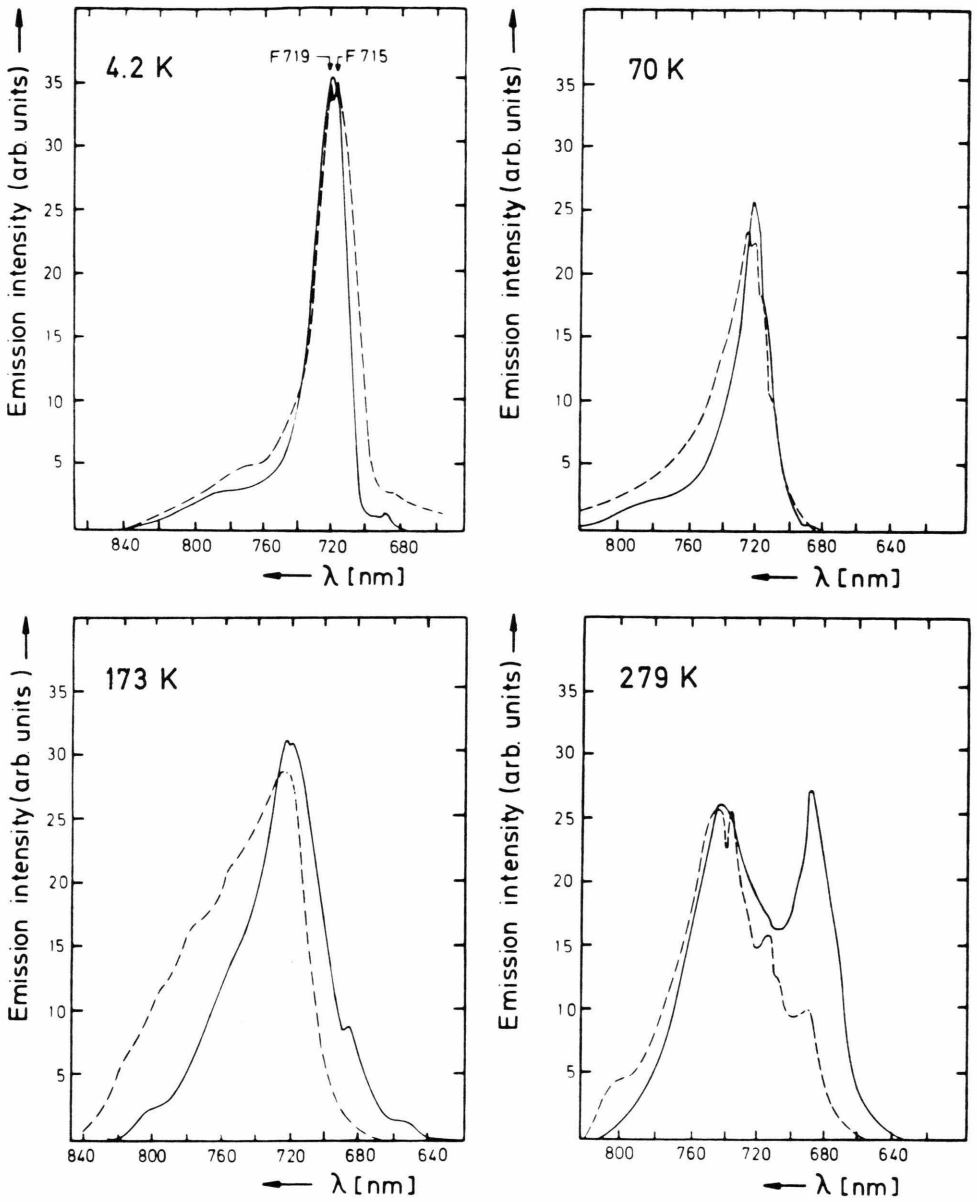

Fig. 8. Comparative sketch of prompt (———) and delayed (– – –) emission spectra at 4.2 K, 70 K, 173 K and 279 K measured during warming up a paste of algae from 4.2 K to room temperature. All spectra are only representative as for their shape and not for the total intensity.

Fig. 6 which shows the prompt fluorescence between 670 nm and 700 nm on an expanded scale. The band *positions* coincide within the limits of error with those from Chlorella pyrenoidosa in dilute suspensions [1, 14, 15]. This correspondence shows that conclusions which are based only on the spectral position of individual bands are not significantly influenced by additional reabsorption effects in the paste.

The structure of the delayed emission spectrum at 4.2 K (Fig. 7) is less clearly resolved than that of the prompt fluorescence. Furthermore, the short-wavelength wing of the main bands is more intense for delayed emission so that its spectrum appears broadened (Figure 8).

To get more information on the subband structure, both types of emission spectra have been measured

at various temperatures between 4.2 K and room temperature (Figure 8). Such investigations indicate clearly a strong change of their shape with temperature. Although the comprehensive analysis of these measurements is still in progress we can state that the positions of the main peaks in the delayed emission spectra are the same as those in the prompt fluorescence. Thus, they are identical with well known fluorescence bands of Chlorella cells.

The *intensity dependence* of the delayed emission on the exciting intensity at low temperatures (4.2 and 77 K) is shown by Fig. 9 in a double logarithmic scale. A straight line of slope 1 fits the measured data points over the entire range of exciting intensities.

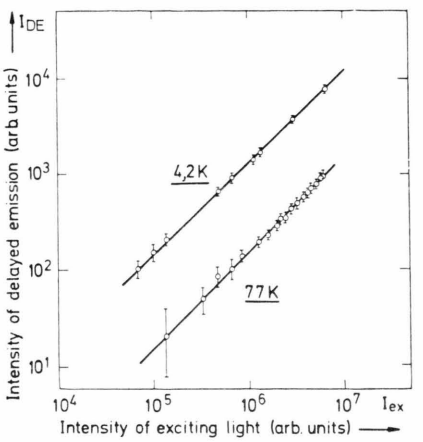

Fig. 9. Dependence of the total intensity $\int I_\lambda^{DE}\,d\lambda$ of delayed emission at 4.2 K and 77 K on the exciting intensity (excitation wavelengths 420—560 nm).

The total intensity of both, prompt and delayed emission, respectively, show a complicated *temperature dependence* between 4.2 and 310 K (Figs. 10 and 11). Prompt emission can be observed over the entire range of temperature; delayed fluorescence, on the other hand, is not detectable between 248 K and 270 K and is, therefore, at least 50 times weaker than at 4.2 K.

Below 200 K the temperature dependencies of both types of emission intensities are similar. In contrast to this, they are basically different above 200 K.

Our investigation of the emission with *different excitation wavelengths* was intended mainly to clarify the qualitative effect of the exciting wavelength on the existence of prompt and delayed fluorescence.

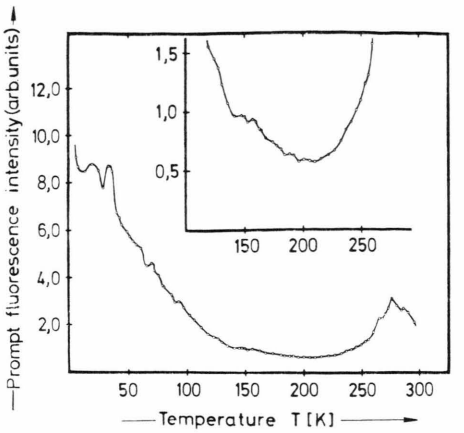

Fig. 10. Total emission intensity $\int I_\lambda^*\,d\lambda$ of prompt fluorescence measured with a paste of cells at various temperatures during warming up from 4.2 K to room temperature.

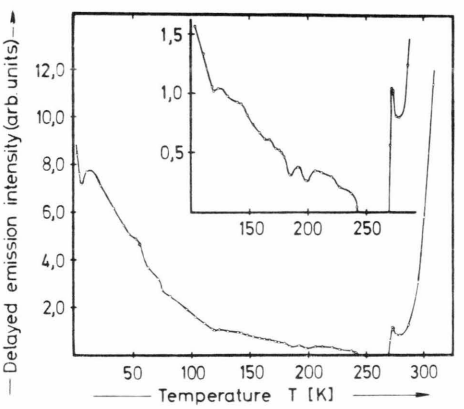

Fig. 11. Total emission intensity $\int I_\lambda^{DE}\,d\lambda$ of delayed fluorescence measured as in Figure 10.

Therefore, various cut-off and interference filters were substituted for filter F in the excitation path. The results obtained are briefly summarized below:

Prompt and delayed emission of living, photosynthesizing cells at room temperature as well as prompt emission at 4.2 K can be observed with all excitation wavelengths in the visible region. In contrast, the delayed emission at low temperatures (4.2 and 77 K) appears only if the excitation wavelength is shorter than roughly 600 nm, the intensity produced with red light being lower than that at blue excitation by at least a factor 50.

4. Discussion

The complete evaluation of our measurements is not yet accomplished. Nevertheless, a few aspects

are discussed below as a basis for further interpretation. For doing this we want to enter into some details of delayed fluorescence production thus dealing with physical interactions which are possibly involved in the primary quantum conversion process of photosynthesis. These questions are well investigated for room temperature conditions [1–12]. Ideas have been developed rendering possible a rather comprehensive insight into the delayed emission properties. In spite of this, it is unclear whether these models are also correct under low temperature conditions ($4.2\,\mathrm{K} < T < 200\,\mathrm{K}$). Therefore we briefly *summarize some physical mechanisms* which are known in general as a source of delayed light even at very low temperatures:

Mechanism I (electron-hole model):

The delayed fluorescence is explained by use of an analogon to the band model of semiconductors. An emission originates from the recombination of free mobile charge carriers within the structure of the photosystems which is assumed to by crystal-like. This idea is well known from investigations of delayed light properties of photosynthesizing samples at room temperature [5, 7, 11, 12].

Mechanism II (triplet-triplet annihilation model):

In organic molecular crystals it is well established that the mutual annihilation of two triplet excitons is able to cause an emission from the lowest excited singlet level [25, 26, 31]. Since triplet states are involved in the creation of radiating singlet states the decay time of this delayed fluorescence is in the order of the triplet lifetime. It is feasible that a similar process might be active as origin for the delayed emission in photosynthesizing systems [6].

Mechanism III (Back reactions within the primary photochemistry):

During the illumination of cells states ($D^+ R A^-$) are produced (where D represents the primary electron donor, while A is the primary acceptor of the particular reaction center R [1, 27, 28]). The recombination of charges which occurs even at low temperatures [27, 28] is possibly accompanied by recombination luminescence:

$$(D R A) + h\nu \rightleftarrows (D R^+ A^-) \rightarrow (D^+ R A^-).$$

Mechanism IV (Trapped electrons):

Electrons from chlorophyll molecules can be trapped during irradiation in the vicinity of their electronic states [22, 23]. During a subsequent thermal detrapping they may enter the manifold of excited chlorophyll states thus producing a delayed emission of light.

Mechanism V (Trapped excitons):

Various investigations with molecular crystals indicate that triplet excitons are able to cause delayed emission of light if they are trapped, thus producing an isolated excited state which is long-lived [29]. The thermal detrapping of the energy as well as the isolated state itself may cause a delayed emission of light.

Mechanism VI (Phosphorescence):

In contrast to the mechanisms I – V which all deal with singlet-singlet transitions, the radiative depopulation of triplet states into the singlet ground state is another possibility for a long-lived emission of light. An experimental distinction of this process from the other ones is, in general, easily possible by studying the spectral properties of the radiation.

In order to get some information about the physical processes which are included in delayed light production at low temperature ranges we will compare our experimental results with the above summarized mechanisms.

Temperatures below 200 K:

The identical spectral shapes of prompt and delayed emission which are observed with living, photosynthesizing cells at room temperature (Fig. 4 and [1, 2]) confirm, that delayed emission originates from the same electronic levels as the prompt. These states are short-lived. The storage of energy, therefore, occurs in other, metastable states, for which a possible way of deactivation is the one via the above mentioned short-lived emitting levels. In agreement with Tollin [18] we want to note that the same statement is true at low temperatures since the main peaks of delayed emission appear with identical spectral position in the prompt fluorescence spectra. This requires that the luminescence originates in the first excited singlet level of the radiating particles thus excluding *mechanism VI (phosphorescence)* to account for the observed long-lived emission.

Mechanism I (electron-hole model) cannot explain the experimental observation that excitation wavelengths shorter than 600 nm must be used in

order to produce delayed emission at 77 K. The electron-hole mechanism, therefore, seems not to be responsible for this type of long-lived fluorescence.

Mechanism II (triplet-triplet annihilation), in general can easily be identified by its characteristic intensity dependence of the delayed fluorescence on the intensity of excitation. In a double logarithmic scale the slope of this curve should be 2 for low and 1 for high intensities. As Fig. 9 shows, our experimental results do not verify this behaviour. Therefore we have no evidence on a triplet-triplet annihilation process. Nevertheless it should be noted, that we cannot exclude this mechanism completely because the slope 2 in Fig. 9 might appear for even lower intensities for which the experimental set-up was not sufficiently sensitive.

The temperature response of the total emission intensities (Figs. 10 and 11) are similar for the short- and long-lived fluorescence *below 200 K*. This suggests that only mechanisms acting in the short-lived emission process are influenced by the temperature since they are involved in both types of emission. No evidence is given for a significant dependency on temperature of the processes populating the emitting singlet levels for *delayed* emission of light. This result favours the assumption of a tunneling process to be involved in the population mechanism of delayed fluorescence. Since such processes do not require a thermal activation, the *mechanisms III and/or IV and/or V* are in agreement with all experimental results if tunneling processes are included for delayed repopulation of the radiative states.

Temperatures above 200 K:

Here we observe basically different temperature responses of the prompt and delayed emission intensity. This can be accounted for by a distinct temperature dependence of the population mechanism since both types of fluorescence are fed by different processes while the emitting events are identical. This conclusion indicates that processes which are not dependent on the temperature (such as tunneling processes) are no longer dominant in the creation of radiative states for delayed fluorescence. The experimental results (e. g. the disappearance of a delayed emission between 248 K and 270 K) can be tentatively explained by assuming that the lifetime of the metastable states is shortened by thermally activated processes thus reducing the lifetime of the delayed fluorescence below the limits of the experimental set-up (0.3 msec). This is in agreement with recently published results [23] concerning a glow peak at 265 K which could be explained assuming a thermally activated reoxydation of primary acceptors Q^- (PS II) by oxydized chlorophylls.

The distinct increase of delayed fluorescence intensity *above 270 K* indicates that additional processes become dominant in this temperature range. It seems that the electronic pathways open at least partially. Therefore, additional states which are included in the transport chain are available now to support delayed fluorescence.

5. Conclusions

Summarizing the results of Chapter 4 we can rule out some of the proposed models (mechanisms I, II and VI). On the other hand we get some evidence for three possible mechanisms of delayed light production at temperatures *below 200 K:* back reactions of the primary photochemistry and/or detrapping processes of electrons or else excitons. All these mechanisms should work via tunneling events to repopulating the radiative states of the chlorophylls.

So far the evaluation of our measurements reveals no evidence for the validity of the electron-hole model and the triplet-triplet annihilation process (this statement is only made for temperatures below 200 K, a time domain of delayed emission longer than 0.3 msec, and experimental conditions as described in Chapter 2).

At temperatures *above 200 K* thermally activated mechanisms become dominant. No evidence is given for an activity of tunneling processes to support the delayed emission in this range of temperature. We assume that thermally activated back reactions of the primary photochemistry are dominant in the population mechanism of delayed fluorescence. In accordance to this, the disappearance of long-lived emission between 248 and 270 K indicates together with the properties of a glow peak at 265 K [23] the oxydation of the primary acceptor Q^- of photosystem II.

Further experiments are planned to test the interpretations given above by studying the response of delayed emission at low temperatures on treatment with artificial donors, acceptors and inhibitors as well as on other experimental conditions of the sample such as pre-illumination or pre-heating.

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