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Temperature Dependence of Antennae Chlorophyll Fluorescence Kinetics in Photosystem I Reaction Centre Protein†

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CP1, the isolated reaction centre (RC) chlorophyll(chl)-protein of plant photosystem I(PSI) containing P700 and *ca.* 40 antenna Chl has been isolated using sodium dodecyl sulphate and gel electrophoresis. It retained the triplet e.s.r. polarisation pattern characteristic of active charge separation and recombination. Low-temperature and time-resolved fluorescence emission spectra showed that at least two discrete antenna chl forms were present, and excitation energy transfer between them and P700 was studied by measuring chl sub-nanosecond fluorescence decay kinetics over a range of temperatures and emission wavelengths, using *ca.* 100 ps Ar-ion laser excitation pulses and single-photon detection, resulting in *ca.* 10 ps time resolution. The two forms are F720, emitting at 720 nm (low-energy sites within the antenna) and F690, emitting at 690–695 nm. The latter form was only observed at short times (<200 ps) and at low temperatures. Decay kinetics were fitted to the sum of three exponentials. The two longer (>1 ns) components were of small amplitude and have no significance for energy transfer. The lifetime of the shortest resolved component varied in a complex way with temperature between 30 and 150 ps, also dependent on emission wavelength. At $T > 200$ K the lifetime was 40 ± 10 ps, independent of wavelength, but on lowering the temperature it developed a strong wavelength dependence with a distinct minimum at 690–695 nm. A model is presented for energy transfer between the discrete chl antenna forms which accounts for the change of the observed lifetimes with temperature. In this model F690 forms a core antenna close to the RC and can transfer energy to P700 even at 10 K. Endothermic energy transfer out of F720, which is inhibited by low temperatures, gives rise to the observed temperature dependence of the F690 and F720 fluorescence lifetimes.

The initial ultrafast events in plant photosynthesis [energy transfer within an antenna pigment bed and subsequent primary charge separation in a reaction centre(RC)] have been the subject of numerous picosecond fluorescence studies.[1] Progress in the study of bacterial photosynthesis has depended on the biochemical extraction of relatively simple and well defined complexes from the photosynthetic apparatus. However, for plant systems these methods have not been successful and the isolated chlorophyll(chl)-protein complexes are relatively large, containing many chl molecules, which are heterogeneous and can be distinguished spectroscopically.[2] This inhomogeneity is inherent for the native complex and is not a result of isolation.[2]

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In the case of *in vitro* chl a similar inhomogeneity within the pigment population can occur and has been studied using both steady-state fluorescence measurements,[3] and time-resolved fluorimetry at low temperature,[4] giving information on energy-transfer processes within the inhomogeneous manifold of the chl molecules in frozen solution.

Time-resolved measurements of the room-temperature fluorescence from one of the plant complexes [the sodium dodecyl sulphate (SDS) prepared CP1 complex (containing *ca.* 40 antenna chl and the RC of photosystem 1)] indeed show a spectral dependence in their kinetics.[5] In the present communication we extend this work and report a systematic study of the wavelength-resolved emission kinetics of this complex in the temperature range 10–270 K. These kinetics are primarily determined by energy-transfer and trapping processes within the complex. Energy-transfer rates are strongly dependent on spectral overlap as well as on donor–acceptor distance and relative orientation. A temperature dependence of fluorescence kinetics could be due to changes in the overlap of the donor emission and acceptor absorption as a result of shifting and narrowing of the bands and also, perhaps more importantly, from the presence of one or more endothermic, 'uphill' reverse energy-transfer steps.

The observed temperature and wavelength dependence of the fluorescence decay kinetics in the emission bands at 690 nm (F690) and 720 nm (F720) from CP1 are analysed, and a model is presented for excitation energy transfer. A preliminary report and analysis has already appeared.[6]

Experimental

Materials

The CP1 was isolated from wild-type barley chloroplasts using SDS polyacrylamide gel electrophoresis under non-denaturing conditions according to the procedure of Machold *et al.*[7] All spectroscopic measurements were carried out on CP1 present in $2 \times 2 \times 4$ mm gel pieces, which had been stored frozen at 77 K. After minimal exposure at room temperature, necessary to manipulate the sample gel, it was rapidly cooled in an optical cryostat (either a Varian E-257 N_2-vapour flow tube, or a liquid-He-cooled Oxford Instruments CF204, depending on the temperature range required). The sample integrity was checked by comparing the emission spectrum before and after a series of measurements.

Methods

A Coherent Radiation CR18 Ar^+ laser (f.w.h.m. 100 ps) served as excitation source for the kinetic experiments, and mode-locking of the 458 nm line was performed as in ref. (8). A pulse-selection system[9] was used to modulate the 76 MHz laser pulse train giving a 300 kHz repetition rate (3 μs interval between pulses). The resulting pulses, with energy of *ca.* 0.5 nJ per pulse, were used to excite the sample. The time-averaged intensity of laser light on the sample is therefore low (*ca.* 75 W m^{-2}) and the energy density per pulse is $<10^{11}$ photons cm^{-2}. Fluorescence was detected at 90° with respect to the direction of excitation through a Jarrel–Ash 82-140 dual monochromator (bandwidth 5 nm) by a Philips PM2254B photomultiplier, placed in a thermoelectrically cooled housing (Products for Research TE104F). The detection chain, and in particular the photomultiplier, determines the time resolution of the system. The frequency of detected photons was kept constant at 15 kHz by means of neutral density filters in the excitation beam to avoid pile-up distortions.[10]

The single-photon responses were directed *via* a Canberra 1428A constant-fraction discriminator to the start input of a Canberra 2043 time-to-amplitude converter (TAC). The stop pulses were extracted from the laser modulator driver as described in ref. (9).

The analogue pulses from the TAC were collected by a Nuclear Data 66 multichannel analyser (MCA) in 1024 channels, giving typically 25 ps per channel time resolution. For recording the time-resolved spectra the TAC was equipped with a single-channel analyser (SCA), discriminating the analogue pulses.[11] Logic output pulses of the SCA corresponded to the photon events occurring inside a time window selected by the SCA level controls (t and Δt). The output of the SCA was connected to the multichannel scaler (MCS) input of the MCA. Synchronous scanning of MCS and monochromator at 0.125 nm per channel using a stepping motor was controlled by laboratory-built electronics.

Data were transferred from the MCA to a mainframe computer for analysis. After applying a correction to remove electronic distortions,[12] the fluorescence decay curves were fitted using a least-squares method[13] to a sum of exponentials convoluted with the instrumental response function. The program minimised

$$\chi^2 = (N_i - N_p + 1)^{-1} \cdot \sum_i (y_{c,i} - y_{e,i})^2 / y_{e,i} \quad (1)$$

and the Durbin–Watson parameter. The quality of the fit was also judged from the plot of residual deviations. N_i is the number of fitted data points, N_p is the number of fitting parameters, $y_{c,i}$ and $y_{e,i}$ are the number of counts in the ith channel of a calculated and experimental decay curve, respectively.

As a test of this set-up and the analytical procedure to determine fluorescence decay components with lifetimes of 20–100 ps we have measured the fluorescence-decay kinetics of bilirubin IX in dimethylformamide and in pyridine under similar conditions, and found 10 ± 5 ps and 45 ± 5 ps, respectively, in good agreement with values reported by Chan and Beddard.[14] Kinoshita and Kushida have recently shown that in principle single-photon counting set-ups, like the one we have used here, have a time resolution of *ca.* 1–2% of the f.w.h.m. of the excitation pulse after convolution with the instrumental response function.[15] Our pulsewidth is *ca.* 300–400 ps, so that we should be able to determine lifetimes as short as *ca.* 10 ps.

Results

The steady-state fluorescence intensity of CP1 at low temperature is located mainly around 720 nm, although the spectrum also contains a pair of minor bands at shorter wavelengths. The 77 K spectrum is essentially identical to that at 10 K, which is shown in fig. 1. The absorption spectrum at room temperature has a maximum at 677 nm (not shown) and the ratio of P700/chl found from absorption data is *ca.* 0.025.

We have measured the light-minus-dark $\Delta m = 1$ triplet e.s.r. spectrum of our preparation of CP1 at $T < 10$ K and found the triplet spectrum of the primary donor to have an AEEAAE spin polarisation pattern, characteristic of a state generated by charge separation and recombination.[16,17] We regard this as sufficient evidence for the integrity of the primary electron-transfer chain in the reaction centre. The fluorescence emission spectra (fig. 1) indicate that inner chl antenna molecules are in their native state, showing a maximum near 720 nm.[18,19]

In this paper we shall concentrate on F690 and F720. The F720 fluorescence is seen in both steady-state and time-gated spectra (fig. 1) and is characteristic of plant PSI complexes, which have no chl a/b-protein attached.[18] The F690 fluorescence, which is obscured by overlapping emission bands in the steady-state spectrum, is clearly seen in short-time emission (fig. 1) at temperatures below 80 K. This band has already been reported by Mullett *et al.* in PSI core antenna preparations[19] and is distinct from the emission component seen in plant PSII.

Oxidation of CP1 by excess ferricyanide bleaches both the 720 and 684 nm bands to reveal the 690 nm band more clearly in the steady-state spectrum. The other minor antenna bands at shorter wavelengths are not important for the energy-transfer model in this paper: the weak chl b emission at 655 nm is probably due to remaining traces of

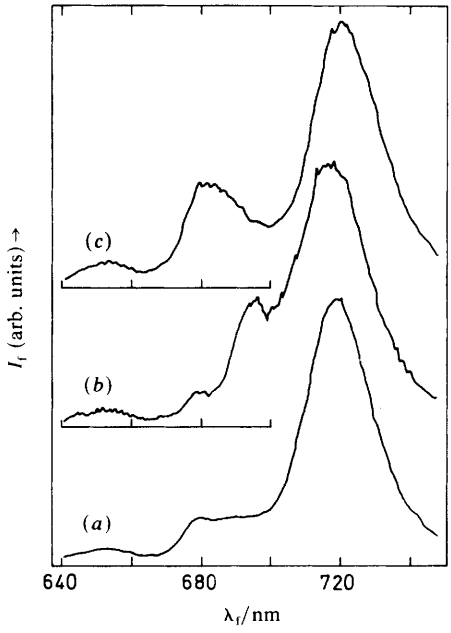


Fig. 1 Time-resolved emission spectra of CP1 at 10 K. (*a*) No time-gating, (*b*) $t = 120$ ps, $\Delta t = 240$ ps and (*c*) $t = 5$ ns, $\Delta t = 8$ ns. Pulse maximum is at $t = 0$. Excitation wavelength = 458 nm.

a denatured chl a/b-protein associated with PSI *in vivo*, and a 680–685 nm emission is ascribed to a small proportion of chl a antenna, whose interaction with protein has been perturbed by the binding of SDS molecules to the surface of the CP1 complex.

The wavelength-resolved fluorescence kinetics of CP1 in the temperature range 10–270 K required three exponential components to obtain satisfactory fits to the experimental decay curves over the entire emission wavelength range of 670–740 nm (fig. 2). In this paper, the two longer (ns) components are left undiscussed, since they do not appear to play a significant role in the energy-transfer process. The lifetime (τ) and relative amplitude (α) of the short component are collected in table 1. This component appears to be very reproducible for different CP1 preparations; *e.g.* fitting fluorescence decays from three samples at 270 K at the emission wavelength 710 nm gave values of 21, 26 and 30 ps, with amplitudes of 0.96, 0.99 and 0.97, respectively. This enables us to estimate the experimental error in the determination of the lifetime to be *ca.* 10 ps. The kinetics remained unchanged within these limits after cooling the sample to 100 K and subsequent reheating to 270 K, confirming that no irreversible changes occur on freezing the samples. It may well be that the lower limit of 20 ps of the lifetimes represents the time-resolution limit of our measurements; however, none of the conclusions of the present work is based on the absolute values of the lifetime, but rather on its relative variation within the range 25–180 ps.

The value of the measured lifetime is clearly emission-wavelength dependent (fig. 3), at least at $T < 200$ K (at 270 K it is independent of wavelength above 690 nm, although this could be due to our time-resolution limit). A definite minimum in the dependence of lifetime on wavelength is already seen in this region at 140 K. This minimum shifts to shorter wavelengths as the temperature is lowered in the range 200–80 K.

The temperatue dependence of the lifetime is presented in fig. 4 for two characteristic emission wavelength regions. At 690–695 nm the lifetime is <100 ps, showing a maximum around 150 K, a minimum around 80 K, and the amplitude is large (>0.9). At

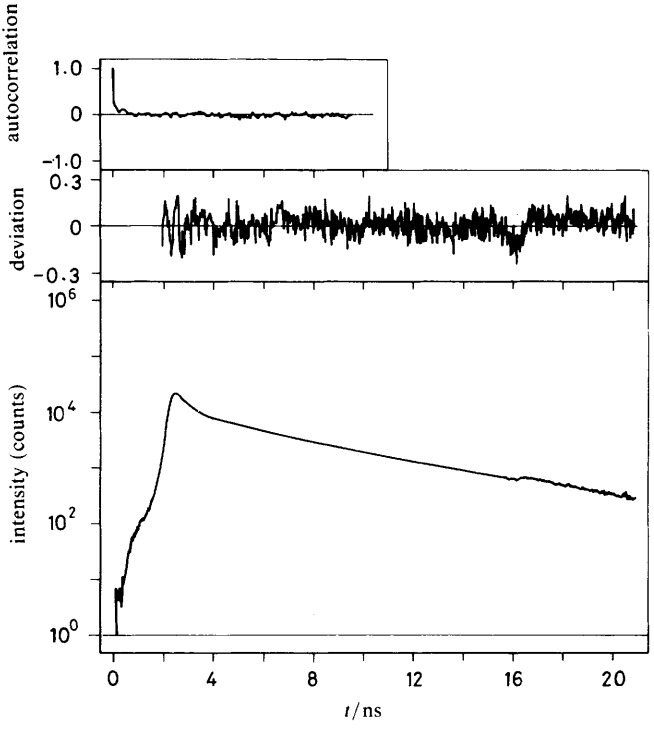


Fig. 2. An example of CP1 fluorescence-kinetics analysis. $T = 10$ K, detection wavelength 690 nm, excitation wavelength 458 nm. The best fit was judged using three criteria: the autocorrelation function (upper curve), the deviation function (middle curve) and the χ^2 value. The values of the lifetimes and the relative amplitudes for the fit shown were: 0.055 ns (0.951), 1.793 ns (0.020) and 5.448 ns (0.029). The excitation pulse, 50 000 counts per channel in the maximum and 300–400 ps broad after detection is for the sake of clarity not shown. MCA time resolution is 0.025 ns per channel. The emission intensity is plotted on a logarithmic scale.

Table 1. The parameters τ_1 (α_1) for the major short component of the deconvoluted fluorescence kinetics

T/K	670	690	695	710	720
270	112 (0.78)	30 (0.96)	30 (0.95)	25 (0.98)	28 (0.97)
200	159 (0.63)	61 (0.91)	58 (0.94)	28 (0.99)	43 (0.98)
140	161 (0.64)	81 (0.89)	67 (0.92)	108 (0.94)	121 (0.92)
80	151 (0.59)	35 (0.94)	32 (0.96)	157 (0.70)	172 (0.57)
40	—	40 (0.96)	58 (0.95)	159 (0.60)	182 (0.49)
10	164 (0.58)	57 (0.95)	79 (0.93)	163 (0.56)	189 (0.46)

wavelengths >705 nm, the lifetime increases fivefold in the temperature range 200–100 K, accompanied by a decrease in the amplitude from >0.95 to *ca.* 0.5.

Discussion

Fluorescence Kinetics

The variation of the lifetime with temperature of the shortest fluorescence decay component for F690 which is presented in fig. 4 and table 1, is a striking feature. These

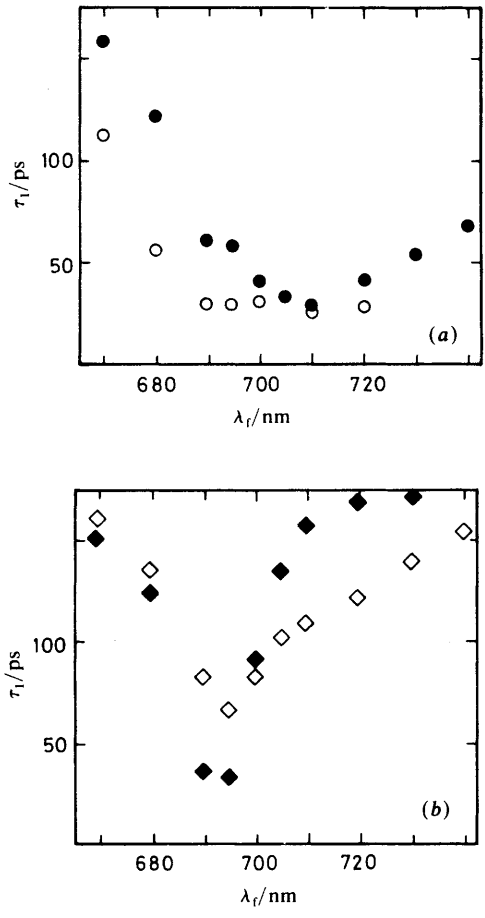


Fig. 3. Dependence of the lifetime of the shortest component in CP1 fluorescence kinetics on the emission wavelength for different temperatures. (*a*) 270 K (○), 200 K (●); (b) 140 K (◇), 80 K (◆).

variations are within the range 30–80 ps. Since we were aware of the limitations to the precision of the determination of lifetimes <100 ps using deconvolution with a broad instrumental response function, we have determined the time resolution of our measurement and data analysis procedure using bilirubin IX, for which the ultrashort lifetime has been determined in different solvents.[15] We thus made sure that the changes in the lifetime of the shortest component of the CP1 particles, resulting from the data analysis, were real and not an instrumental artefact. To minimise errors due to a drift of the pulse in time we have used the average of two pulses, measured before and after the fluorescence decay, in the deconvolution.

Reported lifetimes for PSI fluorescence at room temperature range from 30 to 130 ps; however, most of the sub-100 ps values were later shown to result from excitation annihilation processes due to high incident laser pulse intensities.[20] Low-intensity measurements have provided values of *ca.* 100 ps for a relatively large isolated PSI complex preparation, showing a 730 nm emission maximum at 77 K.[21] For SDS-prepared PSI complexes similar to our CP1, which contain *ca.* 40 antenna chl a per P700, a much shorter lifetime has been reported: 30–50 ps.[22]

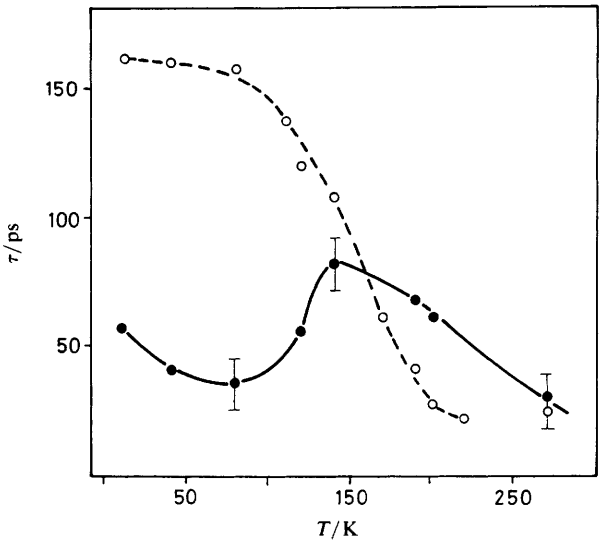


Fig. 4. Dependence of the lifetime of the shortest component in CP1 fluorescence kinetics on temperature for emission wavelengths: 710 nm (○), 690 nm (●). Some typical error bars are indicated.

Since we were mainly interested in resolving the sub-nanosecond decay, we attempted to limit the data-analysis to a 0–5 ns time-window, using up to three exponentials to fit the decay. Although we could find three components, their values were partly outside the range of lifetimes reported by other groups[22,23] and, in addition, depended on the width of the time-window, which is not surprising in view of the non-negligible amplitude of the ns-components (see table 1).

Extending the time-window to 25 ns also yielded three components, two of which were in the ns region and one in the ps region, and these lifetimes were stable, *i.e.* their values did not depend any more on the width of the time-window. Therefore we subsequently used a 25 ns time-window in the data analysis, and consequently we present only one ps component (τ_1). As we will show below, this does not really impair the interpretation of the data using a kinetic model which includes three chl species. At times longer than 25 ns a fourth component with extremely low amplitude is needed for an optimum fit (unpublished results), but this does not influence the determination of the shortest component. We have not attempted to fit the decay with other functions, such as $\exp(-At^{0.5})$.

Butler *et al.*[23] investigated the competition for excitation energy between P700 photochemistry and F735 emission in whole chloroplasts, a very similar situation to that presented here. However, their data should not be compared directly to the values of the short component (table 1), as the poorer signal-to-noise of the single-shot Nd-YAG streak camera set-up would not allow resolution of the three individual components. If a weighted average of all three components is calculated for the 710 nm emission at 80 K for our CP1, a value of *ca.* 1 ns is found, in good agreement with the data of Butler *et al.*[23]

Chlorophyll Heterogeneity

We are aware of the hazards of using SDS for preparing chl-proteins. However, as shown from the spin polarisation pattern AEEAAE, charge separation and recombination still takes place to the full extent in our preparation of CP1, indicating that the

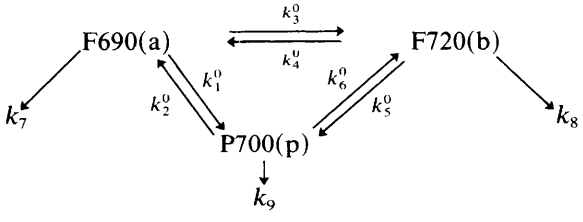
core of the CP1 remains intact. Also the high intensity of the F720 emission band relative to the bands at <690 nm indicates a minimal disturbance of the antenna chl. A recent report[18] confirms that CP1 should be regarded as the minimal PSI reaction-centre complex, with a long-wavelength fluorescence shifted from 735 to 720 nm owing to loss of the outer antenna. The F720 chl forms in CP1 are low-energy sites, distributed in an as yet unknown way within the bulk antenna, probably arising from interactions between chls or between chls and the protein. We assume for the purposes of this paper that the F720 chl is located in that part of the chl-protein molecule which we have called the inner antenna, as distinct from the core antenna and the outer antenna.

It is tempting to ascribe any heterogeneity of the chls in CP1 to the action of SDS during the isolation procedure. However, we believe the heterogeneity to be intrinsic to the PSI reaction-centre protein, since treatment of PSI with the milder non-ionic surfactant Triton X-100 also showed the presence of the F690 and the F720 emission bands.[19] We propose, therefore, the hypothesis that F690 is the core antenna bed, *i.e.* the chl which is located closest to the reaction centre. The hypothesis of a core antenna is analogous to that for antenna pigment organisation in a bacterial system.[24] Thornber and coworkers have suggested from biochemical studies that F690 in PSI is an artefact of chl-surfactant interaction.[25] We regard this as unlikely since the denaturing action of SDS on the chl-protein would result in an emission at shorter wavelength than 690 nm. In addition, the observed short lifetime of F690 is not expected if it were due to partly detached chl.

Proposed Kinetic Model

The pronounced minimum in the wavelength dependence of the fluorescence lifetime at 690–695 nm (fig. 3), together with the evidence for an active RC in CP1, strongly suggests that fluorescence from F690 is quenched by energy transfer to P700, even at the lowest temperatures. We suggest that F690 is situated close to the RC, forming a core distinct from the remaining inner antenna. We show below that a kinetic model based on the presence of a separate species F690, can give an explanation for the experimentally observed temperature dependence of the F690 and F720 fluorescence lifetimes.

We make the following assumptions about the starting conditions: after absorption of the 458 nm excitation, energy would be very rapidly transferred from the bulk inner antenna chl to a low-energy antenna site (F720). Energy absorbed by F690 chl remains in the first few ps within the F690 pigment bed. We adopt the following kinetic scheme for energy transfer:



(2)

This is a series of bimolecular reactions as used for descriptions of energy transfer between isolated molecules in solution, assuming a Coulomic Förster-type process. Energy migration within a given pigment bed probably involves exchange interactions, but we are only concerned here with transfer between the different chl forms. The observation of a multi-banded emission spectrum with different lifetimes for each band is evidence for these forms.

This type of energy transfer is predicted to lead to risetimes in the F690 and F720 emission, but none were detected. We will show below in our numerical results that the risetime of the rising component is calculated to be much less than the lifetime of

the decaying component, and also its amplitude relative to that of the decaying component is calculated to be small. Our data analysis program is not able to resolve the rising component under these conditions.

An essential assumption of our model to explain the temperature dependence of the experimental lifetimes is the endothermic character of the energy transfers from F720 either to F690 or to P700. In addition, excited F720 has temperature-independent decay paths, including fluorescence. Excited F690 is assumed to decay by fluorescence and by temperature-independent (exothermic) transfer to F720 and P700.

The species F690 and F720 are not homogeneous and their emission and absorption spectra are broad envelopes, which is assumed to lead to a temperature dependence of the exothermic transfer processes due to band displacement and/or narrowing on lowering the temperature, as reported for example for purple bacteria.[26]

Excited P700 is assumed to decay through two types of processes: either charge separation leading to the radical pair state from which excitation will not return to P700 within the first ns, or energy back-transfer to the F690 and F720. We further assume that back-transfer to F690 has no activation energy and so is not inhibited on lowering the temperature (even to 10 K) because distance and spectral overlap are favourable.

Analysis of the Model

We shall use the symbols a = [*F690], b = [*F720], p = [*P700], and the corresponding symbols with a subscript to indicate the ground state. We shall also assume that we can write the energy-transfer rate constants as first-order rate constants, so that

$$k_1 = k_1^0 p_0, \quad k_2 = k_2^0 a_0, \quad k_3 = k_3^0 b_0, \quad k_4 = k_4^0 a_0 \quad \text{and} \quad k_5 = k_5^0 p_0. \tag{3}$$

This is justified since under our experimental conditions of low-intensity pulsed excitation the concentration of the ground state of each species is approximately equal to the total concentration of that species, *i.e.* excited singlet, triplet and radical-pair populations are negligible compared with the ground-state population.

This gives rise to a set of differential equations:

$$\frac{da}{dt} = -k_1 a - k_3 a - k_7 a + k_4 b + k_2 p \tag{4}$$

$$\frac{db}{dt} = k_3 a - k_4 b - k_5 b - k_8 b + k_6 p$$

$$\frac{dp}{dt} = k_1 a + k_5 b - k_2 p - k_6 p - k_9 p.$$

The roots (λ_1, λ_2 and λ_3) were found numerically by solving $\det(\mathbf{A} - \lambda \mathbf{I}) = 0$ where

$$\mathbf{A} = \begin{bmatrix} -(k_1 + k_3 + k_7) & k_4 & k_2 \\ k_3 & -(k_4 + k_5 + k_8) & k_6 \\ k_1 & k_5 & -(k_2 + k_6 + k_9) \end{bmatrix} \tag{5}$$

and **I** is the unity matrix.

A program was written in the FORTH-like language ASYST and run on a personal computer to calculate the roots and the amplitudes of the solutions of eqn (4) in the form

$$a(t) = a(0) \sum_{i=1}^{3} a_i \exp(-\lambda_i t). \tag{6}$$

For these calculations, the following assumptions were made.

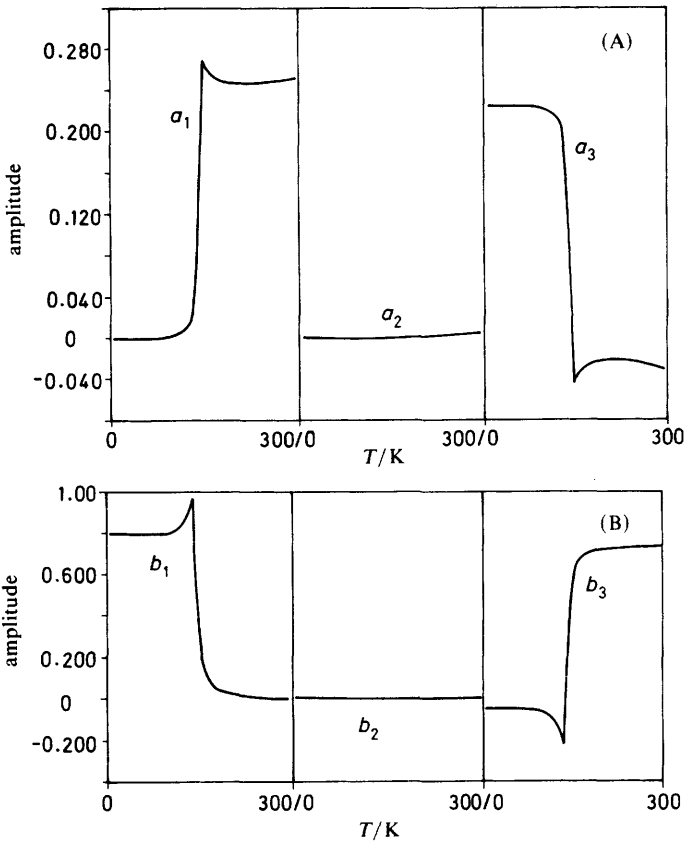


Fig. 5. Calculated amplitudes as a function of temperature of the three characteristic roots in the fluorescence decay of F690 (A) and F720 (B).

(*a*) Starting values of the rate constants, around which some variation was made to arrive at the best agreement of experimental and calculated fluorescence lifetimes, were (all in s^{-1} and at 0 K) (fig. 5 and 6) $k_1 = 8.0 \times 10^9$, $k_2 = 3.0 \times 10^9$, $k_3 = 2.0 \times 10^9$, $k_4 = 8.0 \times 10^8$, $k_5 = 8.0 \times 10^8$, $k_6 = 1.0 \times 10^9$, $k_7 = 6.0 \times 10^9$, $k_8 = 6.0 \times 10^9$, $k_9 = 5.0 \times 10^{11}$. Note that all rate constants represent adjustable parameters, and the values used can be justified as follows: k_1-k_6 correspond to chl-chl donor-acceptor distances of *ca.* 2.5-5.0 nm for Förster energy transfer, and k_7 and k_8 values of $6 \times 10^9\ s^{-1}$ correspond to lifetimes of *ca.* 150 ps in the absence of energy transfer. The large value of k_9 corresponds to a charge-separation time of 2 ps.

(*b*) A linear dependence of the overlap integral in the energy-transfer rate constants k_1, k_2, k_3 and k_6 on temperature was assumed; their values increased two-fold in the range 0-300 K. This assumption is made for reasons of simplicity.

(*c*) A temperature independence of k_7, k_8 and k_9 was assumed.

(*d*) A temperature dependence of the form $f(T) \exp(-\Delta E/kT)$ was assumed for k_4 and k_5, where $f(T)$ is a polynomial in T. The form of this expression will determine to a large extent the temperature dependence of the calculated fluorescence lifetimes, and the form chosen was found to give satisfactory results for the purposes of our simple model. Note that for a temperature-activated uphill energy transfer the most simple

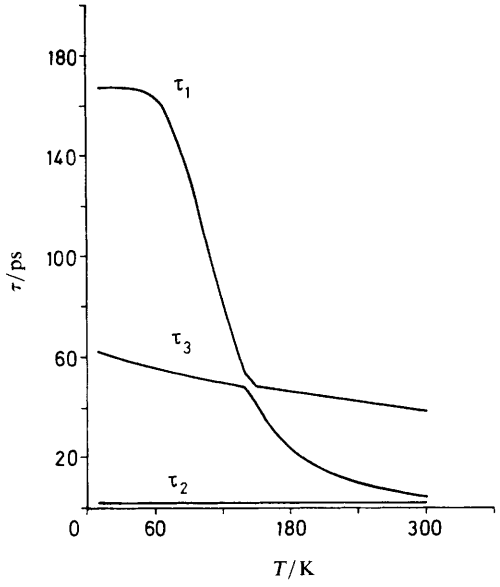


Fig. 6. Calculated characteristic lifetimes of the F690-F720-P700 system as a function of temperature. The lifetimes $\tau_i = -\lambda_i^{-1}$ are in ps, λ_i representing the (three) roots of the kinetic eqn (4). The values of k_1-k_q and $(\Delta E/k)_{4,5}$ used are given in the text.

form would involve a temperature dependence of the form $T \exp(-\Delta E/kt)$. In addition, the rate constants for uphill energy transfer also depend on the value of the overlap integral, which we assumed to be linearly dependent on T[see (*b*), above]. This gives rise to a pre-exponential second-order polynomial.

Of the assumptions (a)-(d), the last has the largest effect on the shape of the calculated curves shown in fig. 6. The form of the temperature dependence of the overlap integral has a minor effect, as compared to the large effect of the exponential temperature dependence in assumption (*d*).

The variation of the calculated lifetimes $(-\lambda_i^{-1})$ with temperature was compared with the experimental data for F690 and F720 (fig. 4). Only the two slowest roots were used because the third is dominated by k_9 and is too fast (*ca.* 2 ps) to be measured with our apparatus.

There is a problem in that we do not know the starting conditions [a(0), b(0) and p(0)] because in plant PSI it is difficult to excite one of the chlorophyll forms exclusively. We would need to know these conditions in order to determine the amplitudes in a weighted sum of the roots, but not if we assume that one root dominates the decay kinetics over the entire temperature range. However, this would require that the curves of the two roots, calculated from the model, against temperature would have to cross (see fig. 4), and our model does not allow for this. Therefore, we assumed that each experimental curve is described by both of the calculated roots, each dominating at either low or high temperatures due to the value of the amplitudes, which are also found to be strongly temperature dependent in the model (see fig. 5). On this basis the temperature dependencies of τ_1 and τ_3 (fig. 6) were calculated as the best fit to the experimental data of fig. 4. The F690 decay kinetics are dominated by τ_3 in the low-temperature (<100 K) range (*i.e.* a_3 dominates), whilst at high temperature (>200 K) τ_1 dominates (a_1 large). The F720 kinetics, on the other hand, are dominated by τ_1 at low temperature and by τ_3 at high temperature. For both F690 and F720 the contribution

of the fast root (λ_2) is negligible, *i.e.* $a_2 \approx 0$ and $b_2 \approx 0$ since this root contains the P700 decay. We have modelled the temperature dependence of the amplitudes. We assume a temperature-independent uniform excitation of all chl forms at time $t = 0$, and the following composition of CP1:

core antenna: inner antenna: reaction centre = 9 : 30 : 1.

The composition of CP1 reflects the number of *ca.* 40 : 1 of core + inner antenna per reaction centre. The assumed ratio core: inner antenna is speculative but inspired by reported values of chl: P700 values of *ca.* 10 in P700-enriched PSI preparations.[27] The calculations show that the latter ratio can be varied over a wide range without affecting the qualitative conclusions of the model. This leads to the starting conditions $a(0) \simeq 0.225$, $b(0) = 0.750$, $p(0) = 0.025$.

The calculated values of the amplitudes are found to be negative (*i.e.* representing rising components) over certain temperature ranges (see fig. 5). The negative value of a_3 at high temperature is, however, small compared with that of a_1, moreover $\tau_1 \gg \tau_3$ so that the effect of mixing τ_3 into τ_1 for the F690 fluorescence kinetics is very small. By the same argument, the negative amplitude for τ_3 in F720 kinetics (b_3) at low temperature would similarly have a negligible effect on the kinetics dominated by τ_1 (b_1).

Our conclusion is therefore that the calculated curves in fig. 6 can describe quite adequately, considering the simplicity of our model, the experimental data of fig. 4. At low and high temperature a single root dominates the decay kinetics of F690 and F720.

In the intermediate temperature range, 100–200 K, the decays of F690 and F720 contain non-negligible amplitudes of both τ_1 and τ_3 so that we would measure in fact a weighted-average lifetime. At 150 K the effect of mixing a rising component and a decay component in F690 fluorescence kinetics is seen as an artefactual lengthening of the decay lifetime obtained from our data analysis to a value well above either τ_1 or τ_3. It should be noted further that the time-resolution limit of our apparatus (*ca.* 20 ps) prevents us from detecting the predicted sub-20 ps lifetimes for F720 at high temperatures.

Although there is a striking similarity between the observed and predicted temperature dependence of the lifetimes, using reasonable values for the kinetic constants in the model, further refinement is desirable. For example, the values of the energy gap ($\Delta E/k$) used to describe the exponential character of the temperature dependence of k_4 and k_5 correspond to 750 and 350 K, respectively. At 300 K k_4 is calculated to have increased to $3 \times 10^9\ s^{-1}$, which is similar to that of k_3 at this temperature. However, k_5 shows a much larger calculated increase as the temperature is raised, reaching *ca.* $7 \times 10^{10}\ s^{-1}$ at 300 K, which is unreasonably large compared to k_6. An improvement is possible, in particular by abandoning the first-order approximation of the kinetics, by optimising the form of the temperature dependence of the energy-transfer rate constants, and by the use of better defined starting conditions, *i.e.* the initial distribution of excitation energy after the laser pulse.

Conclusion

A systematic study has been made of the temperature and wavelength dependences of the lifetime of chl fluorescence from an SDS-prepared PS1 reaction centre chl–protein. We have interpreted these results using the following model for chl–chl energy transfer within this protein: the core antenna F690, whose emission is seen as a discrete band in time-resolved spectra, and the inner antenna low-energy site F720, whose emission is observed as the major long-wavelength band, can transfer energy to each other and also to the reaction centre primary donor, P700. The endothermic transfers from F720 to either F690 or P700 are, however, inhibited by low temperature, causing the observed increase from 30 to 160 ps in the F720 lifetime on going from 200 to 100 K. The experimentally observed multiphase temperature dependence of the F690 lifetime is

suggested to be the result of the mixing of a rising component into the fluorescence decay in the region near 100–200 K: the risetime cannot be observed directly, only its effect on the weighted sum of the fluorescence decay lifetimes.

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