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FLUORESCENCE DECAY KINETICS OF MUTANTS OF CORN DEFICIENT IN PHOTOSYSTEM I AND PHOTOSYSTEM II

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The fast fluorescence decay kinetics of two photosynthetic mutants of corn (*Zea mays*) have been compared with those of normal corn. The fluorescence of normal corn can be resolved into three exponential decay components of lifetime 900–1500 ps (slow), 300–500 ps (middle) and 50–120 ps (fast), the yields of which are affected by light intensity and Mg^{2+} levels. The Photosystem II-(PS II)-defective mutant hcf-3 has similar decay lifetimes (approx. 1200, 450 and 100 ps) but is not affected by light intensity, reflecting the absence of PS II charge recombination. However, yields do respond to Mg^{2+} in a fashion typical of normal corn, which may be correlated with the presence of normal levels of light-harvesting chlorophyll *a* + *b* complex (LHCP). The PS I mutant hcf-50 also shows three-component decay kinetics. In conjunction with the results on the LHCP-deficient mutant of barley presented in a recent paper (Karukstis, K.K. and Sauer, K. (1984) Biochim. Biophys. Acta 766, 148–155), these data suggest that the slow component of normal chloroplasts is kinetically controlled by the decay processes of the LHCP and that the energy comes from one of two sources: (a) charge recombination in the reaction centre or (b) energy transferred within or between LHCP units only. The fast component appears to originate from both PS I and PS II. The complex response of the middle component to cations and light intensity, and its presence in all of the mutants, suggests that it also may have multiple origins.

Introduction

A number of papers have recently appeared in which the decay of room temperature fluorescence of chloroplast membranes has been studied under low light conditions and with good time resolution [1–6]. Using single-photon counting techniques, the decay kinetics can be resolved into three exponential decay components: a slow phase of 1–2 ns, a middle phase of 350–750 ps and a fast phase of around 100 ps [1,4,6]. Both the lifetime and amplitude of the slow phase depend on the redox state of PS II acceptors, so that decay component has been ascribed to fluorescence from antenna chlorophylls which receive energy from charge recombination in the reaction centre. The middle component, which is nearly independent of redox state, was thought to represent energy re-emitted from antenna chlorophylls directly, without an intervening trip to the reaction centre. The fast component has been attributed to some rapidly quenched component of PS II, or to a small amount of room temperature emission from PS I. As we

Abbreviations: PS I and PS II, Photosystems I and II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHCP, light-harvesting chlorophyll *a* + *b*-protein complex of PS II; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid.

show in this paper and in related studies [7], the picture is actually considerably more complicated.

To get a better idea of the origin of the three decay components, several well-characterized mutants lacking different parts of the photochemical apparatus were examined. The mutants are defective in one part of the apparatus and are missing the relevant chlorophyll-protein complexes (Fig. 1), but they make stable thylakoid membranes and retain near-normal activity in the non-mutated parts of the apparatus. Mutants are preferable to detergent-derived membrane sub-fractions, because there is no possibility of artifacts due to the detergent, such as free chlorophyll or partial denaturation of some of the components under study. The mutants we examined were:

(1) hcf-3 (*Zea mays*), which is totally devoid of PS II activity but has normal PS I activity and a normal complement of LHCP [8,9]. It is lacking CPa-1, the reaction centre of PS II [10] and has a reduced amount of CPa-2, which is also part of the reaction centre core particle.

(2) hcf-50 (*Z. mays*), which has very little PS I activity and little or no CPI, but has normal PS II activity and LHCP content [11].

(3) *chlorina f2 (Hordeum vulgare)* which cannot synthesize chlorophyll *b* [12] and therefore cannot make LHCP [13] or CP29, or the recently discovered PS I antenna complex [14–16]. It does have normal PS I and PS II activity, although it requires higher intensities of light for saturation [12].

The corn mutants are discussed in this paper, and the chlorophyll *b*-less mutant of barley in the companion paper [7].

Materials and Methods

The two hcf (high chlorophyll fluorescence) mutants of *Zea mays* were isolated and characterized by Dr. D. Miles, University of Missouri [8,11], who kindly provided the seed. Because the mutations are recessive lethal, the mutant strains are maintained as heterozygotes and the homozygous recessive seedlings detected by their high level of red (chlorophyll) fluorescence under long-wavelength ultraviolet light. Siblings with the normal level of fluorescence were marked at the same time and used as controls. Seedlings with the mutant phenotype grow to the three- or four-leaf stage but die once their seed reserves are exhausted, at 15–18 days under our conditions. Seedlings of the Pioneer cultivar of *Z. mays* were also used as controls and gave the same results as the normal segregants of the hcf strains. The corn seedlings were grown in a greenhouse in a mixture of soil and vermiculite, with supplementary illumination during the wintertime. Class C chloroplasts were prepared by differential centrifugation [6] and suspended in 0.1 M sucrose, 10 mM Hepes-NaOH (pH 7.5), 5 mM NaCl, with or without 5 mM $MgCl_2$. They were diluted just before fluorescence measurements to 10 μg chlorophyll/ml.

The apparatus and procedures for measuring fluorescence decay using single-photon counting have already been described [1,7]. Samples were illuminated at 620 nm with pulses of 8 ps half-maximum full-width duration; 680 nm fluorescence was detected at right angles to the incident beam. The intensity of the incident radiation never exceeded 10^7 photons cm^{-2} per pulse. The fluorescence decay curves were deconvoluted from the excitation curve using the non-linear least-squares method [1]. In all but one case, the decay curve was resolved into three components. The sum of the amplitudes was normalized to 1.00. Relative amplitudes give a measure of the distribution of excitation energy at the beginning of decay. Amplitudes and lifetimes are reproducible within 10% in any one experiment but may differ more than that between experiments. In general, the degree of variability in lifetimes was such that the slow component lifetime was 900–1300 ps in the absence of Mg^{2+} at F_0 or F_{max}, 850–1000 ps in the presence of 5 mM Mg^{2+} at F_0, and 1500–2100 ps in the presence of Mg^{2+} at F_{max}.

Mutant and normal chloroplasts were always isolated and tested on the same day. Overall, there was more variation among the batches of corn which were grown in a greenhouse at various times of the year, than among batches of spinach or peas grown in a controlled environment chamber. These variations did not appear to be related to the chlorophyll *a/b* ratio, and we have no explanation for them. It must be emphasized that the values reported here are representative rather than absolute. What we can show is that there are reproduci-

ble changes in the amplitude, lifetime and yield as a function of light intensity and the presence of divalent cations, and that there are differences between mutant and normal phenotypes.

For visualization of chlorophyll-protein complexes, samples of washed broken chloroplasts were solubilized with 300 mM octylglucoside in 2 mM Tris-maleate (pH 8.0) at a detergent/chlorophyll ratio of 40/1. This was followed by electrophoresis at 0°C on polyacrylamide gels containing 0.1% SDS [17].

Results

The normal chloroplast

In all of our experiments, normal and mutant chloroplasts were compared under four sets of conditions to determine the effects of both light intensity and divalent cations on the decay of 680 nm fluorescence. Chloroplasts were stored and tested in buffer containing either no Mg^{2+} or 5 mM Mg^{2+}. Each sample was tested first under low light intensity (F_0), then under high (saturating) light intensity (F_{max}). In some experiments, 5 μM DCMU was added to samples at F_{max} to make sure that reaction centres were closed.

Normal chloroplasts of corn have the same characteristic fluorescence decay kinetics as those of spinach or pea [1,4,18]. Table I summarizes the results from two types of normal corn chloroplasts: one sample of variety Pioneer and one sample of the normal-phenotype segregants from hcf-3 seedlings. In both cases, the decay curve was resolved into three components, labelled slow, middle and fast. In the presence of Mg^{2+}, increasing the light intensity from F_0 to F_{max} (i.e., closing the reaction centres) caused a dramatic increase in the relative amplitude of the slow component, with corresponding decreases in the amplitudes of the middle and fast components. The lifetime of the slow component was approximately doubled, while that of the middle component increased by as much as 50% in some experiments but showed no increase in others. Closing the reaction centres in the absence of Mg^{2+} increased the amplitude of the slow component but did not increase its lifetime significantly. The experimental variation in the lifetime of the fast component was such that there was no reproducible difference in it between one experimental condition and another; however, the fast decay lifetime is close to the resolution limit of our instrument.

More information can be obtained about the distribution of excitation energy among fluorescing species from an examination of the fluorescence yields (Table II). The yields were normalized so that the total fluorescence yield of the normal chloroplasts at F_{max} in the presence of 5 mM Mg^{2+} was 100. When reaction centres were closed (F_{max}), there was a dramatic increase in the

TABLE I

hcf-3: FLUORESCENCE DECAY AMPLITUDES AND LIFETIMES

Broken chloroplasts were suspended in 0.1 M sucrose, 10 mM Hepes-NaOH (pH 7.5), 5 mM NaCl, with or without 5 mM Mg^{2+}, and exposed to low (F_0) or saturating (F_{max}) light of 620 nm. Amplitudes (α_1, α_2, α_3) normalized to a sum of 1.00 for each experimental condition.

	No. samples	Light intensity	No Mg^{2+}						5 mM Mg^{2+}					
			slow		middle		fast		slow		middle		fast	
			α_1	τ_1 (ps)	α_2	τ_2 (ps)	α_3	τ_3 (ps)	α_1	τ_1 (ps)	α_2	τ_2 (ps)	α_3	τ_3 (ps)
Normal phenotype, cv. Pioneer	1	F_0	0.03	1150	0.42	310	0.55	90	0.10	930	0.44	400	0.45	55
		F_{max}	0.38	1240	0.42	460	0.20	110	0.61	2060	0.17	430	0.23	75
Normal phenotype, hcf-3 segregant	3	F_0	0.10	1260	0.34	330	0.57	110	0.09	840	0.51	350	0.40	110
		F_{max}	0.25	1330	0.44	470	0.31	120	0.53	1560	0.21	520	0.27	80
Mutant phenotype, hcf-3 segregant	3	F_0	0.35	1170	0.32	480	0.34	100	0.50	1270	0.24	520	0.27	85
		F_{max}	0.36	1110	0.33	440	0.32	100	0.53	1160	0.30	510	0.17	105

TABLE II

FLUORESCENCE YIELDS OF NORMAL AND MUTANT SEGREGANTS OF hcf-3

hcf-3 data from same experiment as Table I. $+Mg^{2+}$ buffer contains 5 mM $MgCl_2$, $-Mg^{2+}$ contains none. Yields are normalized to give total = 100 for normal chloroplasts in the presence of 5 mM $MgCl_2$ at F_{max}.

Phenotype	Light intensity	Mg^{2+}	Yield				F_{max}/F_0	
			slow	middle	fast	total	$-Mg^{2+}$	$+Mg^{2+}$
Normal	F_0	−	8	7	4	19	2.3	4.6
		+	5	14	3	22		
	F_{max}	−	25	15	3	43		
		+	86	12	2	100		
Mutant	F_0	−	28	11	3	42	1.3	1.1
		+	58	11	2	71		
	F_{max}	−	37	14	3	54		
		+	59	15	2	76		

slow component yield, which is compatible with the idea that a large portion of this component results from charge recombination in the reaction centre [1,6]. Mg^{2+} caused a further increase in the slow component yield at F_{max}. This could have resulted from more excitation being transferred to another PS II unit rather than decaying by non-radiative processes [1,4], because the effect of Mg^{2+} is to segregate PS II units in the grana regions with the result that they are closer together [19,20]. The increase in lifetime under these conditions is also consistent with this explanation [1,6]. If the effects of Mg^{2+} on steady-state fluorescence and membrane arrangement are mediated via the LHCP of PS II, as is generally believed (e.g., Refs. 19–21), then the slow component must be kinetically controlled by the LHCP and its interactions.

The effects of light and Mg^{2+} on the middle phase are not so easily explained. In most experiments, the yield was increased in going from F_0 to F_{max} in the absence of Mg^{2+}, but was decreased in the presence of Mg^{2+}. In the experiment of Table II, the yield did not decrease as much as it did in most experiments, where it dropped to about half the F_{max} level (e.g., normal chloroplasts in Table IV). Comparing samples in both tables, Mg^{2+} appears to have decreased the yield at F_{max}, but increased it at F_0. This suggests that there may be more than one process contributing to this component, and that the changes in lifetime seen in Tables I and III may reflect different proportions of two or more decays which are near enough in lifetime to be unresolved by our procedures.

Photosystem II-defective mutant hcf-3

This high fluorescence nuclear mutant has no PS II activity and no variable fluorescence [8,9]. It is missing at least six polypeptides associated with PS II [22], including three polypeptides which are known to be chloroplast gene products [23,24]. It appears to be completely lacking the chlorophyll *a* complex CPa-1 (CP 47), (Fig. 1, lane 1), which has been shown to be the PS II reaction centre chloro-

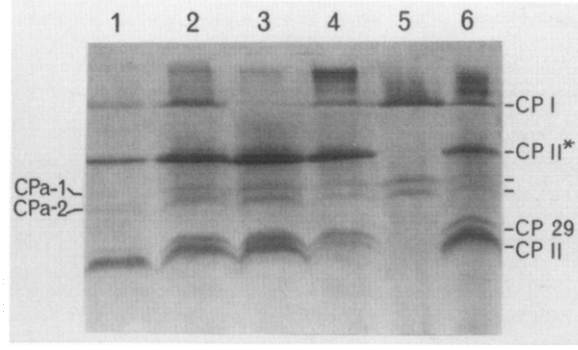

Fig. 1. Chlorophyll-protein complexes of normal and mutant chloroplasts. Complexes were separated on 10% polyacrylamide gels containing 0.1% SDS. Lane 1, hcf-3, mutant phenotype; lane 2, hcf-3, normal phenotype; lane 3, hcf-50, mutant phenotype; lane 4, hcf-50, normal phenotype; lane 5, *chlorina f2* barley; lane 6, normal barley.

phyll-protein complex [10]. This mutant is able to synthesize some of the CPa-1 polypeptide, but it is turned over rapidly because it is not correctly incorporated into the membrane [25].

The major effect of light intensity on normal chloroplasts is to close reaction centres. Because there are no reaction centres to close in this mutant, it might be expected that the chloroplasts would behave as if they were at F_{max} even under low light intensity. Indeed, under both low and high light intensity, the amplitudes and lifetimes of all three components were similar to those of normal chloroplasts at F_{max} (Table I). The total yield at F_0 in the presence of Mg^{2+} was 3-times that of the normal, even though the F_{max} yield was never as high as the normal (Table II). Increased light intensity by itself caused only minor increases in mutant fluorescence yields. However, one way in which the mutant did not behave as if it were permanently in F_{max} is that, in the presence of Mg^{2+}, the slow component lifetime did not show the large increase at F_{max} seen in normal chloroplasts.

Although hcf-3 is missing PS II reaction centres, it does contain the normal complement of LHCP [9]. It might therefore be predicted that those effects of Mg^{2+} which are mediated by the LHCP should be the same in mutant and normal chloroplasts. Mg^{2+} caused a marked increase in mutant yields at both F_0 and F_{max}, most of which was due to a large increase in the slow component. Yields of the middle component remained relatively high under all conditions, with no significant Mg^{2+} effect, in contrast to the situation in normal chloroplasts.

Because of the possibility that young seedlings might contain a very small amount of functioning PS II [25], chloroplasts from 7- and 11-day-old seedlings were studied (data not shown). The fluorescence yields of both were just as insensitive to light intensity as the 14-day-old seedlings on which the rest of our experiments were done. The one small difference in the 7-day-old plants was common to both mutant and normal chloroplasts: they both had slightly higher yields of the middle component, which were insensitive to Mg^{2+}.

Photosystem I-deficient mutant hcf-50

This high chlorophyll fluorescence mutant of corn has little or no PS I activity [11]. Fig. 1 shows that it is lacking CPI, the PS I reaction centre complex (lane 3), but that all of the other complexes are present in normal amount.

Based on previous work, it was predicted that if

TABLE III

hcf-50: FLUORESCENCE DECAY AMPLITUDES AND LIFETIMES

Same conditions as Table I.

	No. samples	Light intensity	Slow		Middle		Fast	
			α_1	τ_1 (ps)	α_2	τ_2 (ps)	α_3	τ_3 (ps)
No Mg^{2+}								
Normal phenotype	2	F_0	0.04	950	0.40	270	0.57	60
	2	F_{max} (+DCMU) [a]	0.29	1000	0.36	350	0.35	75
Mutant phenotype	3	F_0	0.18	1050	0.47	350	0.36	90
	2	F_{max}	0.71	1470	0.29	340	< 0.01	-
	3	F_{max} (+DCMU) [a]	0.73	1500	0.27	310	< 0.01	-
5 mM Mg^{2+}								
Normal phenotype	2	F_0	0.06	920	0.50	320	0.44	70
	2	F_{max} (+DCMU) [a]	0.65	1460	0.21	280	0.15	110
Mutant phenotype	2	F_0	0.14	1060	0.57	350	0.30	100
	2	F_{max}	0.61	1540	0.20	350	0.20	90
	2	F_{max} (+DCMU) [a]	0.66	1490	0.23	350	0.11	115

[a] Sample contained 5 μM DCMU.

TABLE IV

FLUORESCENCE YIELDS OF NORMAL AND MUTANT SEGREGANTS OF hcf-50

Same conditions as Table II. Samples at F_{max} also contained 5 μM DCMU. Yields were normalized to given total = 100 for normal chloroplasts in the presence of 5 mM $MgCl_2$ at F_{max}.

Phenotype	Light intensity	Mg^{2+}	Yield				F_{max}/F_0	
			slow	middle	fast	total	$-Mg^{2+}$	$+Mg^{2+}$
Normal	F_0	−	4	10	3	17	3.1	3.9
		+	6	17	3	26		
	F_{max}	−	35	15	3	53		
		+	93	6	2	100		
Mutant	F_0	−	19	17	3	39	3.2	4.0
		+	13	17	2	32		
	F_{max}	−	115	10	–	123		
		+	117	9	2	128		

some or all of the fast component originated in PS I, the fast component would be missing or diminished in hcf-50. Table III shows that the fast component did disappear at F_{max} in the absence of Mg^{2+}. There was no significant change in the middle phase lifetime under these conditions, which argues against the possibility that the fast phase lifetime had simply increased to the point where it could no longer be resolved. However, the fast component did not disappear at F_{max} in the presence of Mg^{2+}; it had about the same amplitude and yield as in the normal chloroplast. Under conditions where the fast component was present, there was no experimentally significant difference in its lifetime between normal and mutant chloroplasts. These results suggest that the fast phase must involve emission from PS II as well as PS I.

Two features of the mutant's behaviour were somewhat unexpected. In the absence of Mg^{2+}, the lifetime of the slow phase increased by 50% in going from F_0 to F_{max}, as if Mg^{2+} were present. This was not seen in the normal controls. Light intensity had a very large effect on the slow phase yield (Table IV), with an F_{max} yield for the slow phase alone which was greater than 100% of the total normal yield. However, none of the yields was increased by the presence of Mg^{2+} in this mutant.

Discussion

The higher plant photosynthetic membrane contains a number of different chlorophyll-containing units, each of which is able to absorb excitation energy, either directly from light or by transfer from another absorbing species.Once energy is absorbed, it can be emitted as fluorescence, be lost in a radiationless decay process, be transferred to yet another absorbing species or, in the case of a reaction centre complex, be used to drive charge separation and subsequent electron transport [26]. All of these processes are competing and interacting. In this paper, we have shown that mutants totally lacking either PS I or PS II still have three fluorescence decay components. This observation rules out a simple origin for any component. This is particularly true for the middle component, which has a complex response to cations and light in the normal chloroplast, is present but unresponsive in the hcf-3 chloroplast, and responds to light intensity but not Mg^{2+} in chloroplasts lacking LHCP [7,18]. It is probably safe to conclude that it is a composite of several different unresolved decay processes with lifetimes in the 300–500 ps range. Some of these processes will be absent in the mutants or will have altered rate constants.

The yield of slow component fluorescence in normal chloroplasts increases as reaction centres are closed by light or chemical reduction [1,6], supporting the suggestion that a sizeable fraction of this component represents energy derived from charge recombination in the PS II reaction centres, i.e., the back reaction from Q^- [6]. However, charge recombination cannot be necessary for a slow component to be present, because a large part

of the fluorescence from the mutant hcf-3 has a 1000–1200 ps lifetime. In this mutant there can be no PS II charge recombination, because the entire PS II core complex is missing or severely depleted, including CPa-1 and CPa-2 (Fig. 1) and polypeptides of 34, 32, 16 and 10 kDa [8,22]. The fluorescence decay components from such a defective mutant may not arise from the same fluorescing species as in the normal chloroplast. However, the hcf-3 slow component does respond to Mg^{2+} in the same way as the normal (Tables I and II) with the exception that its lifetime is never dependent on light intensity.

Because the slow component yield is increased by both increased light intensity and Mg^{2+} in the presence of functional reaction centres, and by Mg^{2+} alone in their absence, we suggest that two different processes may give rise to decay components with lifetimes in the slow range (900–1500 ps). In the normal chloroplasts, most of the slow component fluorescence would originate from charge recombination in closed reaction centres, with the energy transferred back to associated LHCP units for emission, or transferred to another PS II unit and emitted. In the PS II-lacking hcf-3 chloroplasts, the slow component cannot arise from charge recombination, but could come from excitation energy which is emitted directly by an LHCP unit or is transferred to another LHCP unit and then emitted. This second process could also contribute to the F_0 emission in the normal chloroplast. The effect of Mg^{2+} in increasing the slow component lifetime in the normal chloroplast would reflect its effect on increased transfer of energy between PS II units after charge recombination [1,6].

The results presented here as well as those in the complementary study [7] emphasize the large role played by the light-harvesting chlorophyll *a* + *b* complex in modulating the emission of excitation energy as fluorescence [20,21], particularly with respect to the slow component. The *chlorina f2* mutant of barley, which does not make chlorophyll *b* but makes a small amount of an abnormal LHCP, has only a quarter of the fluorescence yield of normal barley [7]. Furthermore, it does not have a typical slow component (500–1200 ps in normal barley) but only an abnormally slow decay of 1900–2700 ps. The hcf-3 mutant, although completely lacking PS II, has the normal complement of LHCP and its steady-state fluorescence yield is increased by Mg^{2+} [9]. This increase is abolished by a brief trypsin treatment, which selectively affects the LHCP [9]. This again points to the LHCP as being involved in the kinetic control of the slow component. Although the mutant's maximum fluorescence yield in our experiments is never more than 75% of normal at F_{max} even in the presence of Mg^{2+}, this could be due to radiationless decay processes competing more effectively for energy which cannot be trapped by charge separation, rather than a decreased sensitivity to Mg^{2+}.

In contrast to the hcf-3 mutant, the PS I-lacking mutant hcf-50, has an usually large slow component yield. Because this mutant has a normal PS II but is blocked at the level of PS I reaction centre, not only is charge separation possible, but perhaps even a build-up of reducing equivalents in the plastoquinone pool, which could result in an increased probability of charge recombination and a higher yield. Comparison of fluorescence induction curves in the presence and absence of DCMU indicated that the plastoquinone pool was larger in the mutant than the normal (data not shown).

The anomalous behaviour of this mutant with respect to Mg^{2+} is similar to that of the PS I-deficient mutant F14 of *Chlamydomonas* [28]. In that mutant, the addition of Mg^{2+} decreased the F_{max} yield by 10–20% and had a similar effect on the initial level of fluorescence in dark-adapted cells. In the corn mutant under our conditions, Mg^{2+} caused a minor decrease in F_0 yield and no significant change at F_{max} (Table IV).

The fast component of fluorescence decay appears to come from both PS I and PS II. This component has been ascribed to PS I by other workers using chloroplasts [3] or algae [5]. A component of similar lifetime has been reported for PS I particles (Ref. 30; Haworth, P. and Karukstis, K.K., unpublished data). There is an increased yield of this component in the *chlorina f2* barley [7], as would be predicted from the increased amount of PS I chlorophyll-protein complexes and PS I activity reported by Waldron and Anderson [29]. However, in the PS I-deficient mutant hcf-50, the fast component is present under all conditions except at F_{max} in the absence of Mg^{2+}. Therefore, there must be a contribution from PS II [1,2,5],

although we would not go so far as to say that the major portion of the fast component is arising in PS II [5].

It should be pointed out that any given component may not necessarily be identifiable with a particular chlorophyll-protein complex in the way that we have suggested for the slow component and the LHCP. Rather, a particular decay may originate from chlorophylls in the same kind of environment in a number of different complexes. This could explain why the fast component is found to at least some extent in every mutant, and appears to be relatively insensitive to membrane arrangement (Mg^{2+} effects) or to light intensity. However, it is clear that a consistent explanation of the origins of the fluorescent decay components wil have to await the development of kinetic models which take into account all the various transfer and decay processes.

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