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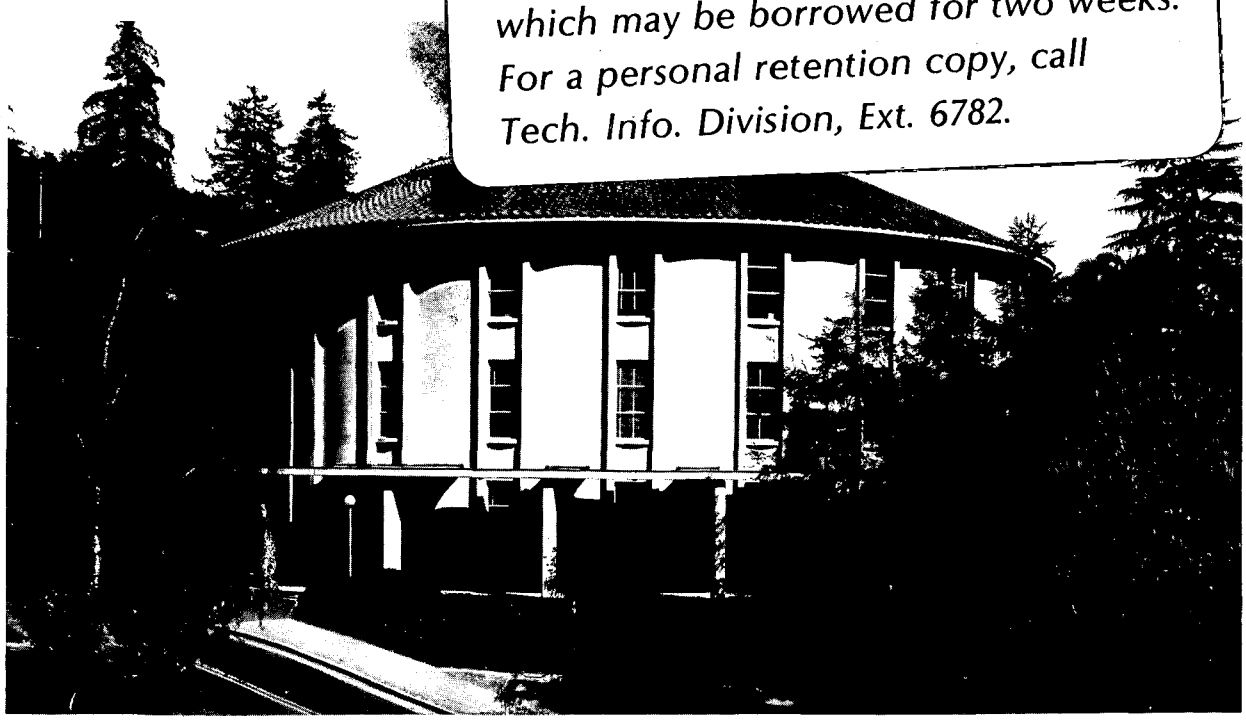

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PICOSECOND FLUORESCENCE KINETICS IN SPINACH CHLOROPLASTS AT ROOM TEMPERATURE: EFFECTS OF MAGNESIUM ION

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Abbreviations:
DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethyl urea;
HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

SUMMARY

Single-photon timing with picosecond resolution is used to investigate the effect of Mg^{+2} on the room temperature fluorescence decay kinetics in broken spinach chloroplasts. In agreement with an earlier paper (Haehnel, W., Nairn, J. A., Reisberg, P., and Sauer, K. (1982) Biochim. Biophys. Acta, in press), we find three components in the fluorescence decay both in the presence and in the absence of Mg^{+2}. The behavior of these components is examined as a function of Mg^{+2} concentration at both the F_0 and the F_{max} fluorescence levels, and as a function of the excitation intensity for thylakoids from spinach chloroplasts isolated in the absence of added Mg^{+2}. Analysis of the results indicates that the subsequent addition of Mg^{+2} has effects which occur at different levels of added cation. At low levels of Mg^{+2} (< 0.75 mM), there appears to be a decrease in communication between Photosystem II and Photosystem I, which amounts to a decrease in the spillover rate between Photosystem II and Photosystem I. At higher levels of Mg^{+2} (about 2 mM), there appears to be an increase in communication between Photosystem II units and an increase of the effective absorption cross-section of Photosystem II, probably both of these involving the chlorophyll a/b light-harvesting antenna.

INTRODUCTION

The addition of cations to broken chloroplasts induces changes in the primary processes of photosynthesis. These changes include: (1) a dramatic increase in the room temperature fluorescence yield of DCMU poisoned chloroplasts [1-4], (2) an increase in the 685 nm fluorescence at low temperature relative to the 735 nm fluorescence [2-4], (3) an increase in the Photosystem II quantum efficiency [2-4], and (4) a decrease in the Photosystem I quantum efficiency [2-4]. Murata [2-4] postulated that cations decrease the rate of spillover from Photosystem II to Photosystem I and that cationic regulation of this rate may be the basis of the state 1 to state 2 transition observed in intact chloroplasts [5,6]. In his model, state 1 (the dark state) is analogous to the state of high cation concentration, with low Photosystem II to Photosystem I spillover; state 2 is analogous to the state of low cation concentration, with high Photosystem II to Photosystem I spillover. More recent work suggests that the cation effect on energy distribution between Photosystem II and Photosystem I is more complicated. Butler and Kitajima [7] concluded from fluorescence induction data at low temperature that, in addition to decreasing the rate of Photosystem II to Photosystem I spillover, Mg^{+2} increases the absorption cross section of Photosystem II. The analysis of fluorescence data by Henkin and Sauer [8] indicated that the only effect of Mg^{+2} is an increase in the absorption cross-section of Photosystem II.

Joliot and Joliot [9] reported that the fluorescence induction curve for intact chloroplasts in whole cells of <u>Chlorella pyrenoidosa</u> displays a sigmoidal rise. They attributed the sigmoidicity to the possibility of energy transfer between Photosystem II units. They proposed a theoretical relation

between the probability of transfer between Photosystem II units, ρ, and the shape of the fluorescence induction curve; the fluorescence induction curve from intact chloroplasts indicates that ρ=0.55 [9]. Experiments with broken chloroplasts show that the fluorescence induction curve is sigmoidal in the presence of Mg^{+2}, but exponential in the absence of Mg^{+2}; the two curves correspond to ρ=0.5 to 0.6 in the presence of Mg^{+2}, with ρ decreasing to a low value in the absence of Mg^{+2} [10-13]. The conclusion is that added Mg^{+2} enables energy transfer to occur between Photosystem II units.

Several possibilities have been presented for explaining the mechanism of the cationic regulation of energy distribution. Izawa and Good [14] found that chloroplasts isolated in low salt medium have unstacked thylakoid membranes and that the addition of salts induces thylakoid stacking. This stacking is correlated with increased light scattering and with the fluorescence increases described above [15,16]. Murakami and Packer [15] and Murata [16] concluded that thylakoid stacking may be the mechanism behind the cation effect and the state 1 to state 2 transition. More recent experiments show that thylakoid stacking and fluorescence yield changes are separable phenomena; that is, one effect can be induced independently of the other [17,18]. Experiments with mutants indicate that a Mg^{+2} effect and a state 1 to state 2 transition are absent in photosynthetic organisms that lack the chlorophyll a/b light-harvesting protein [19]. The conclusion is that an interaction between Mg^{+2} and the chlorophyll a/b light-harvesting protein induces some change which can control the energy distribution between Photosystem II and Photosystem I [19-21].

In a recent paper [22] we described picosecond resolution of the fluorescence decay kinetics in spinach chloroplasts. The fluorescence decay was found to be characterized by three exponential phases. The three phases

can be qualitatively interpreted as follows (See Refs. [22] and [23] for a more quantitative analysis): 1) One slow phase (1-2 ns) is due to radical-pair recombination in Photosystem II of the oxidized primary electron donor, P_{680}^{+}, and the reduced primary electron acceptor, I^{-}, which is believed to be a pheophytin molecule [24-27]. The above recombination occurs with a high yield when the secondary electron acceptor Q [28] is reduced [22-27]. 2) Two faster phases are due to excitation that is lost prior to reaching the reaction center. Of these two faster phases, the fastest (50-100 ps) is kinetically controlled by the rate of excitation transfer from the chlorophyll a antenna of photosystem II (chlorophyll a_2) to the reaction center of Photosystem II, and at least a portion of the slower one (400-750 ps), is kinetically controlled by the rate of excitation transfer from the chlorophyll a/b light-harvesting antenna to the reaction center of Photosystem II. These two types of chlorophyll antennae make up the Photosystem II antenna in Butler's tripartite model [29]. The two faster lifetimes characterize the transfer times from the various parts of the antenna to the reaction center of Photosystem II. 3) The room temperature fluorescence is assumed to be mostly from the Photosystem II antenna; that is, from the chlorophyll a_2 antenna and the chlorophyll a/b light-harvesting antenna. Photosystem I fluorescence may make a contribution, but this should not affect the qualitative interpretation of the above three phases.

In this paper, we report measurements of the three components of the fluorescence decay as a function of the concentration of Mg^{+2}. Experiments were done at the F_0 level and at the F_{max} level in spinach chloroplasts at room temperature. The F_0 level corresponds to the state where all of Q is oxidized (i.e., all Photosystem II reaction centers open), and the F_{max} level corresponds to the state where all of Q is reduced (i.e., all Photosystem II

reaction centers closed). To examine the extent of energy transfer between Photosystem II units, it is necessary to look at the fluorescence decay when some of the Photosystem II reaction centers are open and some are closed. For experiments in the partially closed state, we have measured the intensity dependence of the fluorescence decay kinetics for spinach chloroplasts in the absence of Mg^{+2}. We find that most of our data can be explained by assuming that Mg^{+2} has two effects. The addition of Mg^{+2} to thylakoids from broken spinach chloroplasts isolated in a Mg^{+2}-free buffer first decreases the rate of energy transfer or spillover from Photosystem II to Photosystem I; this first effect saturates at low concentrations of Mg^{+2} (< 0.75 mM). A second effect, saturating at about 2 mM Mg^{+2}, causes an increase in both the absorption cross section and the extent of energy transfer between Photosystem II units.

MATERIALS AND METHODS

Broken spinach chloroplasts were isolated by the method described previously [22]. Fresh spinach leaves, grown either in a growth chamber or in a greenhouse, were ground in a blender for 10 s in 50 mM HEPES-NaOH buffer (pH 7.5) with 0.4 M sucrose and 10 mM NaCl followed by 2 min of centrifugation at 2000 xg. After one wash with fresh grinding buffer, the chloroplasts were kept for 20 min at $0^{o}C$ in 10 mM HEPES-NaOH buffer (pH 7.5) with 0.1 M sucrose and 10 mM NaCl. After centrifugation for 5 min at 2000 xg, portions of the pellet were resuspended in several different buffers. Each buffer contained 10 mM HEPES-NaOH (pH 7.5), 0.1 M sucrose, and 5 mM NaCl. Each buffer also either had no Mg^{+2}, or contained a concentration of $MgCl_2$ equal to the concentration desired for the fluorescence measurement. The chloroplasts were allowed to

equilibrate in these buffers for at least 1 hour. The chlorophyll concentration was adjusted to 18 μg chlorophyll/ml by dilution with the appropriate resuspending buffer. For experiments at the F_0 level, we added 1.25 mM potassium ferricyanide as electron acceptor, 1.25 mM potassium ferrocyanide to control the redox potential, and 2.5 μg/ml gramicidin D as uncoupler. These levels of added K^+ and polyvalent anions do not by themselves affect the room temperature fluorescence properties of broken spinach chloroplasts. The uncoupler was added to prevent the slow formation of a pH gradient across the thylakoid membrane, which has been reported to cause a decline in the fluorescence yield [30]. The chloroplast sample was rapidly stirred in a 1 cm x 1 cm cuvette, and each sample was replaced every 10 min if more data accumulation was needed. For experiments at the F_{max} level, we added 12.5 μM DCMU and 2 mM hydroxylamine hydrochloride. To close the reaction centers the sample was illuminated with about 10 flashes of saturating light immediately before the lifetime measurement. The intensity-dependence experiment was done like an F_0 experiment, except that ferri- and ferrocyanide were omitted from the resuspending buffer. All measurements were carried out at room temperature (20-22°C), and the cuvette was painted black except for a window for the exciting beam and a window in the direction of the photomultiplier. This masking was necessary to eliminate a broadening of the apparent excitation pulse shape due to reflections.

The excitation pulse was provided by a Spectra Physics synchronously pumped mode-locked dye laser which is composed of an SP 171 argon ion laser, an SP 362 mode locker, and a modified SP 375 dye laser. The output pulses of this laser have a full-width half-maximum duration of about 15 ps (as determined by zero background, second harmonic generation [31]). All experiments used the laser dye rhodamine 6G with excitation pulses at 620nm.

The single-photon timing detection system and the methods of numerical analysis are described elsewhere [22,32,33]. All of the data analyses presented here result from a resolution of the fluorescence decay kinetics into a sum of exponentials. That is, the time dependence of the fluorescence decay is given by

$$F(t) = \sum_{i=1}^{3} \alpha_i \exp(-t/\tau_i) \qquad (1)$$

where α_i and τ_i are the amplitude and the lifetime of the ith component respectively. The yield of the ith component is equal to $\alpha_i \tau_i$; this yield represents the total number of photons emitted in the ith phase. We estimate that our fluorescence lifetime measuring system can resolve fluorescence lifetimes as short as 25 ps [22].

RESULTS

Mg^{+2} dependence of F_0 level fluorescence

Table I summarizes the results from our earlier paper [22] which have been renormalized to facilitate comparison of the yields between different experiments. The effects of adding Mg^{+2} to spinach chloroplasts at the F_0 level are: 1) a decrease in the lifetime of the slow phase with no change in its yield, 2) a slight increase in the lifetime of the middle phase accompanied by a doubling of the yield, and 3) a decrease in the yield of the fast phase. The change in the fast phase, however, may be within the uncertainty of our measurements, because the fast phase is the most difficult phase to resolve. The dependence of the three lifetimes on Mg^{+2} concentration is plotted in Fig. 1. The slow phase increases somewhat at low levels of Mg^{+2} and then decreases to its final value by $[Mg^{+2}]$ = 2 mM. We note that, like the

fast phase, the slow phase is a small part of the total F_0 decay and is difficult to resolve. The precise details of the change in the slow phase lifetime will need confirmation, but we generally observe a decrease in the slow phase lifetime upon the addition of 5 mM Mg^{+2}. The lifetimes of the fast and middle phases show only minor changes.

In Fig. 2 is plotted the total yield and the yield of each component versus Mg^{+2} concentration. The total yield increases about 30%, saturating at $[Mg^{+2}]$ = 0.75 mM; this increase is in good agreement with the results of Henkin and Sauer [8]. The changes in the individual decay components are surprisingly complex. The yield of the slow phase increases four-fold, peaking at $[Mg^{+2}]$ = 0.75 mM, and then decreases to nearly its original value. The decrease is complete at about $[Mg^{+2}]$ = 2 mM. Despite difficulty in resolving the lifetime of the slow phase, the rise and fall of its yield was observed to be similar in each sample investigated. It is, therefore, likely that the rise and fall of the lifetime of the slow phase mentioned above is real. The yield of the middle phase remains approximately constant up to 1 mM and then approximately doubles, with the doubling nearly complete by $[Mg^{+2}]$ = 2 mM.

Mg^{+2} dependence of the F_{max} level fluorescence

The effects on the F_{max} level resulting from increasing the concentration of Mg^{+2} added to broken spinach chloroplasts are: 1) an increase in the lifetime of the slow phase, accompanied by a four-fold increase in its yield, 2) an increase in the lifetime of the middle phase, accompanied by a slight decrease in its yield, and 3) changes in the fast phase which probably do not lie outside the uncertainty of our measurement. The fast phase in the F_{max} level is especially difficult to resolve because it is a very small component relative to the other two phases. The lifetimes of the three components versus

Mg^{+2} concentration are plotted in Fig. 3. The lifetime of the slow phase increases from 1170 ps to 1600 ps, saturating at $[Mg^{+2}]$ = 0.75 mM or less. The range of the change, 1170 ps to 1600 ps, is different from the range in Table I (1700 ps to 2000 ps); the discrepancy is probably due to sample variability. Despite the differences in ranges, the saturation of the effect at $[Mg^{+2}]$ = 0.75 mM is reproducible for the slow phase lifetime. The lifetime of the middle phase increases only slightly from 380 ps to 430 ps; the increase occurs at low Mg^{+2} concentration ($<$ 0.75 mM). The smaller change here as compared to Table I could also be due to sample variability. We have always seen an increase in this lifetime at the F_{max} level upon the addition of Mg^{+2}. The increase is sometimes small, and the increase presented in Table I represents about the maximum effect.

The total yield and the yield of each component is plotted versus Mg^{+2} concentration in Fig. 4. The total yield doubles, saturating at $[Mg^{+2}]$ = 2 mM; this increase is in close agreement with the results of Henkin and Sauer [8]. All of the increase is accounted for by a fourfold increase in the yield of the slow phase; this increase also saturates at $[Mg^{+2}]$ = 2 mM. The only other effect is a slight decrease in the yield of the middle phase, saturating at about $[Mg^{+2}]$ = 1 mM.

Intensity dependence of the fluorescence decay kinetics in the absence of Mg^{+2}

The effect of intensity on the three kinetic components of spinach chloroplasts in the presence of Mg^{+2} is plotted in Figs. 6 to 8 in Ref. [22]. The results show a smooth transition from the F_0 values to the F_{max} values given in Table I. Here, we have repeated the same experiment in the absence of Mg^{+2}. The results plotted in Figs 5 and 6 show smooth transitions between

limits similar to the F_0 and F_{max} values given in Table I. The lifetime of the slow phase is nearly constant at about 1350 ps. The slow phase yield increases 8.3 fold. The lifetime and yield of the middle phase increase somewhat, the yield increasing about 60-70%. The lifetime of the fast phase remains constant, and its yield decreases.

DISCUSSION

Comparison with other Mg^{+2} dependent fluorescence lifetime measurements

Because we have resolved three fluorescence decay components where other studies have resolved only two components [34-36] or one component [37], it is difficult to compare our results quantitatively to literature results. A qualitative comparison, however, reveals that our data can be reconciled quite well with other Mg^{+2}-dependent fluorescence lifetime measurements [34-37]. Searle et al. [35] looked at the effect of adding Mg^{+2} to wild-type barley chloroplasts at both the F_0 and F_{max} levels. At F_0, they saw very small changes in the lifetimes of two components and a slight increase in the yield of their slow component (600 to 650 ps). Our result for F_0 agrees with this result, if we note that their slow phase is probably an average of our middle and slow phases. The effect of adding Mg^{+2} at the F_{max} level has been examined for chloroplasts from wild-type barley [35] and from peas [34,36]. All three studies [34-35] recorded increases in yields which predominate in the slow part of the fluorescence. The slow fluorescence lifetime was found either to increase [34,35] or to remain constant [36]. Our results are in essential agreement with these results as well. These previously reported Mg^{+2} effects, however, are generally smaller, because a two-component analysis averages some of the middle phase into the slow phase.

Moya et al. [37], using the technique of phase fluorimetry with a one component analysis, measured the fluorescence lifetime as a function of intensity both in the presence and absence of Mg^{+2}. A plot of their average lifetime versus total yield showed that in the absence of Mg^{+2} the average lifetime is proportional to the total yield, and it increases from 0.4 ns in the all-open state to 1.0 ns in the all-closed state. A plot of an average lifetime calculated from our intensity-dependent data in the absence of Mg^{+2} (see Figs. 5 and 6) by the formula

$$\tau_{mean} = \sum_{i=1}^{3} \tau_i \phi_i \Big/ \sum_{i=1}^{3} \phi_i \qquad (2)$$

is identical to the results of Moya et al. [37] (plot not shown). The deceptively simple linear relation between average lifetime and total yield has influenced the conclusions of several authors.

Model for the origin of fluorescence

The results of two previous papers [22,23] led to the working model for the origin of fluorescence shown in Fig. 7. The basic structure of the model is derived from the tripartite model proposed by Butler [29]. The major addition is the explicit inclusion of the electron transfer processes involving pheophytin, which occur in the reaction center of Photosystem II [24-27]. Figure 7 illustrates the state where the Photosystem II reaction center is closed. Electron transfer beyond pheophytin is blocked, but P_{680}^{+} and Ph^{-} may undergo recombination resulting in either a fast "delayed" fluorescence [24-27], triplet formation or radiationless decay to the ground state. If the Photosystem II reaction center is open, electron transfer will continue beyond Q with a high probability and the "delayed" fluorescence will be quenched. A detailed kinetic analysis of this model [23] shows that the

total fluorescence which is emitted from the chlorophyll a/b proteins and the chlorophyll a_2 proteins can be described by a sum of three exponentials. The origin of each phase is a complex interaction among the rate contants in Fig. 7, but they can be qualitatively described as follows: 1) The fastest phase (about 100 ps) is kinetically controlled by the decay processes of the chlorophyll a_2 antenna, and these processes are dominated by the transfer rate, k_{T20}, from the chlorophyll a_2 antenna to the reaction center of Photosystem II. 2) The middle phase (300-750 ps) is kinetically controlled by the decay processes of the chlorophyll a/b light-harvesting antenna, and these processes are dominated by transfer rates from the chlorophyll a/b to the chlorophyll a_2 antenna or to Photosystem I ($k_{T32} + k_{T31}$). 3) The yield of the slow phase is controlled by the presence of Q^-, and its lifetime is affected by two factors. The first is the rate of charge recombination between P_{680}^+ and Ph^- and the second is the rate of fluorescence quenching from the chlorophyll antenna.

Interpretation of the Mg^{+2} Effect

By investigating the influence of Mg^{+2} on fluorescence induction, Henkin and Sauer [8] found two distinguishable effects of the ion on fluorescence. These effects saturated at 0.5 and 2.5 mM Mg^{+2}. Our results presented above also require at least two separate Mg^{+2} effects saturating at approximatly these same concentrations. This is most obviously apparent in Fig. 2 where the yield of the slow phase increases between 0.0 and 0.75 mM Mg^{+2} and then decreases until 2.0 mM Mg^{+2}. We will discuss these two effects of Mg^{+2} on our data separately.

1) Effects of high concentrations, $>$ 0.5 mM Mg^{+2}

In our previous paper [22] we reported a doubling of the slow phase

lifetime as Photosystem II reaction centers go from all open to all closed in the presence of Mg^{+2} (see Table I and Ref. [22] Fig. 3). We found it difficult to simulate this doubling without including connections between Photosystem II units. If some Photosystem II reaction centers are open and Photosystem II units are capable of intercommunication, excitation returning to the antenna after a charge recombination may get transfered to an open reaction center and become quenched. The net effect is a shortening of the slow phase lifetime in comparison with the behavior in the absence of intercommunication Thus, the intensity dependence of the slow phase lifetime can be used to examine the extent of energy transfer between Photosystem II units. In contrast with the results of the experiment in the presence of 5 mM Mg^{+2} [22] (see also Table I), we find that in the absence of Mg^{+2} the lifetime of the slow phase is essentially constant (Figs. 5 and 6). This suggests that Mg^{+2} is required for communication to occur between Photosystem II units. This conclusion is consistent with the results of fluorescence induction experiments in the presence and absence of Mg^{+2} [10-13].

From the above result, we expect a shortening of the lifetime and a decrease in yield of the slow phase as Mg^{+2} is added at the F_0 level, but no corresponding changes should occur at the F_{max} level. A lifetime shortening and yield decrease in the slow phase does not occur at the F_{max} level because there are no open reaction centers to serve as excitation quenchers, regardless of whether the reaction centers are interconnected. Inspection of Figs. 1-4 shows that in the range 0.5 mM to 2.0 mM Mg^{+2} there is indeed a decrease in the lifetimes and yields of the slow phase at the F_0 level, but there are no decreases at the F_{max} level. We believe, therefore, that the transition from separate Photosystem II units to an interconnected state occurs in this concentration range.

2) Effects of low concentrations, < 0.75 mM Mg^{+2}

Low concentrations of Mg^{+2} cause increases in the slow phase lifetimes at F_0 and F_{max} (see Figs. 1 and 3) which are completed by $[Mg^{+2}]$ = 0.75 mM. Two possibilities for this Mg^{+2} effect are either a decrease in a radiationless decay rate (k_D, k_d, and/or $k_{d'}$) or a decrease in the Photosystem II to Photosystem I spillover rate (k_{T31}).

A connection between the behavior of the two photosystems was proposed by Satoh, Strasser and Butler [38], who found an 18% increase in Photosystem I activity when Photosystem II is inhibited. Although the fractional increase of the Photosystem I rate is independent of Mg^{+2} concentration, deconvolution of absolute rates indicates that the spillover rate is about 50% less in the presence of Mg^{+2} than in its absence [29]. Such a decrease would lessen the Photosystem I contribution to the quenching of the excitation in the Photosystem II antenna. This could then account for the increases we observe in the slow phase lifetimes.

An alternative explanation for their results is a direct effect of Mg^{+2} on the electron transfer kinetics in Photosystem I. In this case, a Mg^{+2}-induced decrease in the radiationless decay rates (k_d and $k_{d'}$), as suggested by Melis and Ow [39], would satisfactorily explain our data. It is not possible to distinguish this effect from changes in spillover rates without parallel measurements of Photosystem I and Photosystem II activities.

A decrease in spillover from Photosystem II to I, k_{T31} in the model Fig. 7, should also be reflected in the middle phase lifetime. This is because, as mentioned above, the middle phase lifetime is approximately equal to $1/(k_{T32} + k_{T31})$; a decrease in k_{T31} should, therefore, result in an increase in the lifetime. At both F_0 and F_{max} in Table I and in Figs. 1 and 3, we see a

slight increase in the middle phase lifetime. The changes are usually small and completed by $[Mg^{+2}]$ = 0.75 mM. It is possible, therefore, that the lifetime increases in the middle and slow phases, both of which are completed at low Mg^{+2} concentrations, are due to changes in the Photosystem II to Photosystem I spillover rate.

3) Additional Mg^{+2} effects

Both mechanisms discussed above, by which low levels of Mg^{+2} induce an increase in the slow phase lifetime, should also cause a concomitant increase in the slow phase yield. However the yield of the slow phase at F_{max} continues to increase up to $[Mg^{+2}]$ = 2 mM, even though the lifetime effects are completed at a lower concentration of Mg^{+2}. A possible explanation for this would be an increase in the absorption cross-section of Photosystem II which would cause the yield of the slow phase to increase without increasing its lifetime. Thus, the Mg^{+2} effects occurring at higher Mg^{+2} levels ($[Mg^{+2}]$ = 1 to 2 mM) may result from a Mg^{+2}-induced increase in the absorption cross-section of Photosystem II. The conclusion that both spillover changes and absorption cross-section changes occur upon the addition of Mg^{+2} is in agreement with the work of Butler and Kitajima [7]. The yield of the middle phase in the F_0 experiment increases with $[Mg^{+2}]$ until about $[Mg^{+2}]$ = 2 mM. This effect is consistent with an increase of the absorption cross-section of Photosystem II. However, we did not observe an increase in the yield of the middle phase in the F_{max} experiment.

Although it is difficult to be precise about changes in the fast phase, we consistently observed a decrease in its lifetime upon the addition of Mg^{+2}. As Mg^{+2} increases communication between Photosystem II units, we postulate that it also strengthens the connection between the chlorophyll a_2 antenna and

the Photosystem II reaction center. A Mg^{+2}-induced increase in the rate constant k_{T20} would then account for the observed shortening of the fast phase.

Conclusion

The addition of Mg^{+2} to broken spinach chloroplasts isolated in the absence of Mg^{+2} has two effects which occur in different concentration ranges. As the Mg^{+2} concentration is increased from 0.0 to 0.75 mM, the rate constant for transfer between Photosystem II and Photosystem I decreases. It is possible that changes occurring in this concentration range may instead or in addition also affect the kinetics of electron transfer or the rate of radiationless decay in the Photosystem II reaction center. As the Mg^{+2} concentration is increased further to 2.0 mM, changes in the chlorophyll a/b light-harvesting antenna occur which both increase the absorption cross-section of Photosystem II and bring about communication between Photosystem II units. There is probably also an increase in the transfer rate between the chlorophyll a_2 antenna and the Photosystem II reaction center. Most of these correlations can be understood as a consequence of effects of Mg^{+2} on the organization of the chlorophyll a/b light-harvesting antenna. This conclusion is in good agreement with the results of Lieberman _et al_. [19] who concluded that the chlorophyll a/b light-harvesting antenna is required for Mg^{+2} effects.

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TABLE I

LIFETIMES AND RELATIVE YIELDS OF THE FLUORESCENCE FROM SPINACH CHLOROPLASTS

These data show the effects of adding 5 mM Mg^{+2} at both the F_0 and F_{max} levels. All results are from chloroplasts isolated from a single set of spinach leaves. The yield figures are normalized such that $\Sigma\phi$ at F_{max} in the presence of Mg^{+2} equals 100 (data from Ref. [22]).

	No Mg^{+2}			+5 mM Mg^{+2}		
Level	τ	ϕ	$\Sigma\phi$	τ	ϕ	$\Sigma\phi$
F_0	130	4.4		100	2.5	
	360	9.4	17	420	19.5	25
	1500	3.2		1200	3.0	
F_{max}	160	3.5		50	1.0	
	530	20.7	44	750	17.0	100
	1700	19.8		2000	82.0	

FIGURE LEGENDS

Fig. 1: Lifetimes of the components of the fluorescence decay in spinach chloroplasts at the F_0 level as a function of the concentration of Mg^{+2}.

Fig. 2: Total yield and yields of the components of the fluorescence decay in spinach chloroplasts at the F_0 level as a function of the concentration of Mg^{+2}. Yields are normalized such that $\Sigma\phi$ in the absence of Mg^{+2} equals 100.

Fig. 3: Lifetimes of the components of the fluorescence decay in spinach chloroplasts at the F_{max} level as a function of the concentration of Mg^{+2}.

Fig. 4: Total yield and yields of the components of the fluorescence decay in spinach chloroplasts at the F_{max} level as a function of the concentration of Mg^{+2}. Yields are normalized such that $\Sigma\phi$ in the absence of Mg^{+2} equals 100.

Fig. 5: Lifetimes of the components of the fluorescence decay in spinach chloroplasts isolated in the absence of Mg^{+2} as a function of laser intensity

Fig. 6: Total yield and yields of the components of the fluorescence decay in spinach chloroplasts isolated in the absence of Mg^{+2} as a function of laser intensity. Yields are normalized such that $\Sigma\phi$ at the highest laser intensity equals 100.

Fig. 7: Working model for the origin of fluoresence in chloroplasts. Symbols

are: k_F, rate constant for fluoresence; k_D, k_d, and k_d', rate constants for radiationless decay; k_{T32}, k_{T23}, k_{T31}, k_{T20}, and k_{T02}, rate constants for energy transfer; k_c, and k_r, rate constants for electron transfer; P680, reaction center of phoytosystem II; Ph, pheophytin; Q, secondary electron acceptor in photosystem II.

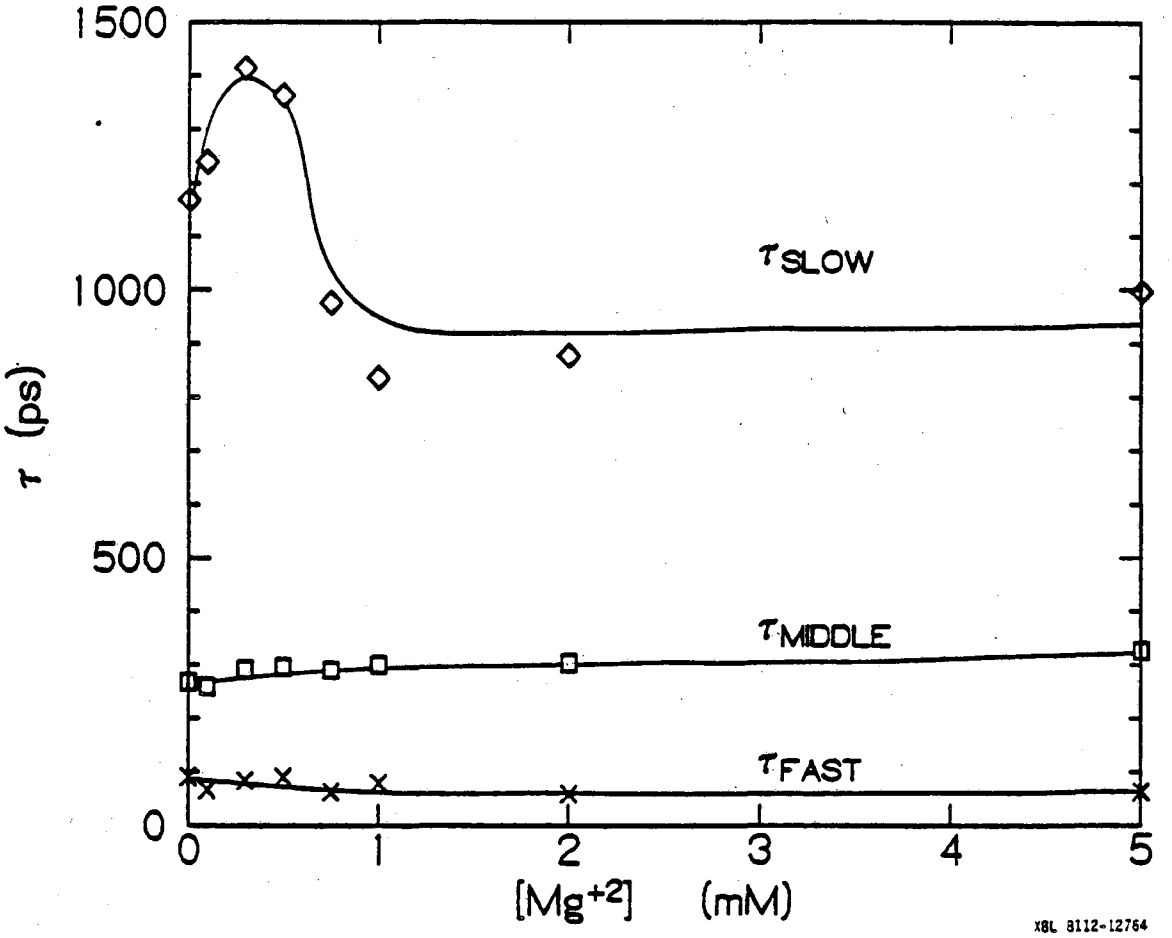

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Fig. 1

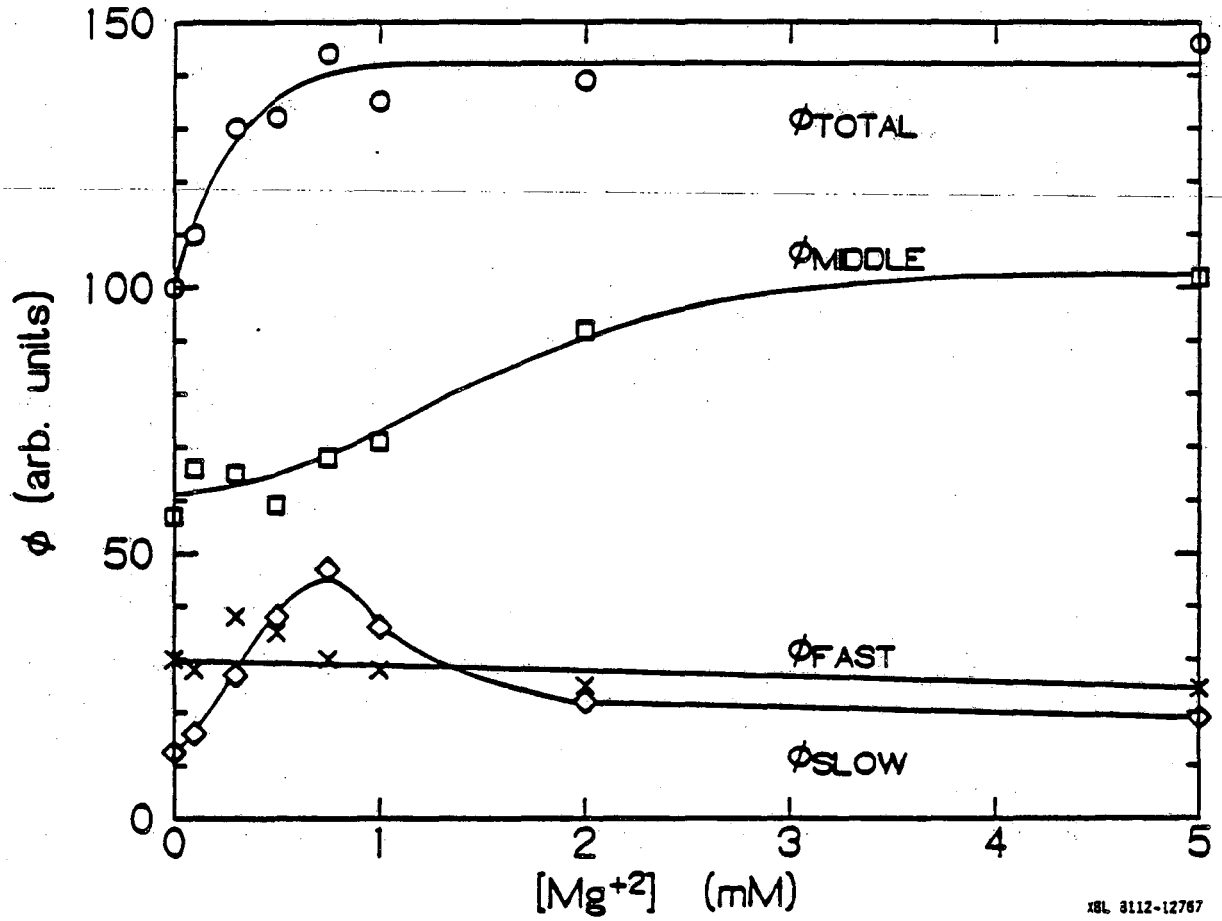

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Fig. 2

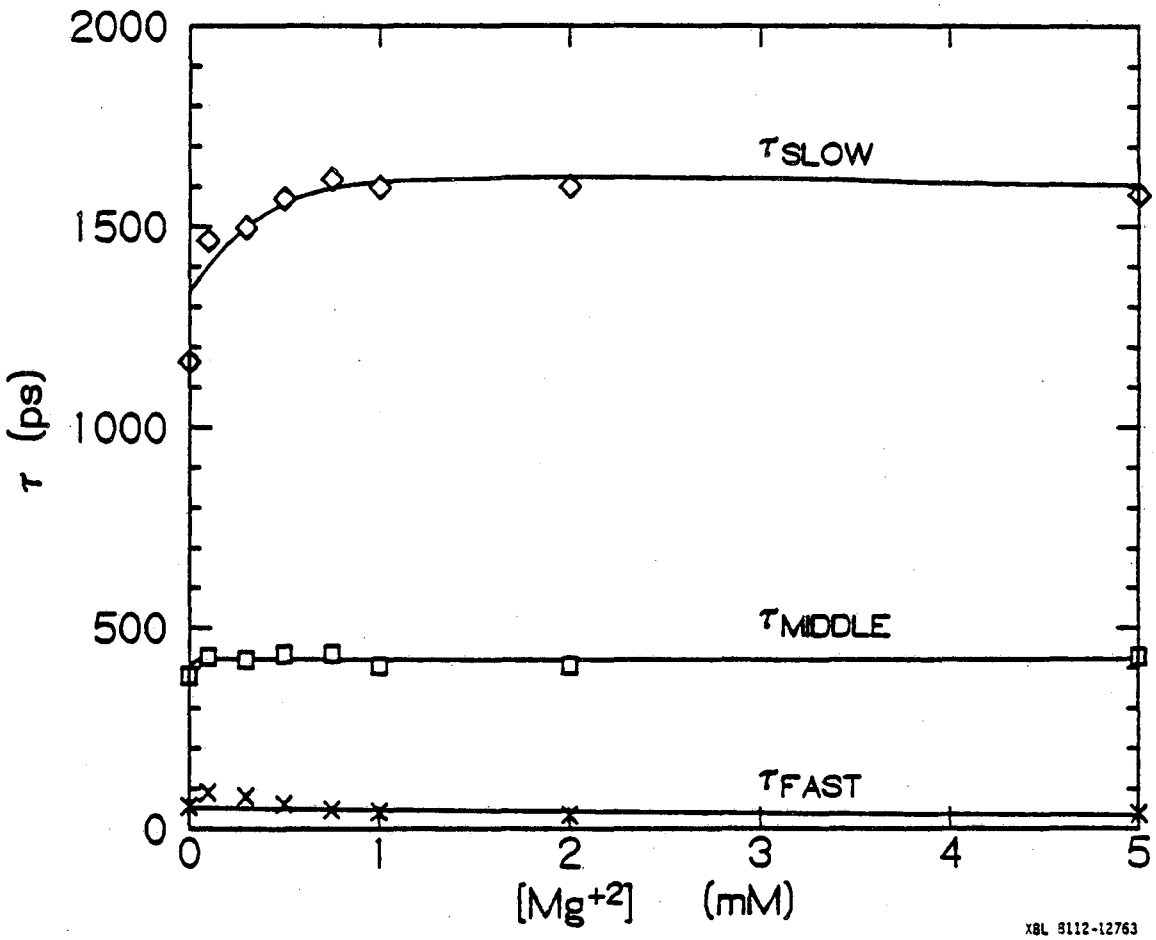

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Fig. 3

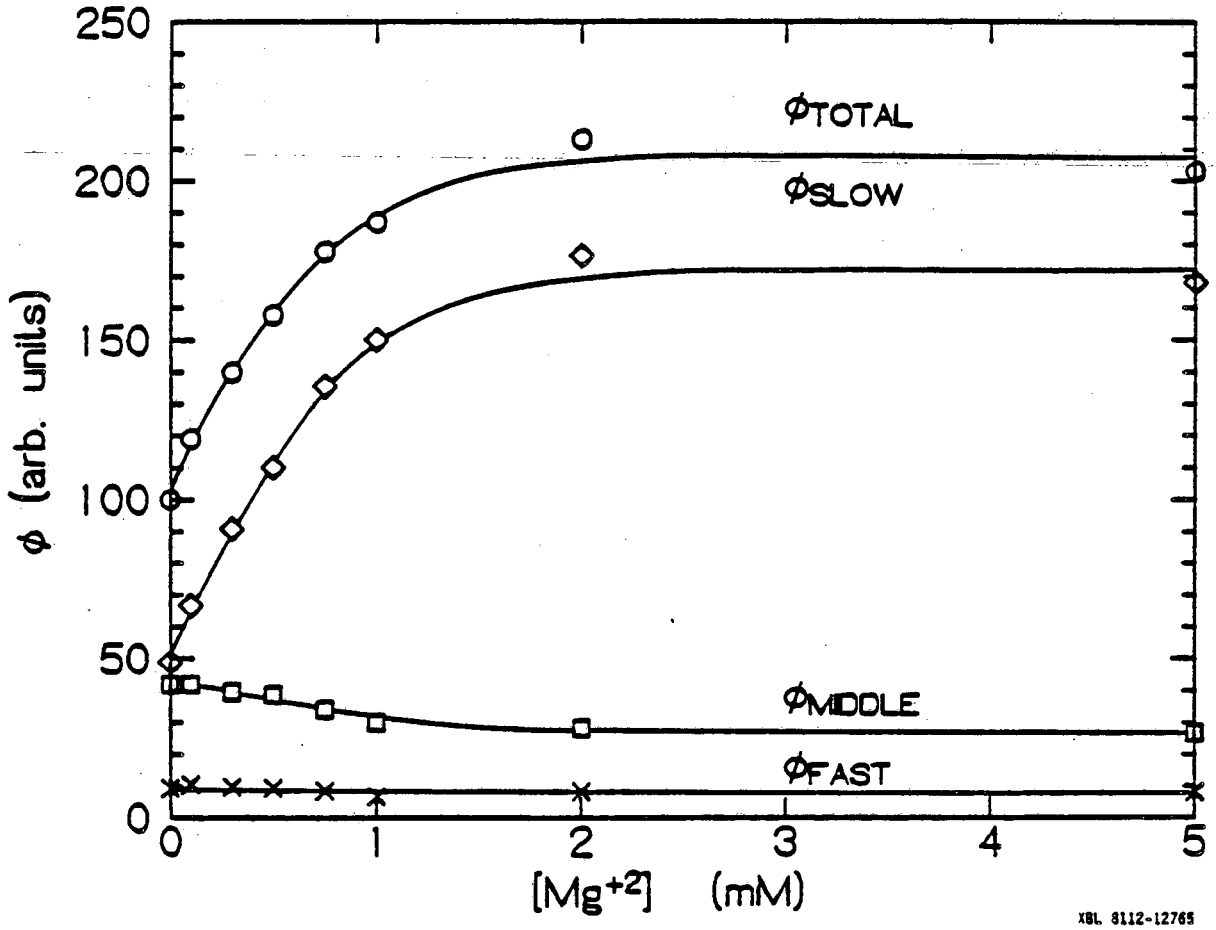

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Fig. 4

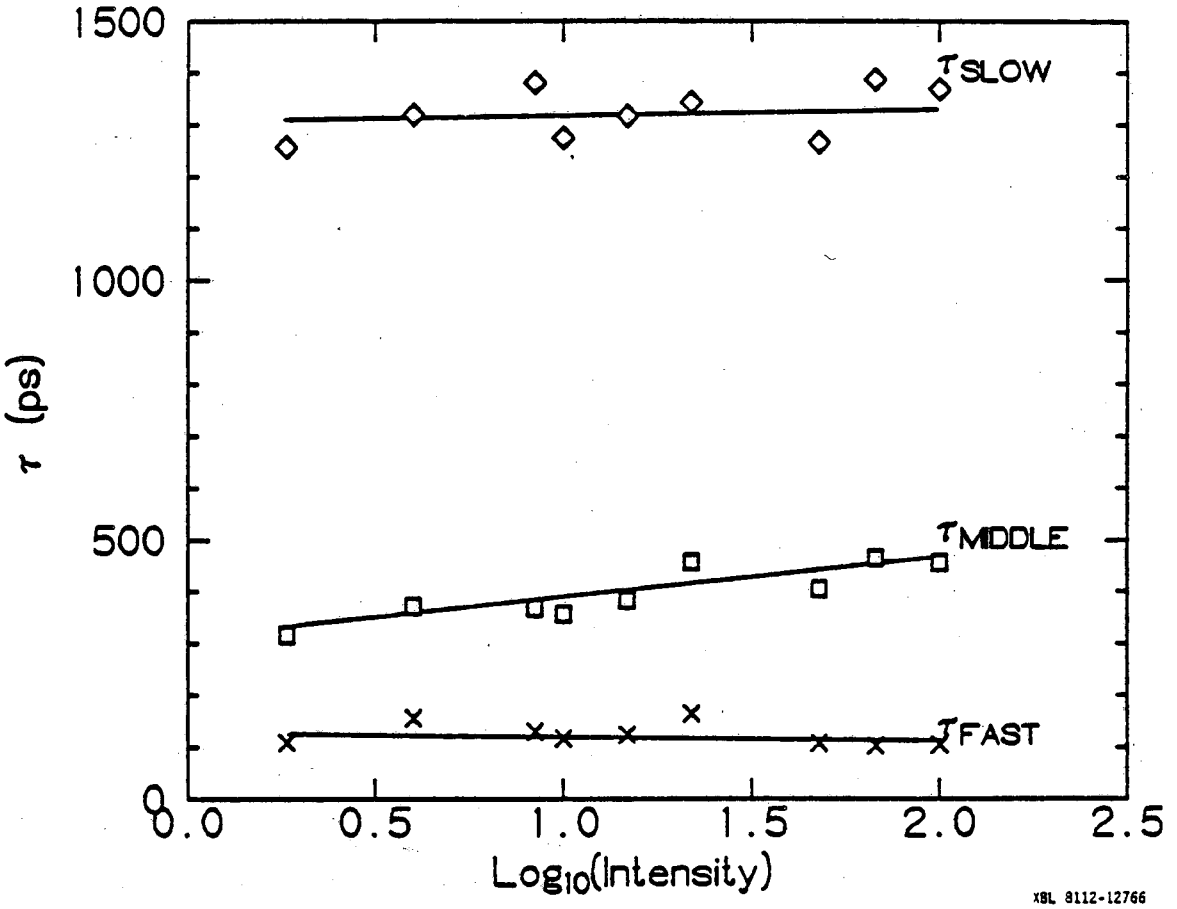

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Fig. 5

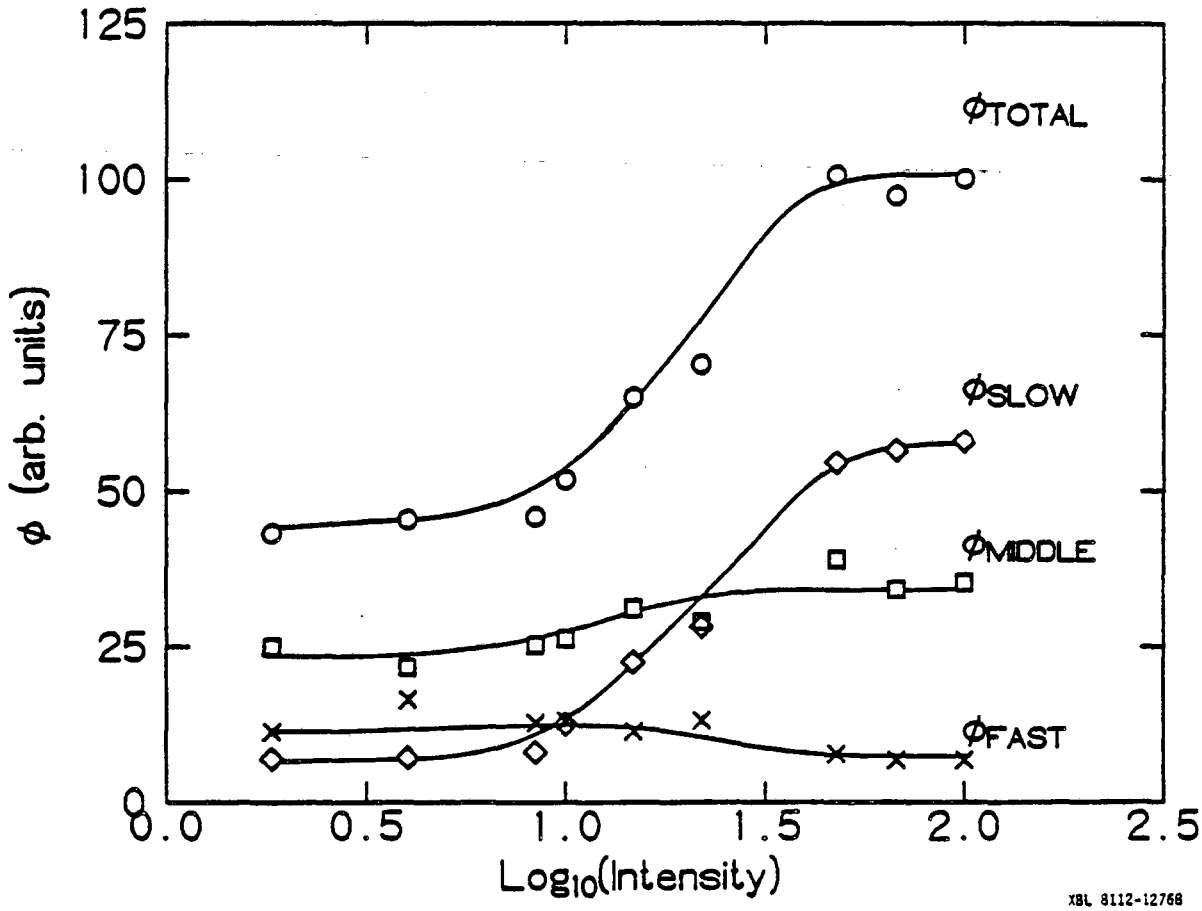

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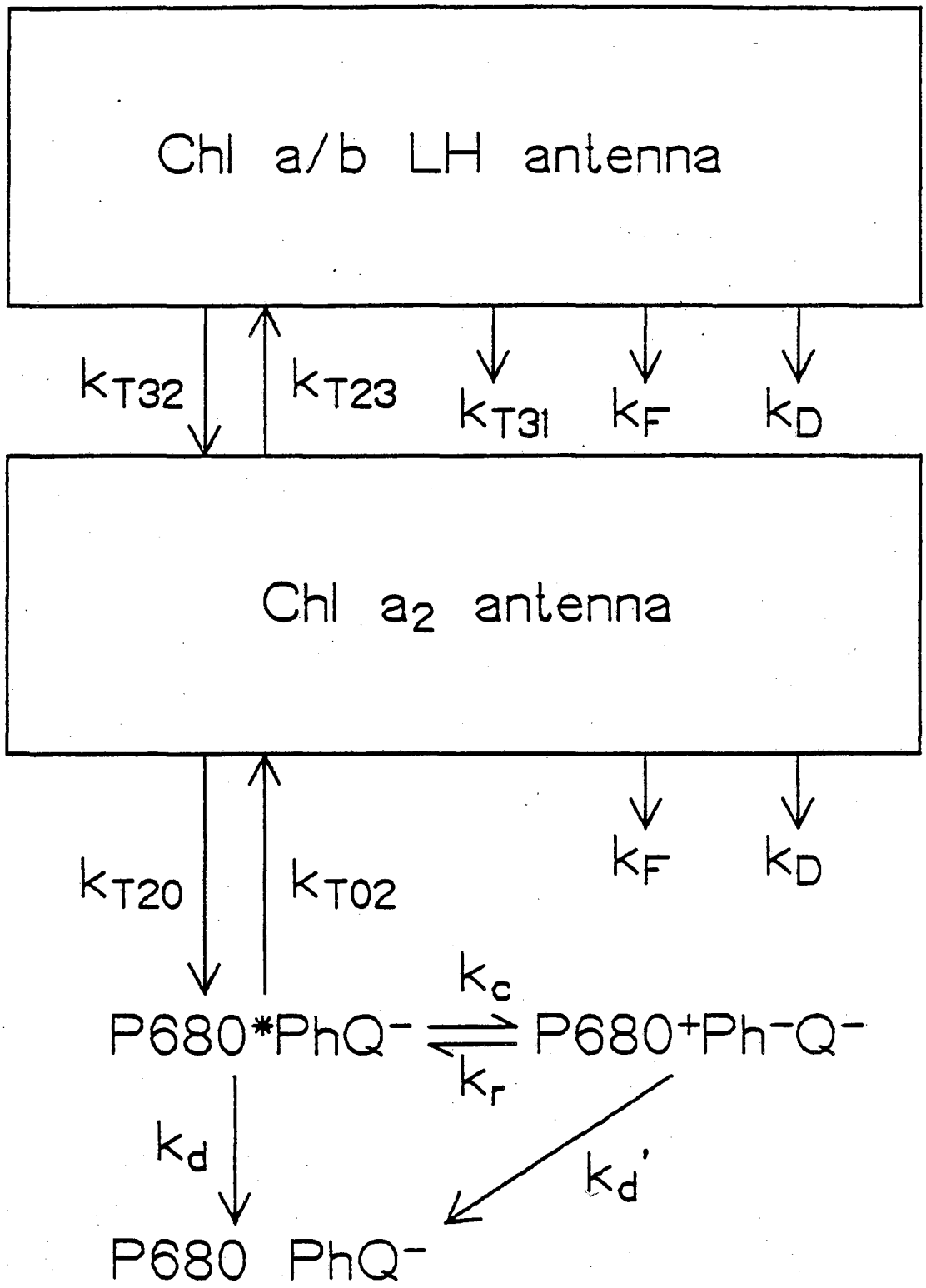
Fig. 6



XBL 8112-12771

Nairn et al.
Fig. 7

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