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THE SLOW COMPONENT OF PHOTOSYSTEM II LUMINESCENCE

A PROCESS WITH DISTRIBUTED RATE CONSTANT?

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The slow phase of Photosystem II luminescence, starting a few seconds after the end of illumination, has often been reported to follow second-order kinetics and was consequently ascribed to a biequimolecular recombination reaction, i.e., a bimolecular reaction with equal concentrations of reactants (holes and electrons). We argue that, given the constraints of the photosynthetic apparatus, the biequimolecular hypothesis is very unlikely. We propose instead that the reacting entities, inherently monomolecular, are continuously distributed over a range of rate constants, k. It is shown that (1) the k-distribution hypothesis results directly in second-order kinetic laws as reported in the literature, provided that the initial k distribution is exponential and (2) a broad class of k distributions (continuous, spanning a large interval of k values) tend to become an exponential function of k at long times. We have reinvestigated experimentally the slow phase of luminescence in *Chlorella* and spinach chloroplasts and submitted its decay to numerical analysis. The best description is given by $L(t) = L(0)(1 + k_1 t + k_2 t^2)^{-1}$. The corresponding initial distribution is of exponential type (with its maximum located close to but not at $k = 0$). This kinetic type is invariant under a number of experimental conditions (light intensity, uncouplers, etc.) which, in the frame of the biequimolecular hypothesis, would be expected to change the kinetic type. If the idea of the k distribution is correct, it means that luminescence in this time range reveals a continuum of kinetic states of unspecified origin. We may only conclude that the above states are stable for at least about 100 s and that they are not primarily associated with the O_2-evolving system.

Introduction

The relaxation kinetics of photoinduced signals in photosynthetic systems fall into two categories. The first is that of monophasic or first-order kinetics. Mechanistically, first order is necessarily equivalent to monomolecular. The frequent occurrence of the first category can be traced back to the property of the functional units in the photosynthetic apparatus in behaving as independent kinetic entities. This is especially true in the area of fast phenomena or when experimenting with simplified systems. The second category is more diverse, but may be broadly spoken of as that of polyphasic kinetics. An almost trivial instance is when the observed species is in a relatively fast equilibrium with one or several other species. Another possibility is currently receiving much attention: polyphasicity may reveal an underlying structural heterogeneity, i.e., several sub populations of the same species contribute to a given

Abbreviations: Tricine, *N*-tris(hydroxymethyl) methylglycine; Chl, Chlorophyll; PS, photosystem.

observable, but cannot be distinguished directly from each other (e.g., see the hypotheses in Refs. 1 and 2).

In this second category, luminescence holds a remarkable position: the slow phase of its decay — starting a few seconds after the end of illumination — can be described with good accuracy by second-order kinetic laws. Consequently, the phenomenon was ascribed to a bimolecular recombination process. There are several reports (reviewed in Ref. 3, see also a more recent instance reported in Ref. 4) that the luminescence intensity $L(t)$ in this time range is given by the expressions:

$$L(t) = L(0)(1 + kt)^{-1} \tag{1}$$

or in some cases

$$L(t) = L(0)(1 + kt)^{-2} \tag{2}$$

where k is an apparent rate constant and t the time of observation. For processes in homogeneous phase, Eqn. 1 is characteristic of biequimolecular reactions, i.e., $A + B \rightarrow C + D$ where the concentrations of the reactants A and B are equal. When this condition is relaxed ($[A] \neq [B]$), the reaction is simply bimolecular and is describd by a different expression. In both cases, however, it may be shown that an equivalent description is an infinite sum of exponential time functions. Hence, a bi(equi)molecular process is eminently polyphasic.

In the present context, the biequimolecular interpretation is not straightforward. We earlier argued [3] that it was difficult to think of the recombining electrons and holes (or equivalents) as free diffusing entities, which is what a bimolecular mechanism requires. In the long time range we are considering this objection is not so strong because the electrons are localized in the plastoquinone pool and, since the latter communicates with several PS II centers [5], recombination should have some bimolecular character. The puzzling point, however, is the observed biequimolecular character. We stress again that this implies that the electron and hole pools — i.e., the reduced quinone molecules and the O_2-evolving complexes with oxidized S states — have exactly the same size. This is very unlikely because, although the electron and hole equivalents are created photochemically in pairs, the mechanisms of their disposal in the two above pools — electron transfer to PS I and O_2 evolution — are not synchronized and do not run at the same speed in general. Therefore, equality of the two pools should be the exception rather than the rule.

We report here a reinvestigation of the slow phase of luminescence in *Chlorella* and in isolated spinach chloroplasts in order to test precisely its second-order behavior by numerical analysis. We propose a simple explanation of the apparent biequimolecular character of the decay, based on the idea of a continuous distribution of rate constants,and present several arguments against the bimolecular hypothesis.

Theoretical Section

The following hypotheses are made : (1) The experimental response arises from a kinetically heterogeneous population: the latter is characterized by a continuous distribution over the rate constant k which determines the transformation of its members. (2) For each member individually, the transformation is monomolecular. (3) Each member contributes equally to the observable monitoring the whole decay.

At any instant t, the fraction of the population $dC(t,k)$ in the range from k to $k + dk$ is given by:

$$dC(t,k) = F(t,k)dk \tag{3}$$

$F(t,k)$ being a time-dependent distribution function. By hypothesis 3, we are only concerned with the whole population; its concentration is found by integration of Eqn. (3) over k:

$$C(t) = \int_{k=0}^{\infty} F(t,k)dk \tag{4}$$

The process being inherently first order (hypothesis 2), the time dependence of $F(t,k)$ can be represented explicitly by:

$$F(t,k) = F(0,k)e^{-kt} \tag{5}$$

where $F(0,k)$ is the initial k distribution. Knowing the latter, the time course of the population decay

is given by substituting Eqn. 5 into Eqn. 4, i.e.

$$C(t)=\int_{k=0}^{\infty} F(0,k)e^{-kt}\,dk \tag{6}$$

Let us consider as a simple case a rectangular distribution from k_{min} to k_{max} (Fig. 1A): $F(0,k)=(k_{max}-k_{min})^{-1}$. In this case Eqn. 6 gives:

$$C(t)=\frac{\exp(-k_{min}t)-\exp(-k_{max}t)}{(k_{max}-k_{min})t} \tag{7}$$

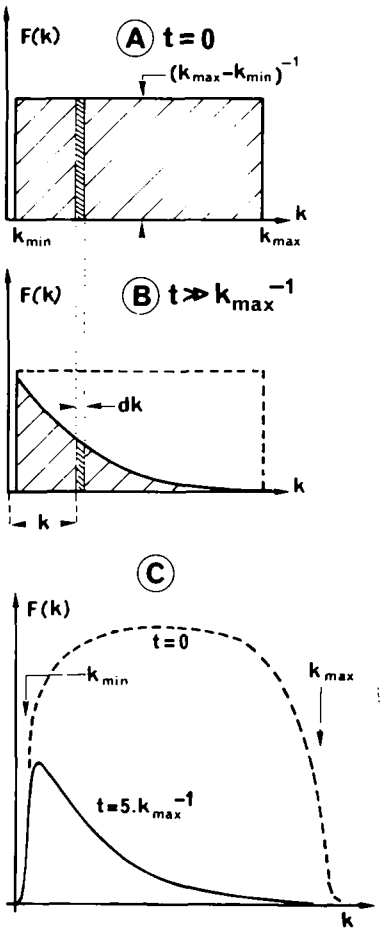

Fig. 1 Rectangular initial distribution of a rate constant and its evolution in time. The distribution function $F(k)$ defines the frequency of occurrence in the population of reacting species of members with a given rate constant, k (see text). (A) Initial rectangular distribution defined from k_{min} to k_{max}; the lightly shaded area is a measure of the initial concentration of the whole population ($=1$, with given value of $F(k)$). (B) At later time t, any elementary subpopulation ($k, k+dk$; heavy shading) is reduced in size compared to A by a factor $\exp(-kt)$; for $t \gg k_{max}^{-1}$, $F(k)$ is a pure exponential. (C) The taking on of an exponential form of an arbitrary distribution. At $t=0$, the distribution extends practically from k_{min} to k_{max}; it is continuous but otherwise arbitrary. For $t \gg k_{max}$, the distribution becomes exponential according to the mechanism outlined in B.

Assuming that k_{min} is very close to the origin on the k-axis and for $t \gg k_{max}^{-1}$, we see that $C(t) \approx t^{-1}$, a behavior very similar to the second-order biequimolecular type. Note also that, under the above conditions, the distribution becomes exponential (Fig. 1B). If the observation is started at t_0 when the distribution is already exponential, taking t_0 as a new time origin and normalizing $C(t)$ with $C(t_0)$, one obtains:

$$\bar{C}(t)=\frac{C(t_0+t)}{C(t_0)} \cong \frac{1}{1+k_{ap}t} \tag{8a}$$

$$k_{ap}=t_0^{-1} \tag{8b}$$

which is of the standard second-order biequimolecular type. The luminescence data presented below have been normalized and time scaled as in Eqn. 8a.

Two points should be noted. Firstly, a quasi-exponential form of the k distribution at the observation time t_0 and, consequently, appearance of biequimolecular-type kinetics are expected for any well behaved initial distribution, i.e., a continuous, smooth distribution over an extended k range such as $k_{max} \gg k_{min} \cong 0$. Fig. 1c illustrates this property: the generating initial distribution is of the above-defined type (but otherwise arbitrary); for $t \cong 5k_{max}^{-1}$, it is already well established in exponential form. This effect tends to obliterate the exact shape of the initial distribution, thus producing a kind of universal-type kinetics. Secondly, the basic Eqn. 6 shows that $C(t)$ is the Laplace transform (with respect to k) of $F(0,k)$. Therefore, from an actual k-distributed decay curve one may always recover, by an inverse Laplace transform, the generating distribution. The latter might not be of the simple types considered in the foregoing text. We shall make use of this property below.

Material and Methods

Algae (*Chlorella pyrenoidosa*) were grown and harvested as previously described [6]; they were resuspended in fresh growth medium for use. Chloroplasts (from *Spinacia oleracea*) were prepared according to Ref. 7 and kept at 0°C; they

were diluted in various buffered media (see legends). Tris washing for inactivating the O_2-emitting system was done as described in Ref. 8.

A 25 ml sample (usually 70–100 μg Chl/ml) was transferred to the luminescence set up consisting of a light-tight, thermostatically controlled and stirred glass vessel. White light from a microscope-type incandescence lamp (30 W) was focused onto a plastic light guide (2.5 mm diameter) fitted to the cover of the vessel. Similarly, a glass light guide (10 mm diameter) transmitted the luminescence light to a photomultiplier (EMI 9558). Two independent shutters in front of the actinic and of the observation-light guides were operated manually in sequence to protect the photomultiplier during the actinic illumination. In a typical experiment, the actinic light was given for 20 s, the lamp shutter was turned off and 5 s later the photomultiplier shutter was turned on for monitoring the slow luminescence phase. The amplified signal was stored in a digital recorder (TRACOR) and subsequently transferred to an HP-9845 computer for processing. Variation of the actinic light intensity was achieved by adjusting the lens diaphragm of the lamp housing; its relative magnitude was estimated from light scattered by the suspension viewed by the photomultiplier at low sensitivity.

Processing of the data included baseline correction, normalization to the first recorded point of the signal (i.e., at t_0) and unweighted least-squares fitting to several models. Decision as to the quality of fitting was always confirmed by visual inspection of the graphical display of the experimental and model curves (see Fig. 3). For better discrimination between models, some of the data were also analyzed in terms of a sum of exponentials by another program (see Ref. 9, in which a smoothing algorithm has been incorporated).

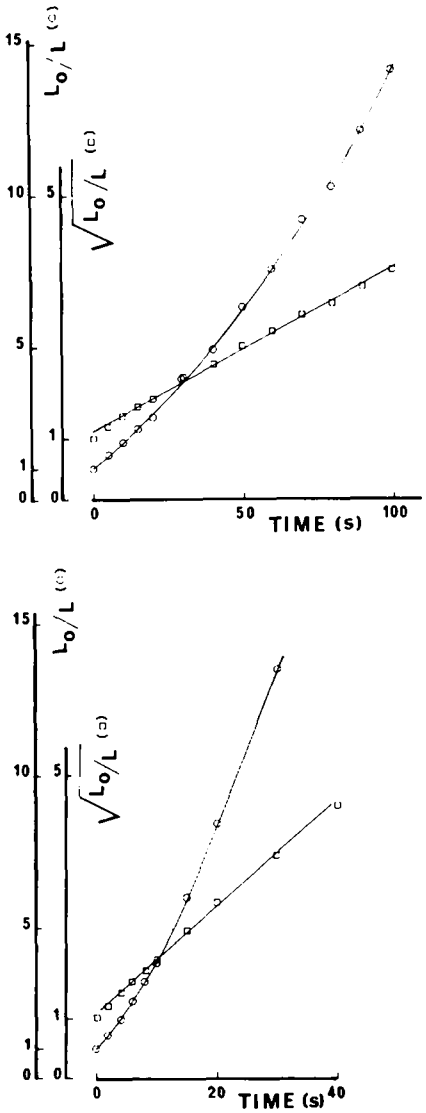

Fig. 2 Reciprocal plots of luminescence of chloroplasts according to the linear-1 model (L_0/L, ○) and the linear-1/2 model ($\sqrt{L_0/L}$ □). (A) Normal chloroplasts, 67 μg Chl/ml, 50 mM phosphate, pH 5.5, 1 mM $MgCl_2$. (B) Tris-washed chloroplasts, 29 μg Chl/ml, 50 mM Tricine, pH 7.5, 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$. Samples were illuminated during 20 s; luminescence was recorded at $t_0 = 5$ s after the end of illumination; L_0, luminescence intensity at t_0; temperature 20°C.

Results

General

We categorized earlier the kinetic laws, Eqns. 1 and 2, which were termed linear-1 and linear-1/2, respectively [3]. We shall adhere to this terminology. In the preliminary stage of the present work and on some occasions, we have tested the above kinetic laws by a direct graphical procedure. For example, Fig. 2 depicts reciprocal plots of L_0/L and $\sqrt{L_0/L}$ corresponding to Eqns 1 and 2, respectively. With this graphical test, we experienced some variations concerning the second-order laws;

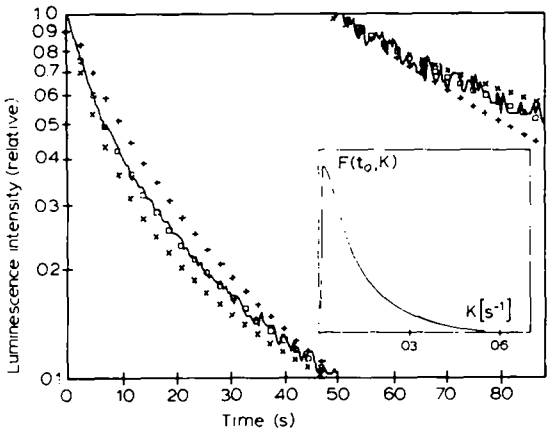

Fig. 3 Semilogarithmic plot of luminescence slow phase of *Chlorella*(————). Symbols represent least-squares fitting according to three models: (□) mixed type, (×) linear-1, and (+) linear-1/2, with root-mean-square errors $=3.9\cdot10^{-4}$, $2.1\cdot10^{-3}$ and $2.7\cdot10^{-3}$, respectively. 78 μg Chl/ml. Illumination during 20 s, luminescence was recorded at $t_0=5$ s after the end of illumination. Data scaled at t_0 and normalized to L_0 (luminescence intensity at t_0). Temperature 20°C. Inset: computed distribution $F(k)$ at $t_0=5$ s by inverse Laplace transform (see text) of the mixed type: $(1+k_1t+k_2t^2)^{-1}$; $k_1=0.124\ s^{-1}$ and $k_2=7.08\cdot10^{-4}\ s^{-2}$ are representative values. The curve is $(\alpha-\beta)^{-1}[\exp(-k/\alpha)-\exp(-k/\beta)]$; $\alpha=0.118\ s^{-1}$ and $\beta=0.006\ s^{-1}$.

fitting with the linear-1/2 law was often as good or even better than with the linear-1 law; in fact, in general, both fittings were not optimal. The curvature of the reciprocal plots suggested trying a mixed-type law:

$$L/L_0=\left(1+k_1t+k_2t^2\right)^{-1} \tag{9}$$

in essence generalizing the above two primary types. As Eqn. 9 cannot be tested graphically, we then resorted to computer-assisted least-squares fitting. A comparison of the three second-order laws (linear-1, linear-1/2 and mixed) is given in Fig. 3, showing that the mixed type provided an optimal description of the data. This result is general: over a total of 38 recordings, we have found that the mixed type was the best in 26 cases, as good as one of the other types in 10 cases and inferior in 2 cases.

We leave for the moment the question as to the meaning of the mixed-type law in the frame of k-distributed kinetics. On a computational ground, its superior performance may not be too surprising inasmuch as the mixed type has two free parameters to adjust (k_1 and k_2), whereas the linear-1 and linear-1/2 types have only one. Therefore, it might be that we are only approximating with the mixed type the true, unknown kinetic law. In an attempt to solve this problem, we also tried to fit our data to a sum of exponentials, this being the more general description of a decay curve. A sum of order $n(\Sigma\alpha_i\exp(-\lambda_i t),\ 1\leq i\leq n)$ involves $2n$ free parameters or actually $2n-1$ because of normalization of data at t_0. The rationale of this test is

TABLE I

COMPARISON OF FITTING OF DATA TO MIXED TYPE AND SUMS OF EXPONENTIALS

Chlorella: same conditions as for Fig. 3. k_1 and k_2 are the time coefficients in Eqn. 9; α_i and λ_i are the amplitudes and reciprocals of lifetimes, respectively, in a sum of exponentials: $\Sigma\alpha_i\exp(-\lambda_i t)$.

Model	Computed parameters		Root-mean-square error[a]
Mixed type (two parameters)	$k_1=0.115\ s^{-1}$ $k_2=1.4\cdot10^{-3}\ s^{-2}$		$2.3\cdot10^{-3}$
Two exponential (three parameters)	$\alpha_1=0.68$ $\alpha_2=0.32$	$\lambda_1=0.115\ s^{-1}$ $\lambda_2=0.022\ s^{-1}$	$2.4\cdot10^{-3}(2.9\cdot10^{-3})^b$
Three exponential (five parameters)	$\alpha_1=0.29$ $\alpha_2=0.48$ $\alpha_3=0.23$	$\lambda_1=0.278\ s^{-1}$ $\lambda_2=0.08\ s^{-1}$ $\lambda_3=0.019\ s^{-1}$	$2.1\cdot10^{-3}(3.1\cdot10^{-4})^b$

[a] Defined as $\sqrt{\Sigma\left(1-(Y_i/y_i)^2\right)/N}$; where $N=$number of points: $1\leq i\leq N$; Y_i, model function; y_i, experimental data.

[b] Same as footnote a but noise in y_i values has been reduced by computer smoothing.

TABLE II

EFFECT OF SEVERAL FACTORS ON KINETIC TYPE OF SLOW LUMINESCENCE DECAY IN *CHLORELLA* AND CHLOROPLASTS

Except where otherwise indicated, relative light intensity (I) = 100, temperature 20°C, 20 s illumination, data scaled and normalized at t_0 and L_0 (see Figs. 2 and 3). k_1 and k_2 are the time coefficients in Eqn. 9 (mixed type).

Material and No.	Conditions	t_0 (s)	Mixed type		
			Fit[a]	k_1 (s^{-1})	k_2 (s^{-2})
Chlorella					
1	$I = 100, L_0 = 338$	2	(+)	0.218	$4.2 \cdot 10^{-5}$
	$I = 50, L_0 = 230$	2	+	0.179	$1.2 \cdot 10^{-3}$
	$I = 33, L_0 = 218$	2	+	0.186	$6.4 \cdot 10^{-4}$
2	Ethanol: Control	5	+	0.179	$1.8 \cdot 10^{-3}$
	5% ethanol	5	(+)	0.377	$8.2 \cdot 10^{-4}$
3	Heating: 45°C, 4 min	5	+	0.183	$4.4 \cdot 10^{-3}$
4	Temperature (°C):				
	15	5	+	0.116	$7.3 \cdot 10^{-4}$
	20	5	+	0.140	$8.2 \cdot 10^{-4}$
	25	5	+	0.140	$1.0 \cdot 10^{-3}$
	30	5	+	0.155	$9.2 \cdot 10^{-4}$
Chloroplasts					
5	(a) Control[b]	8	(+)	0.114	$-1.2 \cdot 10^{-5}$[d]
	$+10^{-7}$ M nigericin	8	(+)	0.094	$-5.9 \cdot 10^{-5}$[d]
	(b) Control[c]	7	+	0.037	$3.1 \cdot 10^{-4}$
	+0.7 mM NH_4Cl	6	+	0.028	$1.4 \cdot 10^{-4}$
	(c) Control[c]	5	+	0.070	$2.2 \cdot 10^{-2}$
	$+10^{-5}$ M gramicidin D	5	+	0.035	$1.7 \cdot 10^{-2}$

[a] Quality of fit: +, mixed type better than linear-1 or linear-1/2 models; (+), mixed type as good as one of the other models.
[b] 50 mM phosphate, pH 5.5.
[c] 50 mM phosphate, pH 7.5, 0.1 M KCl.
[d] Negative values are not significant; in this case, the fit by the mixed type is no better than that by the linear-1 type.

that a two-exponential model (three parameters) not fitting the data better than the mixed type (two parameters) would constitute an issue favorable to the latter model (mixed type). Table I shows that this is the case; note that, for the same reason, the fact that a three-exponential model gives a still better fit (especially for the smoothed data) is not surprising.

In Table II, where the effect of several experimental conditions are compared (see below), we have included the computed values of k_1 and k_2 (mixed type). They exhibit some numerical scatter for the following reason. It is known that the apparent rate constant in a second-order process depends much on the zero time of observation (t_0, see Eqn. 8b). Because of manual operation of the shutters and discrete sampling of the signal (0.467 s per point, see Materials and Methods), t_0 was poorly defined in our experiments. Obviously, however, for the determination of the kinetic type, which was our goal, this uncertainty is entirely irrelevant.

Evidence against the biequimolecular hypothesis

We have studied the effect of several experimental conditions (detailed in Table II) in an attempt to modify the initial makeup of the process. We argue that if the biequimolecular hy-

pothesis were true, we should be able by suitable manipulations to alter independently the initial concentrations of reactants, thereby modifying the kinetic type of the decay (see Introduction), whereas, if the k-distributed hypothesis were true, we should at best by so doing change the shape of the initial generating distribution, with little effect on the decay type because its becoming exponential (see Theoretical Section). In Table II, we show that none of the treatments we tried were able to alter the decay type, which always stayed close to the mixed type. The treatments were as follows. (1) Light intensity (*Chlorella*, No. 1). This is likely to affect, at steady state, the PS II to PS I balance and therefore the ratio of electron to hole equivalents giving rise to luminescence. Note that the rate constants were independent of the initial amplitude L_0. A similar situation was already met in the second-order decay of Q^-, the reduced primary acceptor of PS II [10]. (2) Ethanol (*Chlorella*, No 2). This substance was shown to affect in parallel the 515 nm electrochromic change and the phosporylation activity [11]. (3) Uncouplers (chloro-plasts, No. 5). Addition of nigericin, NH_4^+ or gramicidin D was likewise intended to modify either component (ΔpH or $\Delta\psi$) of the proton-motive force. With the above two treatments, our purpose was to interfere with the electron-hole recombination, for instance, by modifying the intrathylakoid H^+ concentration, since the latter is known to control the luminescence process [12]. (4) Moderate thermal denaturation (*Chlorella*, No. 3). By this we were aiming at specifically modifying the concentration of hole equivalents in the O_2-evolving system [13]. Tris washing in chloroplasts [18] does the same thing even more drastically. It is seen, however (Fig. 2B), that Tris-washed chloroplasts exhibit the same second-order luminescence decay type as does the control (Fig. 2A). Additionally, Table II (No. 4) shows that temperature, although not altering the decay type, reveals a thermal activation on k_1 (apparent activation energy of approx. 0.4 eV). The k-distributed theory outlined above does not predict such an effect: k_1, approximating k in Eqn. 8b, should only depend on t_0. A more refined treatment discussed below provides an explanation.

Discussion and Conclusion

The idea of a distributed rate constant that we propose as an alternative to the classical interpretation of an apparent biequimolecular process in luminescence is by no means new. We have found that this hypothesis has been proposed long ago in other areas of research, notably solid-state phenomena and biological reactions at low temperature (see a review by Goldanskii [14] where this type of kinetics is elegantly termed polychromatic). Our interest in this idea was also renewed by the work of Ke et al. [15] who resorted to k-distributed kinetics to interpret the decay of P-700$^+$, the oxidized primary donor of PS I, at very low temperature.

There is, however, an important difference between our theoretical derivation and that of the above-quoted authors. Although the points of view are equivalent, we have explicitly considered a k distribution instead of an E_a distribution (E_a, activation energy), as the former authors did. The advantages of our phenomenological approach is that: (1) it yields by straightforward derivation the formal description of a second-order process that exactly mimicks a biequimolecular reaction, as observed particularly in the slow phase of luminescence; and (2) it also yields the important conclusion of a tendency towards becoming exponential of the k distribution at long times, which naturally explains why this particular type of kinetics is observed among slow phenomena. We take this property, as well as our inability to modify by any experimental conditioning the luminescence decay type, as strong arguments in favor of the hypothesis of k-distributed kinetics. The fact that the exact kinetic law which we have found to prevail (mixed type) is not the simple linear-1 or linear-1/2 type presents no difficulty. We noted in the foregoing text (see Theoretical Section) that $F(t_0,k)$ could be calculated from the $\bar{C}(t)$ data by performing an inverse Laplace transform. The result of such an operation is shown in Fig. 3 (inset). The computed distribution may be schematically described as an exponential with initial offset; this function is not a step function at $k = k_{min}$, as in Fig. 1B, but rises smoothly from zero to a finite value at some range around k_{min}. This looks a priori a very realistic situation; we therefore expect to find the mixed

type in most cases of k-distributed processes. An alternative derivation of the mixed type can be obtained by refining the approximation in Eqn. 8a *:

$$\bar{C}(t)=\left[1+\left(t_0^{-1}+k_{min}\right)t+t_0^{-1}k_{min}t^2\right]^{-1} \quad (10)$$

Eqn. 10 provides a reasonable explanation for the temperature dependency of $k_1(=t_0^{-1}+k_{min})$ which was noticed in Table II.

In the present study, we did not intend to reach beyond a formal characterization of k-distributed kinetics in a limited domain, luminescence. We simply believe that we are dealing with a continuum of kinetic states, be it with respect to conformation in a membrane complex or to distance between and mutual orientation of reactants, etc. Our results concerning the O_2-evolving system (thermal denaturation, Tris treatment) suggest that this system is not specifically involved in this spectrum of kinetic states. Another conclusion is that the putative distribution is stable or metastable, except for its own spontaneous evolution, in the time range of our study ($\cong$ 1–100 s). By this we mean that any member of the subpopulation $(k, k+\mathrm{d}k)$ at time t_1 will still belong to the same sub population at $t_2 > t_1$, unless it has already reacted and disappeared.

To substantiate the k-distribution hypothesis further, at least three questions arise. (1) How general is this description? To which responses of the photosynthetic apparatus can it be applied? We mentioned above the low-temperature reduction of P-700$^+$ [15]. Instances of second-order or strongly polyphasic kinetics have been reported in various areas; to mention a few cases: the decay of fluorescence yield at low temperature in the presence of DCMU (Ref. 16, where the role of the k distribution is alluded to), the deactivation of S_2 and S_3 states [17], etc. (2) Are there besides the polyphasicity of decays other manifestations of k distribution? We believe that the once well known hyperbolic light-saturation curve of photosynthesis [18] is a case in point. It may simply be shown that, given an exponential distribution of the photochemical rate constant, such a hyperbolic relation results. It is interesting that Jursinic [19], when analyzing light-saturation curves of flash-induced O_2 evolution in *Chlorella* and chloroplasts using Poisson statistics, had to assume a continuous exponential-like distribution of the antenna's cross-sections as a function of light intensity in order to fit his data best*. As another consequence of a photochemical k distribution, we may anticipate frequency-selection effects: by flashing light in the frequency range from k_{min} to k_{max} one should selectively activate a fraction of the population. Earlier results of ours in the field of millisecond luminescence [9] or of others dealing with the electrochromic decay rate constant and phosphorylation rate [20] hint at such a possibility. (3) In the case of electron transfers, what are the steps affected by k distribution and what are the mechanisms? Concerning the localization problem, we mentioned that this phenomenon arises in a PS II segment including presumably plastoquinone but excluding the O_2-evolving system. Further kinetic restriction of this segment is required. The problem of mechanism is probably more difficult: at room temperature, tunneling (e.g., see Ref. 14) is certainly out of consideration as a limiting factor and a dispersion of conformational states, or of mutual orientations and distances may not be easily and unequivocally studied with our present means of structural investigation.

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* In Eqn. 8a, $\exp(-k_{min}t)$ of Eqn. 7 is approximated by 1; in Eqn. 10, the better approximation $1-k_{min}t$ is used.

* We observe that his light-saturation curve (Fig. 1 in Ref. 19) closely follows a hyperbola.

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