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INTRODUCTION: ULTRAFAST REACTIONS IN PHOTOSYNTHESIS

The present issue of *Photochemistry and Photobiology* deals with the ultrafast reactions in the primary process of photosynthesis. The definition of what one calls "ultra-fast reactions" was left to the individual authors; it ranged from picoseconds (ps) to microseconds (μs). All of the papers were invited for this issue; these include those presented by the various speakers (to be cited at appropriate places) at the symposium on "*Primary Photoprocesses in Photosynthesis: Ultrafast Reactions*" held at the 5th annual meeting of the American Society for Photobiology, May 11–15, 1977, in San Juan, Puerto Rico. All the papers in this issue were reviewed by three authorities in the field, and the authors have incorporated as many of their suggestions as possible. I thank all the reviewers for their help and their names will be included in the annual list of the reviewers of the entire journal of *Photochemistry and Photobiology*.

I shall first present a general introduction to the primary photo-processes in photosynthesis so that the rest of the papers may be placed in the context of the overall process. This will be followed by a brief historical review of the ps fluorescence studies, the main theme of most of the papers.

Primary processes in photosynthesis

Photochemists and photobiologists have been struggling for a long time to understand the secrets of photosynthesis, the process by which green plants and photosynthetic bacteria convert solar energy into chemical energy. In green plants, the products of this conversion are food and O_2, and the reactants are CO_2, H_2O and photons. In photosynthetic bacteria, H_2O is replaced by other electron donors, and thus no O_2 is evolved.

Light energy is absorbed (within 10^{-15} s) by an assembly of pigment molecules, called the *photosynthetic unit* (Emerson and Arnold, 1932), and transferred, in all likelihood, by the Förster resonance mechanism, to specialized reaction center Chls within a nanosecond (ns). These reaction center molecules, termed "special pairs," are believed to contain two Chl *a* or B-Chl molecules joined by water molecules or other ligands (see e.g. a suggested model by Shipman *et al.*, 1976). Most of the pigments (whether Chl *a*, or other accessory pigments such as Chl *b*, carotenoids, or phycobilins) are called antenna or bulk pigments. The ratio of the antenna to the reaction center (or the trap) molecules is ~300:1 in green plants and ~50:1 in photosynthetic bacteria.

At the reaction center molecules, light energy or excitation energy (transferred to it from an antenna molecule) is conserved in the form of a charge separation with the formation of a Chl *a* (or BChl) cation and the reduction of a primary electron acceptor. In the bacterial system, it has been possible to isolate purified reaction center complexes, i.e. free from antenna pigments. With these preparations, the early ultrafast reactions of the reaction center BChl have been investigated (see various chapters in Clayton and Sistrom, 1978; Dutton *et al.*, this issue; Holten *et al.*, this issue). It appears that the following set of reactions occur:

$$(BChl)_2 \cdot I \cdot QFe \xrightarrow{h\nu} (BChl)_2^* \cdot I \cdot QFe \xrightarrow{<10\,ps} (BChl)_2^+ I^- \cdot QFe \xrightarrow{100\text{–}200\,ps} (BChl)_2^+ \cdot I \cdot Q^- Fe \xrightarrow[\text{cyt } c \rightarrow \text{cyt } c^+]{1\,\mu s\text{–}1\,ms} (BChl)_2 \cdot I \cdot Q^- Fe \xrightarrow[UQ \rightarrow UQ^-]{100\text{–}500\,\mu s} (BChl)_2 \cdot I \cdot QFe, \quad (1)$$

where $(BChl)_2$ is the "special pair", I is an intermediate (most probably, a bacteriopheophytin) QFe is a quinone-iron complex, (BChl)* is a singlet excited state of the special pair, $(BChl)^+_2$ is the bacteriochlorophyll cation, cyt *c* is cytochrome *c*, and UQ (ubiquinone) is a secondary two-electron acceptor (see e.g. Wraight, 1977). The numbers above the arrows indicate the halftimes of the reactions.

In green plants, there are two pigment systems and two light reactions (see the pioneering work of Emerson, 1958; various chapters in Govindjee, 1975; Barber, 1977; Trebst and Avron, 1977). Light reaction II leads to oxygen evolution and reduction of plastoquinones (PQ), and light reaction I leads to the reduction of nicotinamide adenine dinucleotide phosphate ($NADP^+$) and oxidation of plastoquinones. The bottleneck reaction in the rate of overall electron flow is in the reoxidation of PQ (~20 ms). As purified reaction center Chl *a* complexes have not yet been isolated, it has not been possible to study the events shown for bacterial photosynthesis in Eq. 1. I hope that this gap will ultimately be filled and will be the subject of a future symposium. However, we do know that charge separations in both pigment systems I and II occur in <20 ns (the limit of the instrument used; see Witt, 1975).

The suggested reactions for pigment system II (PSII) are (also see Wong *et al.*, this volume)

$$P680 \cdot Q \xrightarrow{h\nu} P680^{*}Q \xrightarrow{<20\,ns} P680^{+}Q^{-} \xrightarrow[Z \rightarrow Z^{+}]{30\,ns} P680Q^{-} \xrightarrow[R \rightarrow R^{-}]{100\text{–}600\,\mu s} P680Q, \qquad (2)$$

where P680 is the reaction center Chl *a* of PS II, which is, in all likelihood, also a Chl *a* dimer, and Q is a quinone molecule (van Gorkom, 1974; Knaff *et al.*, 1977). Whether or not pheophytin precedes Q remains to be seen (Klimov *et al.*, 1977). $P680^{+}$ is a Chl *a* cation (see e.g. Mathis *et al.*, 1976), Z is a secondary electron donor, and R is a secondary two-electron acceptor, also a quinone (see e.g. Velthuys and Amesz, 1974; Govindjee *et al.*, 1976; Pulles *et al.*, 1976). The time of ~30 ns is based on some new measurements on the decay of $P680^{+}$ to P680 by van Best and Mathis, 1978. The doubly reduced R^{2-} reduces the plastoquinone. The Z^{+} transfers its charge, we believe, to a manganese complex labeled M, which upon accumulation of four positive charges, oxidizes H_2O to molecular oxygen (see e.g. Diner and Joliot, 1977; Govindjee *et al.*, 1977).

The suggested reactions for pigment system I (PSI) are (see e.g. Sauer *et al.*, 1977):

$$P700 \cdot A_1 \cdot A_2 \cdot P430 \xrightarrow{h\nu} P700^{*} \cdot A_1 \cdot A_2 \cdot P430 \xrightarrow{<20\,ns} P700^{+} \cdot A_1^{-} \cdot A_2 \cdot P430 \longrightarrow P700^{+} \cdot A_1 \cdot A_2^{-} \cdot P430 \longrightarrow P700^{+} \cdot A_1 \cdot A_2 \cdot P430^{-} \xrightarrow[pc \rightarrow pc^{+}]{\sim 20\,\mu s} P700 \cdot A_1 \cdot A_2 \cdot P430^{-} \xrightarrow[Fd \rightarrow Fd^{-}]{\sim 5\,ms} P700 \cdot A_1 \cdot A_2 \cdot P430, \qquad (3)$$

where P700 is the reaction center Chl *a* dimer of PS I, A_1 and A_2 are, perhaps, some iron-containing centers, P430 is, most likely, a bound ferredoxin, and pc is a copper protein plastocyanin. Whether or not a pheophytin precedes A_1 remains to be seen. Also, the kinetics of these events remains to be fully explored. The reduced ferredoxin (Fd) reduces $NADP^{+}$ producing the reducing power (NADPH).

During the flow of electrons from H_2O to $NADP^{+}$, protons (H^{+}) are released to the interior of the membrane (thylakoids) on which photosynthesis occurs. This proton gradient, along with an associated membrane potential, could then be used to make adenosine triphosphate (ATP) (Junge, 1977). With ATP and NADPH available, CO_2 can be converted into food.

Chlorophyll a fluorescence lifetimes

The "ultrafast" reaction of photosynthesis is excitation energy transfer within the photosynthetic unit, and its trapping at the reaction center. These events precede the oxidation–reduction reactions discussed above. These times can be obtained by studying the lifetime of Chl *a* fluorescence under various conditions. Much work remains to be done in this field, but the present status of this area, as obtained by ps laser excitation, is presented here.

When I came to the University of Illinois at Urbana in 1956, Steve Brody, in Eugene Rabinowitch's group, had just completed his measurements on the lifetime (τ) of Chl *a* fluorescence *in vitro* and *in vivo* using a hydrogen flash lamp, developed mainly by Mr. J. Malmberg of the University of Illinois' Betatron Laboratory, which provided ~ 1 ns pulses (see Brody, 1957). Brody and Rabinowitch (1957) reported a τ of 5 ns for Chl *a* in ethyl ether and 1.6 ns in the green alga *Chlorella*. These were ultrafast events in photosynthesis. We soon learned of the independent measurements in A. N. Terenin's group in the U.S.S.R. of τ *in vivo* and *in vitro* by the phase delay method (see Dmetrievsky *et al.*, 1957). One of the conclusions of these studies was that the quantum yield (ϕ) of Chl α fluorescence, *in vivo*, calculated from $\tau = \tau_0 \phi$, where τ_0 is the intrinsic lifetime when all de-excitation is by fluorescence, was higher than the measured quantum yield of fluorescence suggesting the existence of some non-fluorescent Chl *a* complexes *in vivo* (later understood to be due to the weakly fluorescent so-called PS I). In most of the early experiments, with ns flashes, τ had to be extracted from the data by mathematical methods (see e.g. Tomita and Rabinowitch, 1962). Thus, it was a major excitement when Seibert *et al.* (1973) provided the first measurements on *in vivo* Chl with ps laser flashes. It was immediately obvious that the new τ values, obtained with sophisticated instruments, were 10–100 times lower than those obtained earlier even with the phase shift method which has the capability of measuring τ down to 0.1 ns. The research groups of G. Porter and J. Barber (in London), A. Campillo and S. Shapiro (at Los Alamos), A. Rubin (in Moscow) and R. Alfano (in New York) also reported, in several papers, low lifetimes of fluorescence which did not agree with the early measurements with ns flashes or with the phase method. Mauzerall (1976) showed, using 7 ns flashes, that ϕ dropped with increasing in-

tensities and suggested that this may be the reason why low τ's were obtained by the ps method as these involved the use of very high intensities. Campillo *et al.* (1976) showed, for the first time, that the τ indeed increased as the intensity of ps laser flashes were decreased. Another group, which included N. Geacintov (of New York), J. Breton (of France) and C. Swenberg (also of New York) began to provide an understanding of the molecular process which led to these decreases in τ and ϕ.

It was with the above background information that a symposium on ultrafast reactions was put together with the hope of learning the latest advancements in the field and to present a forum for a direct dialogue between the various researchers in the field. In my judgment, the dialogue was successful because each speaker seeemed to appreciate each other's viewpoints at the end of the symposium. Campillo and Shapiro (this volume) have provided a very thorough review of the entire field beginning from the early ns work and have provided a summary of their latest work. Mauzerall (this volume) presents his views on how he can understand the decrease in ϕ and τ due to multiple excitations of the photosynthetic unit. Swenberg *et al.* (this volume) provide an analysis of decrease in ϕ and τ at higher intensities in a theory involving singlet–singlet annhilation processes in single ps pulses and singlet–triplet annihilation processes in a train of multiple pulses. Pellegrino *et al.* (this volume) review their research with the optical Kerr method starting with the early work of Seibert *et al.* To complete the arena, I have also invited the group of Porter (see Tredwell *et al.*, this volume), and of Rubin (this volume) to present summaries of their results and views. Also, DeVault and Kung (this volume) were invited to summarize their latest observation of a fluorescence from excited states higher than the first singlet excited state; this observation provides further evidence for the non-linear effects at high intensities and seems to be the first demonstration of "blue" fluorescence when *in vivo* systems are excited with high intensity "red" light. It is now well established that low intensity single ps pulses provide τ's in the same range as that observed by early observers (see e.g. Mar *et al.*, 1972; Briantais *et al.*, 1972) and, high light intensity effects are beginning to provide information on the topology of the photosynthetic pigment systems. Kinetics of fluorescence, with ps lasers, remains to be investigated during the transition from the open (O) to the closed (P) reaction center.

Brody and Rabinowitch (1957) (also see Brody, 1960; Tomita and Rabinowitch, 1962) had provided the first measurements on the times of energy transfer from one pigment to the other in the red alga *Porphyridium cruentum* based on the rise of Chl *a* fluorescence when the samples were excited in the accessory pigments. The time resolution was poor and no kinetic information could be obtained. The ps method provides the only means to obtain information on the kinetics of energy transfer as the decay of donor fluorescence can be measured in parallel with the rise in acceptor fluorescence. It would be very instructive to study energy transfer from phycoerythrin to phycocyanin to allophycocyanin in isolated phycobilisomes from red algae (Gantt *et al.*, 1976) and from Chl *b* to Chl *a* in light-harvesting pigment protein complex from higher plants. Such an investigation has already begun in intact cells of red algae and phycobilisomes in Porter's group in collaboration with Barber's group at the Imperial College (London) (Porter *et al.*, 1978; Searle *et al.*, 1978). This is an expanding field, and one hopes to learn about the mechanism(s) of energy transfer and migration among the various photosynthetic systems by the use of ps laser techniques.

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