

PHOTO-INITIATED ION FORMATION FROM OCTAETHYL-PORPHYRIN AND ITS ZINC CHELATE AS A MODEL FOR ELECTRON TRANSFER IN REACTION CENTERS

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ABSTRACT Ion formation from the reaction of triplet (T) and ground state (P) octaethyl-porphyrin (OEP) and zinc octaethyl porphyrin (ZnOEP) and the corresponding cross-reactions have been measured in dry acetonitrile. A uniquely sensitive and fast conductance apparatus and a pulsed dye laser allowed the measurements to be made at the necessarily very low concentrations of T. The homogeneous reaction of T (ZnOEP) and P (ZnOEP) occurs with rate constant $k_1 = 2.0 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ and an ion yield of 67%. The similar homogeneous reaction of OEP has $k_2 = 1.3 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ but an ion yield of only 3%. The cross-reaction of T (OEP) with P (ZnOEP) has $k_3 = 1.5 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ and an ion yield of 27%, while the inverse cross-reaction of T (ZnOEP) with P (OEP) has $k_4 = 3 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ and an ion yield of 20%. Thus, the rate constants are only slightly affected but the yields are sensitive to the porphyrin. The possible formation of the heterogeneous ions $\text{ZnOEP}^+ + \text{OEP}^-$, thermodynamically favored by 0.3 V over the homogeneous ions, has little influence on the observed yields. The data are explained by electron transfer and Coulomb field-electron spin-controlled escape of the initial ion-pair.

INTRODUCTION

Much progress has been made in our understanding of the primary photochemical reactions of bacterial photosynthesis (1). The availability of purified reaction centers has allowed the elucidation of the path of the electron transfer reactions and their description at the level of quantum mechanics. Thus, after excitation, the electron leaves the donor, a dimer of bacteriochlorophyll, in $<10^{-11}$ s and remains for 10^{-10} s on a bacteriopheophytin (2), before passing on to a ubiquinone molecule. The bacteriopheophytin anion acts as a bridge to the more stable quinone anion, and thus contributes a high-energy path through the barrier between the quinone acceptor and bacteriochlorophyll dimer donor. This barrier amounts to 30 ms at low temperatures (3). The thermodynamically highly favored reverse electron transfer reaction to the ground state is slowed sufficiently to allow useful work to be obtained by the biochemical electron transfer machinery. The usefulness of a primary charge transfer between the pigment molecules was pointed out by Kamen (4). The possibility of favoring this reaction by use of the metalloporphyrin-free base porphyrin to bridge the gap

was also anticipated (5). Our work has shown that even in free solution well over 50% of the photon energy can be stored (for up to ~ 0.1 s) in the reactive free radicals after electron transfer reactions to the free base porphyrins or from the metalloporphyrins (6-7). These reactions have quantum yields near unity and so are very efficient. However, evidence for ion formation in the direct reaction of porphyrin and metalloporphyrin was lacking. Using an extremely fast and sensitive conduction apparatus (8), we have now obtained direct kinetic rate and yield measurements on precisely these reactions.

METHODS

A detailed description of the conduction apparatus (8) and its application to the study of the photochemistry of lumiflavin (9) have been published. The apparatus detects changes of 10^{-9} M of photogenerated univalent ions with a time constant of $0.3 \mu\text{s}$ and changes of 10^{-12} M at longer times. The anaerobic conductivity cell is operated by a voltage clamp in a positive pulse, negative pulse mode which assures long-term stability of the conductance. Gating and timing circuits allow the photogenerated conductance change to be measured after pulsing transients have relaxed. A high-speed digitizer and signal averager allow efficient data collection. The results were plotted by an x, y recorder and analyzed "by hand."

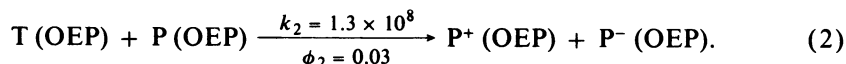
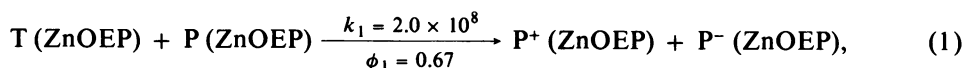
The light pulse is obtained either directly from a nitrogen laser emitting 7-ns full width at half maximum pulses, or via a dye laser tuned with an interference filter. The beam was spatially filtered and expanded to achieve homogeneous illumination ($<5\%$ variation) of the cell. Direct photoeffects at the platinum electrodes were always negligible. Absolute energies were determined at the position of the conductance cell by a YSI model 65 radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). The conductance cell was absolutely calibrated with tetraethyl ammonium perchlorate.

The ion yield is defined in an absolute sense: ions formed per quantum absorbed. The former is obtained from the instrument sensitivity, X : $X = 1.8 \times 10^{-3} \cdot V \cdot s \cdot \lambda$, in millivolts per nanomolar concentration, where V is the polarizing voltage (typically 10 V), s is the cell constant for homogeneous illumination (2.14 cm), and λ the ion equivalent conductance. The latter value is assumed equal for P^+ and P^- and was calculated, with minor correction for oblateness, from the Stokes, Einstein, and Walden relations: $35 \text{ mho cm}^2 \text{ mol}^{-1}$ in acetonitrile. These relations are accurate for large ions immersed in small solvent molecules. The quanta absorbed were obtained from the absolute light intensity and the absorption of the solution at that wavelength. The absolute value of the ion yields thus defined may be in error by $\pm 10\%$ because of the systematic errors. However, the relative error, important in the present context, is far less, and is determined by the signal-to-noise ratio.

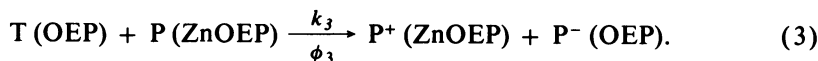
Since oxygen quenches the triplet state at encounter-limited rates, and the reaction studied was 100 times slower, the requirement $O_2 < 0.01 P_0$ was achieved by vigorous purging with O_2 -free (<0.5 ppm) N_2 . A typical concentration of O_2 was 10^{-9} M. Since the triplet-triplet reaction is also encounter-limited, it was necessary to use extremely weak excitation (saturation parameter, $1 - e^{-\sigma I}$, $< 10^{-3}$). Thus the ion concentration was 10^{-9} - 10^{-11} M. This conductance measurement is the only feasible method of detecting such low concentrations of ions. It appears that most, if not all, data on porphyrin and chlorophyll photoreactions in the literature are highly contaminated with the voracious triplet-triplet reaction. The solvent was of spectroscopic quality and was doubly distilled, first from P_2O_5 , then from highly activated molecular sieves directly into the reaction cell for several flushings before mixing in the small amount pigment. The background conductance typically corresponded to $< 10^{-6}$ M ions.

RESULTS

The determination of the rate constants and yields for the homogeneous reactions, i.e., T (OEP) + P (OEP) and T (ZnOEP) + P (ZnOEP), was relatively straightforward since the yields were independent of excitation wavelength. OEP is octaethylporphyrin, ZnOEP is its zinc chelate, T is the triplet state, and P the ground state of the pigment. Since P was in large excess ($\sim 5 \times 10^{-6}$ M) over T ($< 10^{-9}$ M) the kinetics of formation of (uncorrelated) ions was accurately first-order. Although the ion recombination rate is encounter-limited, the very low concentration of ions made the recombination rate negligible in the ion formation time range (> 0.1 s vs. 0.5 ms). The order of the reaction was determined by varying the pulse energy (I) and the pigment concentration (P). It was accurately first-order in each and thus the second-order rate constant could be obtained from the measured pseudo first-order ion formation constant. Details will be given in a complete report of the photoreactions of the pigment (P + T, T + T, and T*) in a wide variety of solvents.¹ Evidence for electron tunneling through some 7 Å of solvent and for the striking effect of electron spin states (ion yield of P + T > T + T) was obtained (10). The results of these measurements are that the free base porphyrin reacts at about one-half the rate of the zinc porphyrin, but with a far smaller yield (ϕ) of ions:



The use of a rhodamine B dye laser, emitting at 618 nm, allowed the selective excitation of the free base porphyrin in a mixture of OEP and ZnOEP:



The product ions are written in their thermodynamically favored form, but we have no independent proof of this assumption. In any case, kinetic and yield analysis are not dependent on this assumption. The rate constant and yield are calculated from the observed values of the pseudo first-order formation rate constant, k , and the observed ion concentration C :

$$k \text{ (obs)} = k_2 \text{P (OEP)} + k_3 \text{P (ZnOEP)}, \quad (3a)$$

$$C \text{ (obs, mixture)} / C \text{ (obs, OEP)} = (k_2 \phi_2 + k_3 \phi_3) / (k_2 + k_3) \phi_2. \quad (3b)$$

The data and results are listed in Table I. The rate constant and yield for reaction 3 are intermediate to that of reactions 1 and 2.

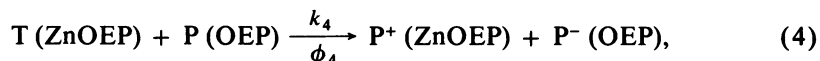
¹Ballard, S. G., and D. Mauzerall. 1978. Manuscript in preparation.

TABLE I
DATA OF REACTION: T (OEP + P (ZnOEP) → P⁺ (ZnOEP) + P⁻ (OEP)

Pigment	<i>k</i> (obs)	<i>C</i> (obs)	<i>k</i> ₃	φ ₃
5 μM OEP	<i>s</i> ⁻¹ × 10 ⁴	nM	<i>M</i> ⁻¹ <i>s</i> ⁻¹ × 10 ⁻⁸	
	6.3	0.059		
5 μM OEP + 5 μM ZnOEP	13.9	0.32	1.5	0.27

Excitation wavelength, 618 nm.

The problem of determining the characteristic parameters for the second heterogeneous reaction:



is more complicated, since there is no region of the ZnOEP absorption spectrum where OEP absorption is negligible. Consequently, all four reactions proceed simultaneously.

We write the general equation for the concentration of ions as a function of time following impulse excitation:

$$C = T_Z^0(A'/A)[1 - \exp(-At)] + T_O^0(B'/B)[1 - \exp(-Bt)], \quad (5)$$

$$A = k_1 P_Z + k_4 P_O$$

$$B = k_2 P_O + k_3 P_Z,$$

$$A' = \phi'_1 k_1 P_Z + \phi'_4 k_4 P_O$$

$$B' = \phi'_2 k_2 P_O + \phi'_3 k_3 P_Z,$$

$$T_Z^0 = F_Z I_{\text{abs}} \theta_Z$$

$$T_O^0 = F_O I_{\text{abs}} \theta_O,$$

$$F_Z = \epsilon_Z P_Z / a$$

$$F_O = \epsilon_O P_O / a,$$

$$I_{\text{abs}} = I_0 [1 - \exp(-al)]$$

$$a = \epsilon_Z P_Z + \epsilon_O P_O.$$

The derivation is straightforward from the definitions. The quantum yield of triplet

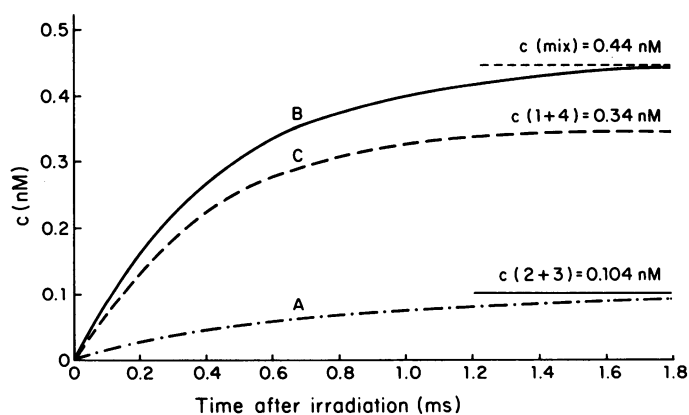


FIGURE 1 The increase in ion concentration (*c*) versus time for the mixed reactions of ZnOEP + OEP after pulse illumination (curve *B*). Curve *A* is the calculated component from reactions 2 and 3. Curve *C* is the difference of curve *B* and curve *A*, caused by reactions 1 and 4.

TABLE II
DATA OF REACTION: T (ZnOEP) + P(OEP) → P⁺ (ZnOEP) + P⁻ (OEP)

	OEP	ZnOEP
P_i	$5 \times 10^{-6} \text{ M}$	$5 \times 10^{-6} \text{ M}$
$\epsilon_i(337)$	3.09×10^4	2.32×10^4
F_i	0.571	0.429
I_0		7.68 nM
C_{tot}		0.444 nM
C_i	0.104 nM	0.340 nM
	$B = 1.39 \times 10^3 \text{ s}^{-1}$	$A = 2.48 \times 10^3 \text{ s}^{-1}$
	$k_4 = 3.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$	
	$\phi_4 = 0.21$	

formation for ZnOEP is θ_Z and for OEP is θ_O , and the ion yield from the triplet for the specific reactions 1–4, is ϕ'_i . These factors are combined in the previous definition of ion yield, i.e., $\phi_1 = \theta_Z \phi'_1$, $\phi_2 = \theta_O \phi'_2$, $\phi_3 = \theta_O \phi'_3$ and $\phi_4 = \theta_Z \phi'_4$. T^0 refers to the initial triplet concentration, ϵ to the absorbancy index, l to the light path length, and I_0 is the einsteins of photons in a pulse passing through the cell volume.

Now the second term on the right of Eqs. 5 (ion formation from $T_O = C_O$) contains only known parameters. It may thus be computed (curve A, Fig. 1) and subtracted from the measured ion formation (curve B, Fig. 1). The resulting curve (C, Fig. 1) is just the first term on the right of Eq. 5 (ion formation from $T_Z = C_Z$) and thus is reduced to the previous case for the calculation of k_4 and $\theta_Z \phi'_4 = \phi_4$. The parameters of the calculation are given in Table II, and the results are summarized in Table III. The errors are best estimates of relative errors.

DISCUSSION

In principle the variation in the ion yield (Table III) could be caused by changes in the yield of triplet state (θ_O, θ_Z) or in the yield of ions (ϕ'_i). However, the yield of triplets for porphyrins is very high and quite constant. The triplet yield of etioporphyrin was 0.83 and of zinc etioporphyrin was 0.94 when measured by Gradyusko and Tsvirko (11, 12) under conditions similar to ours.

The redox properties of porphyrins have been well summarized by Fuhrhop (13).

TABLE III
SUMMARY OF RATE CONSTANTS AND ION YIELDS OF
T-P REACTIONS IN ACETONITRILE

Reaction	$M^{-1} s^{-1} \times 10^{-8}$	ϕ_i
1. T (ZnOEP) + P (ZnOEP)	2.0 ± 0.05	0.67 ± 0.01
2. T (OEP) + P (OEP)	1.3 0.1	0.03 0.002
3. T (OEP) + P (ZnOEP)	1.5 0.15	0.27 0.01
4. T (ZnOEP) + P (OEP)	3.0 0.2	0.21 0.02

The difference in the one-electron oxidation and reduction potentials of many porphyrins is constant, near 2.2 V, the energy of the lowest optical transition. This supports our contention that little "reorganization energy" is required for electron transfer reactions in these systems (7). Entropy effects prevent a simple comparison of absorbed photon energy and redox energy of the resulting photochemical reaction. We will discuss these effects elsewhere, and here turn to the relation between formation of porphyrin cations and anions and their redox potential. The oxidation potential of a free base porphyrin is about 0.2 V more positive (oxidizing) than that of a zinc porphyrin. The oxidation potential increases with decreasing electronegativity of the central substituent. Conversely, the reduction potential of a free base porphyrin is about 0.2 V less negative (reducing) than that of a zinc porphyrin. Thus the pair $\text{ZnP}^+ + \text{P}^-$ is favored over the pair $\text{ZnP}^- + \text{P}^+$ by 0.3 ± 0.1 V, and the cross-reactions to this pair (Eq. 3, 4) should be favored over the homogeneous reactions (Eq. 1, 2) by 0.15 V. We see from Table III that these thermodynamic expectations are poorly realized. Only the rate constant for the T (ZnOEP) cross-reaction is slightly larger than that of the corresponding homogeneous reaction, and the yields of both cross-reactions are intermediate to the high (ZnOEP) and low (OEP) yields of the homogeneous reactions. We believe the data are best explained on purely kinetic grounds.

Our detailed studies of the homogeneous reactions¹ show that both diffusion in the Coulomb field and the spin state of the electrons are crucial in determining the fate of the initial loose encounter complex. Similar considerations have been invoked by several workers (14–16). In the present case, the approach of triplet (T) and ground state (P) molecules will allow an electron to tunnel between the pair to form a triplet charge transfer species T ($\text{P}^+ \text{P}^-$). Since this spin state cannot decay to the singlet ground state, the ion pair will tend to diffuse apart. It may regenerate T + P at the crossover point between ionic "charge transfer" and molecular "triplet" states. Thus the relatively slow rate constant for ion formation from T + P is caused by these unproductive collisions. A general mechanism for the decorrelation of the electron spins is through coupling with nuclear spins (14). Although the result is in general complex, for large molecules with many protons and nitrogens the relaxation from triplet to the equilibrium mixture of triplets and singlets can be approximated with a single time constant. Once a singlet ion pair, S ($\text{P}^+ \text{P}^-$) is formed, the ions may diffuse together and collapse to the ground state at the reaction radius, or diffuse apart to be measured as uncorrelated ions. Using the Smoluchowski concept, the problem is that of diffusion of two interconverting species in the presence of a Coulomb potential and with boundary conditions different for each species. We have numerically integrated these equations for various conditions.¹ A quantitative calculation in the present case would require more information than we have available, e.g., ion yield as a function of dielectric constant. Our extensive data on the homogeneous reaction of ZnOEP can be fit with spin decorrelation times of about 20 ns and a formation radius of 20–24 Å(10).¹ This radius is larger than the measured reaction radius of $\text{P}^- + \text{P}^+$, which is about the sum of the molecular radii, 15 Å. The excess distance is attributed to electron tunneling in the excited state reaction. These calculations suggest that the 20-fold

lower yield of OEP cannot be attributed only to a faster spin decorrelation time. The collision of OEP with its partner must be "sticky", i.e. the complex must have a sufficient lifetime for considerable spin decorrelation to occur, and the singlet state ion pair rapidly collapses to the ground state at this short (contact) distance.

Inspection of Table III yields some interesting information on the mechanism of the electron transfer reactions. The ion yields are far more sensitive to the particular reaction than are the rate constants. This accords with our interpretation of the rates as being determined by the spin decorrelation to the singlet charge transfer state. The twofold increase in rate constant when the triplet is ZnOEP instead of OEP, reactions 1 and 4, may be caused by increased spin orbit coupling in this excited state. The difference in rate constants and possibly in yields of reactions 3 and 4 show that the trival mechanism of triplet energy transfer to transform reaction 4 to 3 occurs to only a limited extent. This agrees with our hypothesis that electron transfer occurs over greater than nearest-neighbor distances.

These considerations suggest that the pheophytin in the reaction center of photosynthetic bacteria plays not so much a thermodynamic role as one of electron spin decorrelation. This is particularly important if, as is believed (1), the first electron transfer occurs from the singlet state, and thus is highly susceptible to loss to the ground state. Although this loss may be slowed by the energy level gap between the first excited and ground states of porphyrins (17), rapid spin decorrelation is critical. This will be favored by the increased distance and the increased contact with protons in the pheophytin reaction. Because of the anisotropy of the magnetic interactions, the orientation of the molecules in the rigid complex will be important. The presence of a high spin Fe^{+2} ion nearby at the quinone acceptor will also greatly facilitate spin uncoupling. It is known that if the quinone is reduced, the triplet state of bacteriochlorophyll (dimer) is rapidly formed from the charge transfer state involving bacterio-pheophytin (1). Thus a consistent explanation of the detailed mechanism of the energy conversion step in photosynthesis is emerging, and is remarkably well based on quantum mechanics.

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NOTE ADDED IN PROOF

Hoff, Rademaker, Grondelle, and Drysens (*Biochim. Biophys. Acta.* **460**:547. 1977) and Blankenship, Schaafsma, and Parson (*Biochim. Biophys. Acta.* **461**:297. 1977) have shown that magnetic fields of the order of 10^2 G decrease the yield of triplet bacteriochlorophyll from the bacteriochlorophyll cation, pheophytin anion state when further electron transfer to the quinone is blocked by previous reduction. Hoff et al. claim that a preparation low in iron shows a larger and more magnetically sensitive effect than reaction centers with iron or than chromatophores. All workers in this field have found a large variability of results among different preparations. We suggest that the replacement of iron by manganese, as observed by Feher, Isaacson, McElroy, Ackerson, and Okamura (*Biochim. Biophys. Acta.* **368**:135. 1974), may contribute to these heterogeneous results. As far as we know, the quantum yield of the formation of the initial ion-radical pair in the "iron-free" preparations has not yet been determined.

DISCUSSION

SWENBERG: Since you have created ion pairs, did you look for a magnetic field effect on the overall yield of the reaction?

MAUZERALL: Yes, but not on the yield of the free-base porphyrin, only of the metalloporphyrin (see ref. 1, below). We do see magnetic field effects as expected and we interpret them in a way similar to that discussed by Professor Weller.

SWENBERG: You have mentioned some extensive data on the homogeneous reaction of ZnOEP, which you say can be explained by extended correlation times of 20 ns. Your theoretical