Similarly, chemical reduction of 1^+ with a trace of Na/Pb alloy afforded quantitative yields of 2^+.

The ETC process occurs as shown in Scheme I. The homogeneous cross reaction between **2** and 1^+ to give 2^+ and **1** is highly favored and accounts for most of the conversion of **2** to 2^+. Of primary interest in this paper is the mechanism of CO substitution at the 19-electron center, reaction $1 \rightarrow 2$. In order to determine the rate, it is necessary to observe a cathodic current for the reduction of *both* 1^+ and 2^+. Figure 1 shows that temperature variation can be utilized to slow the CO substitution so that both cathodic waves can be observed. Thus, at 25 °C (Figure 1B) the rate is rapid compared to the CV time scale and all **1** formed at the electrode surface is rapidly converted to **2** (and then 2^+), so that the only observed cathodic wave is due to 2^+. Conversely, at −65 °C (Figure 1D) the only cathodic wave is due to 1^+ because the rate of $1 \rightarrow 2$ is too slow for a substantial amount of **2** to form. At −43 °C (Figure 1C) the reaction rate and CV time scale are competitive and a reduction wave is observed for both 1^+ and 2^+; it was found that variation of the nucleophile concentration or external CO pressure had *no effect* on the CV under these conditions, indicating a rate independent of [L] and [CO]. Furthermore, CVs obtained at −43 °C with a variety of P-donors were very similar, which implies the *same* rate for all L. In other words, the mechanism is strictly dissociative, with the rate-determining step being CO loss from **1** to give the 17-electron intermediate, (MeCp)Mn(CO)NO, which is trapped rapidly and completely by nucleophile L.[9]

A digital simulation program[10] was used to determine the rate of $1 \rightarrow 2$. The simulations showed the CVs to be very sensitive to the rate constant for CO dissociation (k_1) but insensitive to the rate constant for the homogeneous cross reaction,[11] provided the latter is greater than ca. 10^4 M^{-1} s^{-1}. The rate constant k_1 was measured over the temperature range −30 to −50 °C with the following results: $\Delta H^{\ddagger} = 72 \pm 8$ kJ; $\Delta S^{\ddagger} = 90 \pm 15$ J K^{-1}; $k_1 = 11$ s^{-1} at −43 °C. The entropy term clearly signals a dissociative process. The enthalpy term seems reasonable for the loss of $^1/_2$ bond order[1d,e,2a–e] in going from 19 e^- to 17 e^-; by comparison, $\Delta H^{\ddagger}$ for CO dissociation from 18-e^- complexes (loss of 1 bond order) is much higher: $Cr(CO)_6$, 162 kJ; $Fe(CO)_4PPh_3$, 178 kJ; $Co(CO)_2(NO)AsPh_3$, 142 kJ; $CpV(CO)_4$, 230 kJ.[12] It is therefore established that CO substitution in the 19-electron **1** is dissociative.

It is reasonable to inquire if **1** is really a 19-electron complex or whether the odd electron is localized in ligand orbitals (especially the nitrosyl). An ESR study[13] of $CpM(CO)_2NO^-$ (M = Cr, Mo), which is isoelectronic with $CpMn(CO)_2NO$, is directly relevant to this question. This study showed that the NO ligand bends and acquires ca. 40% of the unpaired spin density when $CpM(CO)_2NO$ is reduced to the anion. However, a large amount (≥50%) of the unpaired spin density is localized on the metal, so that the radical anions can be reasonably described as 19-electron complexes. It is highly likely that $(MeCp)Mn(CO)_2NO$ (**1**) is similarly best formulated as a 19-electron complex. The simple observation that complex **1** reacts by a dissociative mechanism also argues against the possibility that **1** is a 17-electron complex (with the NO ligand being a 1-electron donor) because 17-electron complexes always react by an associative mechanism unless steric factors dominate.[2a,g,14]

In order to assess the increase in substitution rate in 19-electron compared to 18-electron complexes, the direct reaction of 1^+ with several phosphines was studied. It was found that the reaction to give 2^+ is clean, but the rates were difficult to reproduce. Sometimes, but not always, an induction period was observed and the rates (in the dark under N_2) varied by a factor of 10 for ostensibly the same reaction conditions (e.g., 1 h ≤ $t_{1/2}$ ≤ 10 h with $[1^+] = 10^{-3}$ M and $[PPh_3] = 10^{-2}$ M). This suggests the possibility that the rate of conversion of 1^+ to 2^+ may be determined by the presence of adventitious reductants that initiate an ETC process. In accordance with this is the observation by McCleverty[15] that normally slow CO substitution in 1^+ becomes rapid in the presence of a catalytic amount of reducing agent NEt_3.

Finally, we note that one of the important features of organometallic reactions is the reactivity dependence on the metal in a triad. Within the context of this paper, it was found that $CpRe(CO)_2NO^+$ is reversibly reduced at the same potential as 1^+, but unlike **1**, $CpRe(CO)_2NO$ is *completely unreactive* (on the CV time scale) toward P-donor nucleophiles. This shows that the reactivity with respect to CO dissociation in these 19-electron complexes follows the order Mn ≫ Re, which likely reflects the stronger M–CO bond in the heavier transition metal.[16]

Acknowledgment. This work was supported by a grant from the National Science Foundation (No. CHE-8821588). We are grateful to Prof. P. H. Rieger for helpful discussions.

(9) With the weak nucleophile $AsPh_3$, a rate retardation occurred when the atmosphere was changed from N_2 to CO, showing that CO can compete with $AsPh_3$ for the reaction intermediate. When no nucleophile is present, it is known from previous work that the chemical reduction of 1^+ with NEt_3 or $NaBH_4$ leads to simple dimerization to afford $[(MeCp)Mn(CO)(NO)]_2$: James, T. A.; McCleverty, J. A. *J. Chem. Soc., Dalton Trans.* **1970**, 850. King, R. B.; Bisnette, M. B. *Inorg. Chem.* **1964**, *3*, 791.

(10) (a) Gosser, D. K.; Rieger, P. H. *Anal. Chem.* **1988**, *60*, 1159. (b) Gosser, D. K.; Zhang, F. *Talanta* **1991**, *38*, 715.

(11) Multiple scan CVs showed that the rate constant must be greater than 10^4 M^{-1} s^{-1}.

(12) Howell, J. A. S.; Burkinshaw, P. M. *Chem. Rev.* **1983**, *83*, 557.

(13) Geiger, W. E.; Rieger, P. H.; Tulyathan, B.; Rausch, M. D. *J. Am. Chem. Soc.* **1984**, *106*, 7000.

(14) Kowaleski, R. M.; Basolo, F.; Trogler, W. C.; Ernst, R. D. *J. Am. Chem. Soc.* **1986**, *108*, 6046.

(15) James, T. A.; McCleverty, J. A. *J. Chem. Soc., Dalton Trans.* **1970**, 850.

(16) The same reactivity order obtains with $(arene)M(CO)_3$ (M = Mn, Re): Neto, C. C.; Baer, C. D.; Chung, Y. K.; Sweigart, D. A. Submitted for publication.

Sensitive Detection of Catalytic Species without Chromophoric Substrates

James W. Lane, Xingfang Hong, and Alan W. Schwabacher*

Department of Chemistry
Iowa State University
Ames, Iowa 50011

Received December 2, 1992

The development of antibodies as catalysts[1a,b] has allowed preparation of enzymelike materials that catalyze reactions with predetermined specificity. Monoclonal antibodies to transition-state analogues[1c] catalyze reactions as enzymes do: by preferentially binding and stabilizing the transition state.[1d] These catalysts are selected from a large repertoire of structures, initially by screening for antibodies that bind antigen, and then, usually after expensive scaleup, for activity.[2] Screening directly for activity, rather than binding, would be particularly valuable, considering that many of the most efficient antibody catalysts operate by mechanisms for which the eliciting antigens were not good transition-state analogs.[3]

Efficient screening of antibody activity is critical to the identification of catalysts,[2] and sensitive assays can greatly facilitate

(1) (a) Tramontano, A.; Janda, K.; Lerner, R. *Science* **1986**, *234*, 1566–1570. (b) Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science* **1986**, *234*, 1570. (c) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; p 288. (d) Pauling, L. *Am. Sci.* **1948**, *36*, 51.

(2) (a) *Catalytic Antibodies, Ciba Foundation Symposium 159*; Wiley: Chichester, 1991. (b) Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659–667. (c) Tarasow, T.; Hilvert, D. In *Protein Engineering and Design: An Introduction*; Wrede, P., Schneider, G., Eds.; Walter de Gruyter: New York, in press.

(3) (a) Martin, M. T.; Napper, A. D.; Schultz, P. G.; Rees, A. R. *Biochemistry* **1991**, *30*, 9757–9761. (b) Wirsching, P.; Ashley, J. A.; Benkovic, S. J.; Janda, K. D.; Lerner, R. A. *Science* **1991**, *252*, 680–685. (c) Janda, K. D.; Lerner, R. A.; Tramontano, A. *J. Am. Chem. Soc.* **1988**, *110*, 4835–4837.

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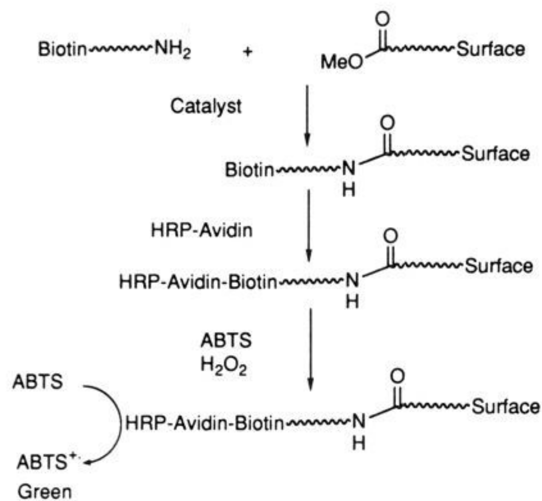


Figure 1.

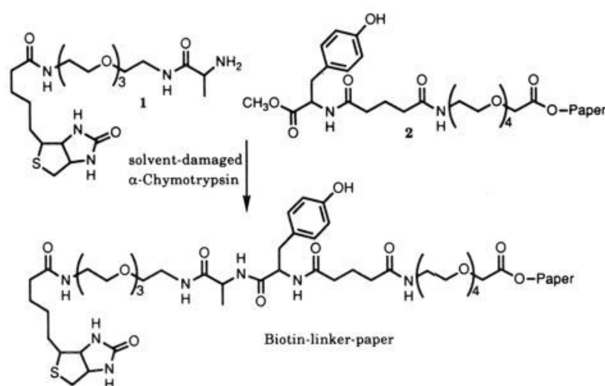


Figure 2.

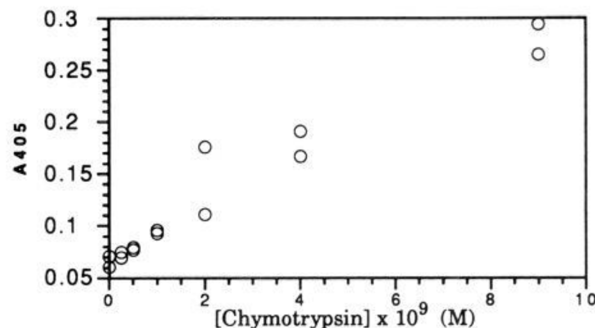


Figure 3. Peptide coupling catalyzed by chymotrypsin in 5 μL of 40% aqueous CH_3CN. Paper disks of **2** (4.8-mm diameter), bearing 6.2 × 10^{-8} mol of tyrosine methyl ester, are exposed to the chymotrypsin catalyst and 72 mM **1** in 5 μL of 60:40 CH_3CN/pH 9 H_2O. After 20 min, the paper is thoroughly rinsed with 1% SDS and PBS, blocked with 3% BSA, incubated with streptavidin–HRP, thoroughly rinsed with PBS, 0.1% Tween 20 in PBS, and H_2O, and then exposed to 5 mM H_2O_2 and 0.8 mM ABTS in pH 4 citrate. The signal is detected at 405 nm.[13]

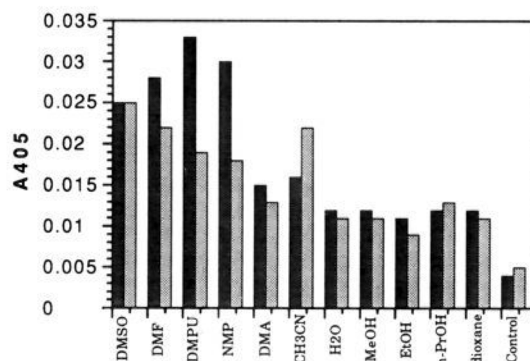


Figure 4. Chymotrypsin-catalyzed simultaneous couplings in various 40% aqueous solvents.

the process.[4] Existing methods depend on release of a chromophore by the reaction assayed, which limits the molecules that may be studied. Among the most important classes of catalyst are those that concatenate molecules. Several such antibodies have been reported.[3c,5] Detection of the coupled product, as opposed to a cleaved substrate, is usually done by HPLC and is not readily used as a screening procedure. A general assay that could detect a coupling catalyst at low concentration by screening directly for catalytic activity would be of great utility. We have developed such an assay that is as easy to carry out as the ELISA[6] used to detect antibody binding. It can be carried out in parallel, in small volume, using existing reagents and instrumentation.

The idea, shown in Figure 1, is simple: to attach a tag to one molecule, and to tether the other molecule to a surface. A catalyst that couples the two molecules may be detected by observation of tag bound to surface. Biotin is used as the tag: it may then be detected using a standard streptavidin/horseradish peroxidase enzyme amplification procedure. We illustrate our method by monitoring the action of Wong's cosolvent-damaged chymotrypsin[7] as a useful, but not particularly stable, catalyst for kinetically controlled peptide synthesis.

We studied the reaction shown in Figure 2.[8] The assay is quite sensitive. A signal clearly above background was observed at chymotrypsin concentrations of 5 × 10^{-10} M. As shown in Figure 3, assay response is linear up to 10 nM.[10] All compounds are needed to detect chymotrypsin activity. Paper without ester substrate is ineffective, and biotin tethered to *t*-Boc-alanine, without the nucleophilic amine, also leads to no signal above background.

We have used our assay to explore the activity of chymotrypsin in other mixed aqueous–organic solvents. Figure 4 shows our results. We have successfully screened a number of cosolvents for efficacy in peptide coupling and have found several that appear to be equivalent to the acetonitrile system identified by Wong et al.[7] Duplicate experiments reveal a fairly large scatter in some cases, but such a rapid and convenient assay is clearly capable of identifying catalysts at low levels.

In contrast to the use of a chromophoric leaving group for such an acylation reaction, our assay detects coupling of substrates and would not be misled by hydrolysis of the active ester. A significant aspect of this procedure is that there are no chemical requirements at the reacting center. No chromophoric substrate need be produced *by the reaction assayed*. A tether for linkage to a surface or reagent is needed, but as the position of the tether is not important, much greater flexibility of design is obtained. The ease

(4) (a) Lewis, C.; Krämer, T.; Robinson, S.; Hilvert, D. *Science* **1991**, *253*, 1019–1022. (b) Gong, B.; Lesley, S. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 1486–1487. (c) Pollack, S. J.; Hsiun, P.; Schultz, P. G. *J. Am. Chem. Soc.* **1989**, *111*, 5961–5962.

(5) (a) Benkovic, S. J.; Napper, A. D.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5355–5358. (b) Cochran, A. G.; Pham, T.; Sugasawara, R.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 6670–6672. (c) Hilvert, D.; Hill, K. W.; Nared, K.; Auditor, M.-T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9261–9262. (d) Schultz, P. G.; Braisted, A. C. *J. Am. Chem. Soc.* **1990**, *112*, 7430–7431.

(6) (a) Engvall, E.; Perlmann, P. *Immunochemistry* **1971**, *8*, 871. (b) Tijjsen, P. *Practice and Theory of Enzyme Immunoassays*; Elsevier: Amsterdam, 1985; Vol. 15. (c) Maggio, E. T. *Enzyme-Immunoassay*; CRC Press: Boca Raton, 1980.

(7) Barbas, C. F.; Matos, J. R.; West, J. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 5162–5166.

(8) Preparation of these materials, by methods related to those of Bednarski,[9] will be described elsewhere.

(9) Bednarski, M. D.; Bertozzi, C. R. *J. Org. Chem.* **1991**, *56*, 4326–4329.

(10) At higher chymotrypsin concentration, an unexplained leveling off of absorbance was seen. This appears not to be a saturation of bound substrate, because an insoluble purple color develops in the paper, the intensity of which increases with chymotrypsin concentration. This purple color is dependent on ABTS.

with which parallel assays may be carried out is also significant. This assay is appropriate as described for activity screening of hybridomas in place of ELISA screening for binding. Minor modifications would allow use in other formats.

The same convenient procedure, with very similar reagents, may be used to follow diverse classes of concatenation reactions. For example, while we have demonstrated the detection of peptide coupling, there is no apparent reason that a Diels–Alder or other reaction would not also be amenable to this approach.

Recent advances in combinatorial antibody technology[11] and other strategies[12] promise to provide an even greater repertoire of potential catalysts, placing greater demands on the screening procedures. We have shown that a highly sensitive and generally applicable assay is now available for the detection of low concentrations of catalysts for bimolecular reactions.

(11) (a) Chiswell, D. J.; McCafferty, J. *Tibtech* **1992**, *10*, 80–84. (b) Barbas, C. F., III; Bain, J. D.; Hoekstra, D. M.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4457–4461. (c) Marks, J. D.; Griffiths, A. D.; Malmqvist, M.; Clackson, T. P.; Bye, J. M.; Winter, G. *Bio/Technology* **1992**, *10*, 779–783.

(12) (a) Famulok, M.; Szostak, J. W. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 979–988. (b) Scott, J. K.; Smith, G. P. *Science* **1990**, *249*, 386–390. (c) Devlin, J. J.; Panganiban, L. C.; Devlin, P. E. *Science* **1990**, *249*, 404–406. (d) Geysen, H. M.; Rodda, S. J.; Mason, T. J. *Mol. Immunol.* **1986**, *23*, 709–715. (e) Brenner, S.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5381–5383.

(13) Abbreviations: ABTS, diammonium 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate); HRP, horseradish peroxidase; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PBS, 10 mM pH 7.2 phosphate, 150 mM NaCl; SDS, sodium dodecyl sulfate.

Mimicking Carotenoid Quenching of Chlorophyll Fluorescence

Roel M. Hermant, Paul A. Liddell, Su Lin, Rhett G. Alden, Ho Kwon Kang, Ana L. Moore,* Thomas A. Moore,* and Devens Gust*

Department of Chemistry and Biochemistry and Center for the Study of Early Events in Photosynthesis Arizona State University, Tempe, Arizona 85287-1604

Received November 23, 1992

Carotenoid pigments are ubiquitous in photosynthetic membranes. They act as antennae by transferring singlet energy into the chlorophyll manifold to drive photosynthesis and as photoprotective pigments by transferring triplet energy out of the chlorophyll system, thereby preventing chlorophyll-sensitized singlet oxygen formation.[1-3] Carotenoids also quench chlorophyll fluorescence in vitro[4] and in vivo.[5] This quenching presents a paradox: carotenoids inject excitation energy into the chlorophyll singlet manifold, but they then dissipate it before it can be used for photosynthetic work. Synthetic carotenoporphyrins mimic carotenoid energy transfer and exhibit carotenoid quenching of tetrapyrrole fluorescence.[3,6] We now report that in a specially designed carotenoporphyrin, quenching results from electron transfer from the carotenoid to the excited singlet porphyrin followed by fast charge recombination.

Although work reporting the singlet lifetimes and fluorescence and energy-transfer properties of carotenoids has recently appeared, the mechanisms whereby these pigments quench the excited singlet states of cyclic tetrapyrroles remain obscure.[7-16]

(1) Cogdell, R. J.; Frank, H. A. *Biochim. Biophys. Acta* **1987**, *895*, 63–79, and references therein.

(2) Siefermann-Harms, D. *Physiol. Plant.* **1987**, *69*, 561–568, and references therein.

(3) Gust, D.; Moore, T. A. *Adv. Photochem.* **1991**, *16*, 1–65.

(4) Beddard, G. S.; Davidson, R. S.; Trethewey, K. R. *Nature* **1977**, *267*, 373–374.

(5) Deming-Adams, B. *Biochim. Biophys. Acta* **1990**, *1020*, 1–24.

(6) Gust, D.; Moore, T. A.; Moore, A. L.; Devadoss, D.; Liddell, P. A.; Hermant, R.; Nieman, R. A.; Demanche, L. J.; DeGraziano, J. M.; Gouni, I. *J. Am. Chem. Soc.* **1992**, *114*, 3590–3603.

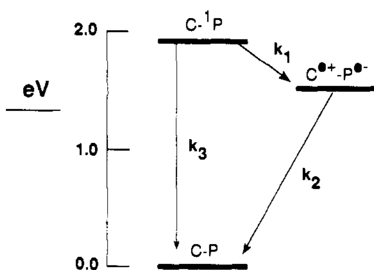


Figure 1. Energy levels and relaxation pathways for the porphyrin first excited singlet state of **1**. Charge separation k_1 and recombination k_2 are electron-transfer processes; k_3 refers to the sum of the other relaxation processes from the first singlet excited state (measured in **2**).

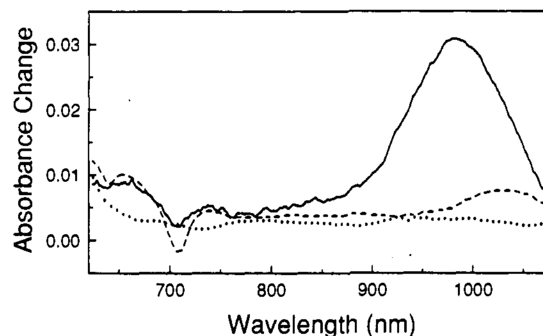
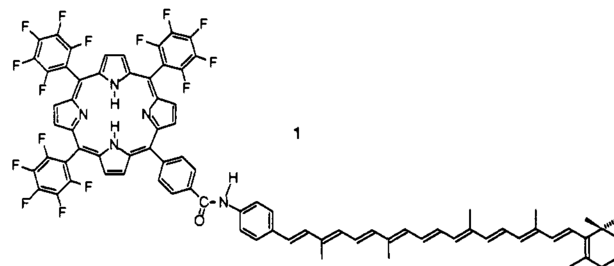


Figure 2. Transient absorption spectra in butyronitrile of **1** (—) and **2** (---) taken 10 ps after a ca. 150-fs, 5-mJ laser flash (108K flashes) and **3** (···) taken 3 ps after the flash. The absorbance of **1** was 2 per cm (ca. 3.6×10^{-4} M) at the excitation wavelength of 590 nm, where 90% of the light is absorbed by the porphyrin. The absorbance of **2** was 1.8 per cm, and that of **3** was 0.2 per cm, so the populations of excited porphyrin and carotenoid in **2** and **3** are the same as those produced in **1**.

Thermodynamically, quenching could occur by electron transfer from the carotenoid (C) to the cyclic tetrapyrrole (P) first excited singlet state to yield a charge-separated (CS) species.[4] Singlet energy transfer from the cyclic tetrapyrrole to the electric-dipole-forbidden S_1 state of the carotenoid is also a possibility, although the energetics of that process are unknown.[13-16]

Carotenoporphyrin dyad **1** was designed to maximize the



possibility of observing the CS species from electron-transfer

(7) Wasielewski, M. R.; Kispert, L. D. *Chem. Phys. Lett.* **1986**, *128*, 238–243.

(8) Gillbro, T.; Cogdell, R. J. *Chem. Phys. Lett.* **1989**, *158*, 312–316.

(9) Shreve, A. P.; Trautman, J. K.; Frank, H. A.; Owens, T. G.; Albrecht, A. C. *Biochim. Biophys. Acta* **1991**, *1058*, 280–288.

(10) Trautman, J. K.; Shreve, A. P.; Owens, T. G.; Albrecht, A. C. *Chem. Phys. Lett.* **1990**, *166*, 369–374.

(11) Katoh, T.; Nagashima, U.; Mimuro, M. *Photosynth. Res.* **1991**, *27*, 221–226.

(12) Wasielewski, M. R.; Liddell, P. A.; Barrett, D.; Moore, T. A.; Gust, D. *Nature* **1986**, *322*, 570–572.

(13) Snyder, R.; Arvidson, E.; Foote, C.; Harrigan, L.; Christensen, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4117–4122.

(14) Cosgrove, S. A.; Guite, M. A.; Burnell, T. B.; Christensen, R. L. *J. Phys. Chem.* **1990**, *94*, 8118–8124.

(15) Osuka, A.; Yamada, H.; Maruyama, K.; Mataga, N. Asahi, T.; Yamazaki, I.; Nishimura, Y. *Chem. Phys. Lett.* **1991**, *181*, 419–426.

(16) Jones, P. F.; Jones, W. J.; Davies, B. H. *J. Photochem. Photobiol. A: Chem.* **1992**, *68*, 59–75.

0002-7863/93/1515-2080$04.00/0 © 1993 American Chemical Society