Photodissociation of Carbon Monoxide and Dioxygen Forms of Synthetic Heme Complexes Studied by Using Picosecond Absorption Spectroscopy. Evidence of a Pseudo-Four-Coordinate Intermediate

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Abstract: We have studied the photodissociation of the CO and O_2 forms of a number of synthetic heme complexes by using 531-nm, 8-ps pulses from a mode-locked Nd–phosphate glass laser employing the standard pump–probe technique. These complexes closely mimic the "R" and "T" states of hemoglobin depending on whether the imidazole–heme steric interaction is strain free or not. Such variations in the proximal imidazole–heme geometry allow one to explore the effects of tertiary constraint of this type on the dissociation. The results of this work are complementary to our earlier work on the natural compounds, hemoglobin and myoglobin, and show that the effects of strain on the heme are manifest in the dynamics and mechanism of the photodissociation. For both the natural and synthetic compounds, we have identified certain predissociative and postdissociative photointermediates as they sequentially evolve by monitoring the $\pi\pi^*$ porphyrin Soret absorption changes as a function of time. Our kinetic analysis indicates that it takes from 2 to 16 ps for the synthetic complexes to dissociate depending on whether the Fe–imidazole bond is strain free or not. They also suggest that a pseudo-four-coordinate complex may be present as a photointermediate in the compounds having the strained Fe–imidazole linkage.

The results of our picosecond photodissociation experiments on the CO and O_2 forms of a number of synthetic reversible oxygen carriers are presented in this report.[1] Earlier picosecond absorption work on the same derivatives of the natural forms of hemoglobin and myoglobin provided a better understanding of the details of the photodissociation in terms of the sequential evolution of four photointermediates which were experimentally isolated, characterized, and kinetically analyzed.[2a] On the basis of this analysis we assigned the first two intermediates to excited undissociated states which differed electronically, whereas the latter two were assigned to dissociative states that could each undergo geminate recombination. Two phases of geminate recombination were observed: a rapid recombination occurring during the first 20 ps concurrent with the third intermediate and a slow recombination concurrent with the fourth intermediate that extended to the nanosecond regime. The rapid recombination was more prevalent for the O_2 derivatives than for the CO compounds and was more apparent in hemoglobin (Hb) than in myoglobin (Mb). These experiments were done by using standard pump–probe picosecond methods following $\pi\pi^*$ Soret absorption changes localized to the plane of the porphyrin. The absorption characteristics of this band, ϵ_{max}, λ_{max}, and the band shape, are very sensitive to both the coordination and geometry of the heme complex. The results on the natural complexes revealed that the tertiary heme geometry, or constraints imposed on the heme by the quaternary structure, affected the dynamics of dissociation. This is particularly evident in comparison of the lifetime of certain photoproducts between Hb and Mb and the α and β chains of Hb.

Thus, we became interested in examining the effects of tertiary constraint of the proximal imidazole on the course of the dissociation, effects that could more easily be examined by studying appropriate synthetic analogues. Additionally, our interest in elucidating the role that the imidazole plays in modulating the dissociation process was prompted by the results of recent picosecond resonance Raman (RR) work.[3] For example, Findsen et al.[3a,d] present results that show the Fe in MbCO has relaxed out of place by 20–30 ps based on analysis of Fe–His resonantly enhanced modes using Raman-shifted Soret excitation, whereas in HbCO other RR work reveals that such relaxation does not occur to the fullest extent possible before about 20 ns.[3b–d] These studies show the importance of the tertiary structural constraints, particularly ones involving the Fe–His linkage, in the out-of-plane relaxation process.

So far two conclusions have emerged from our comparative studies on several dozen natural and synthetic compounds: that the effects of strain on the heme are important in terms of variations found in the dynamics and mechanism of early events of the picosecond dissociation and that it takes some picoseconds for these complexes to dissociate, although the process begins in the subpicosecond regime. The synthetic compounds used in this study mimic the R and T states of hemoglobin depending on whether the imidazole–heme geometry is strain free or not.[4] As our results will show, this particular structural variation dramatically effects the course of the dissociation. We have assigned an intermediate found early in the dissociation of the strained CO complexes to a pseudo-four-coordinate complex. This particular intermediate is absent in the unstrained CO complexes as well as in all the O_2 complexes, strained or not. Our experimental procedure used to characterize these photointermediates as they evolve employs the standard pump–probe method by following absorption changes in the $\pi\pi^*$ Soret band after initiating the dissociation with a 531-nm pulse directed to the Q band. A mode-locked Nd^{3+}–phosphate glass laser is used for the experiments. Our analysis

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of the results is somewhat different than usual in that we obtain modeled spectra for comparison to the experimental spectra over a wide range of pump–probe delays using a numerical integration procedure.[2] In this procedure we convolute the pump and probe pulses with a kinetic model for the photodissociation, which is consistent with the sequential evolution of the intermediates observed experimentally, as these pulses pass through the sample.

As the picosecond dynamics of the CO and O_2 ligand photodissociation in heme complexes is a topic of current theoretical and experimental interest, certain other work having a bearing on the results in this report should be mentioned. The question of what orbital promotion(s) in the Fe(II)(d^6) six-coordinate manifold lead to photodissociation of the CO or O_2 complexes is more easily answered for the CO than the O_2 parent molecules. The extended Hückel calculations of Zerner et al.[5a] have been used for many years to interpret spectroscopic results, particularly with reference to the heme–CO complexes. Their calculation, for example, suggests that population of the $d_{xy,yz}$ (or d_π) → d_{z^2} transition (octahedral symmetry) will lead to photodissociation of the CO complex. This Hückel calculation was updated by Eaton et al.[5b] with particular emphasis on the d–d metal and d–ligand charge-transfer transitions for the HbO_2, HbCO, and deoxy complexes. These theoretical results combined with their experimental CD and MCD spectra allowed spectral assignments to be made for certain d–d and d–ligand transitions. More recently, Waleh and Loew[5c–e] using INDO–SCF–MO–LCAO–CI calculations concluded that both the $d_\pi \rightarrow d_{z^2}$ and the $d_{x^2-y^2} \rightarrow d_{z^2}$ transitions (pyrrole N atoms bisect the *xy* axes) are the best photodissociative candidates since these transitions decrease in energy as a function of increasing Fe–CO distance. The $d_\pi \rightarrow d_{z^2}$ is the preferred transition over the other possibility because it has higher oscillator strength and better "bond-weakening characteristics". These investigators also suggest that the singlet $d_\pi \rightarrow d_{z^2}$ transition is the best candidate for the photodissociating state, rather than intersystem crossing to lower triplet states or through $\pi\pi^*$ porphyrin transitions as Hoffman and Gibson suggest.[5f]

There has also been a number of experimental investigations detailing the picosecond photodissociation of the O_2 and CO forms of the natural complexes using absorption techniques. Representative citations and discussion may be found elsewhere.[2] Recent femtosecond absorption experiments on HbCO, MbCO, and CO–protoheme reported by Martin et al.[6] provide evidence that each of these species forms a deoxy photoproduct with a time constant of 350 fs after excitation with 250-fs pulses at 307 nm. Support of this particular experimental result is provided by a molecular dynamics trajectory simulation study which suggests that it is possible for the iron to relax out of the plane of the porphyrin by 350 fs.[7] Our results and conclusions differ somewhat from these investigations, although we also have evidence that the first stable photoproduct appears in the subpicosecond regime.

Experimental Section

The double-beam phosphate–glass Nd^{3+} laser apparatus used in these experiments has been described elsewhere.[2] Experiments were of the pump–probe type done at a rate of 1/min using a 531-nm (8-ps FWHM, TEM_{∞}, and ≤1-mJ) pulse to initiate the photodissociation by pumping the Q band of the parent six-coordinate CO or O_2 complex. A continuum light pulse, generated from a D_2O cell and appropriately filtered to examine the Soret region, provided the absorbance vs. 42-nm assay of sample–photoproduct concentrations at specific probe–pump maxima pulse delays ranging from −20 to 50 ps. Negative delay settings refer to experiments where the maximum of the probe pulse reaches the sample cell (1-mm optical path) before the maximum of the pump pulse, a configuration in which the tail of the probe pulse interrogates excited sample on the leading edge of the pump pulse. These negative delay experiments, conducted over a suitable range, are critical to the detection and kinetic analysis of photointermediates that appear early in the dissociation, ones that have lifetimes considerably shorter than the 8-ps fwhm pulse width.

To establish the absorbance change $\Delta A(\lambda,t)$ at a particular delay setting, at least 10 experiments are signal averaged taking into account the energy of the 531-nm pulse. With respect to the sample (I) and reference (I_0) beams, each experiment consists of two laser shots, one with the sample pumped and one without: $\Delta A(\lambda, t) = \log (I_0/I)_{\mathrm{pump}} - \log (I_0/I)_{\mathrm{no\ pump}}$. The 42-nm I_0 and I signals are detected by using a pair of wide-area (512-element, channel-plate intensified) diode arrays after first passing these pulses through a subtractive monochromator to remove the excitation light followed by a 1-m spectrograph equipped with a 300 groove/mm grating. These signals are processed by a Tracor Northern OMA/computer.

The equilibrium ground-state spectra of the parent six-coordinate and deoxy five-coordinate (or four-coordinate) species were obtained by using a tungsten lamp placed before the I_0/I beam splitter. Earlier work in our laboratory[2] has established that the experimental spectra of transient photoproducts can be resolved as they sequentially evolve during the dissociation process. At relatively long delays >20 ps after excitation, the spectrum of the stable photoproduct HB* is obtained by adding the ground state to the ΔA difference curve: $\Delta A(\mathrm{HB^*{-}HBCO}) + A(\mathrm{HBCO}) = A(\mathrm{HB^*})$. At earlier delays the procedure of isolating a particular photoproduct is the same except that spectra of other intermediates produced must be taken into account in a manner consistent with the quantum yield of dissociation and conservation principles. Thus the band shape and the experimental parameters $\lambda_{\max}$, fwhm, and an estimate of $\epsilon_{\max}$, as well as an estimate of photoproduct lifetime, are obtained by a workup of the experimental data. In order to establish these parameters more accurately, we first smooth the 512-point experimental difference spectra by using a 31-point algorithm. The smoothed ΔA spectra also facilitate the comparison of the kinetically modeled spectra to establish the rate constants and lifetimes of the intermediates.

The experiments on CO forms of the synthetic compounds were done with sample concentrations adjusted to ~50 μM to give an absorbance of 1 over a 1-mm optical path at the Soret maximum. For the oxygen experiments, the samples were prepared as the CO complexes and covered with a balloon containing a ~3:1 mixture of O_2 to CO. An additional photographic strobe lamp having a flash duration of ~25 μs was integrated into the system and adjusted to pump the sample 0.45 ms before the picosecond pump pulse arrived, allowing sufficient time for the production of the heme–dioxygen complex by means of the following reactions: $\mathrm{HBCO} + h\nu \rightarrow \mathrm{HB} + \mathrm{CO}$ and $\mathrm{HB} + O_2 \rightarrow \mathrm{HBO_2}$. The 0.45-ms period allows sufficient time for the formation of HBO_2, with only a minor fraction of this complex returning to the more stable CO form.[4a]

The compounds were received from T.G.T.'s laboratory in the iron(III) chloride form. Approximately 0.4 mg of this crystal was dissolved in absolute methanol, degassed, and brought back to atmospheric pressure with argon. Aqueous solutions of cetyltrimethylammonium bromide (CTAB) were prepared, filtered, and bubbled with carbon monoxide gas to saturate the system. Sodium dithionite was added for the reduction of Fe(III) to Fe(II), and the methanol solution of the heme was added to the CTAB to create an aqueous solution of $\sim 10^{-5}$–10^{-4} M heme–CO. CTAB produces a micellar system with a hydrophilic exterior and a hydrophobic interior pocket in which the heme complex is contained. Because of the possibility of reactions in which the heme complex binds water, experiments were also done on compounds prepared with benzene or toluene in place of CTAB. All experiments were done at room temperature, ~20 °C. At the CTAB concentrations used, precipitation of the micelles occurs at temperatures below 20 °C.

Results

To examine the effects of imidazole strain on the picosecond dissociation of reversible oxygen carriers, the CO and O_2 forms of four synthetic hemes shown in Figure 1 were studied: **1**, **1-ST**, **1-ET**, and **1-AD**. The first three compounds were studied in aqueous solution, and the last was studied in toluene. Compound **1** is a chelated protocheme containing vinyl side chains. The imidazole is attached to the porphyrin through an amide linkage that minimizes the interaction or strain between the imidazole and the porphyrin–Fe(II) heme. This molecule serves as a model for R-state hemoglobin.[4a,b] **1-ST** is a chelated protoheme in which face strain has been introduced to the complex by means of a methylimidazole. The compound **1-ET** is one of two unchelated

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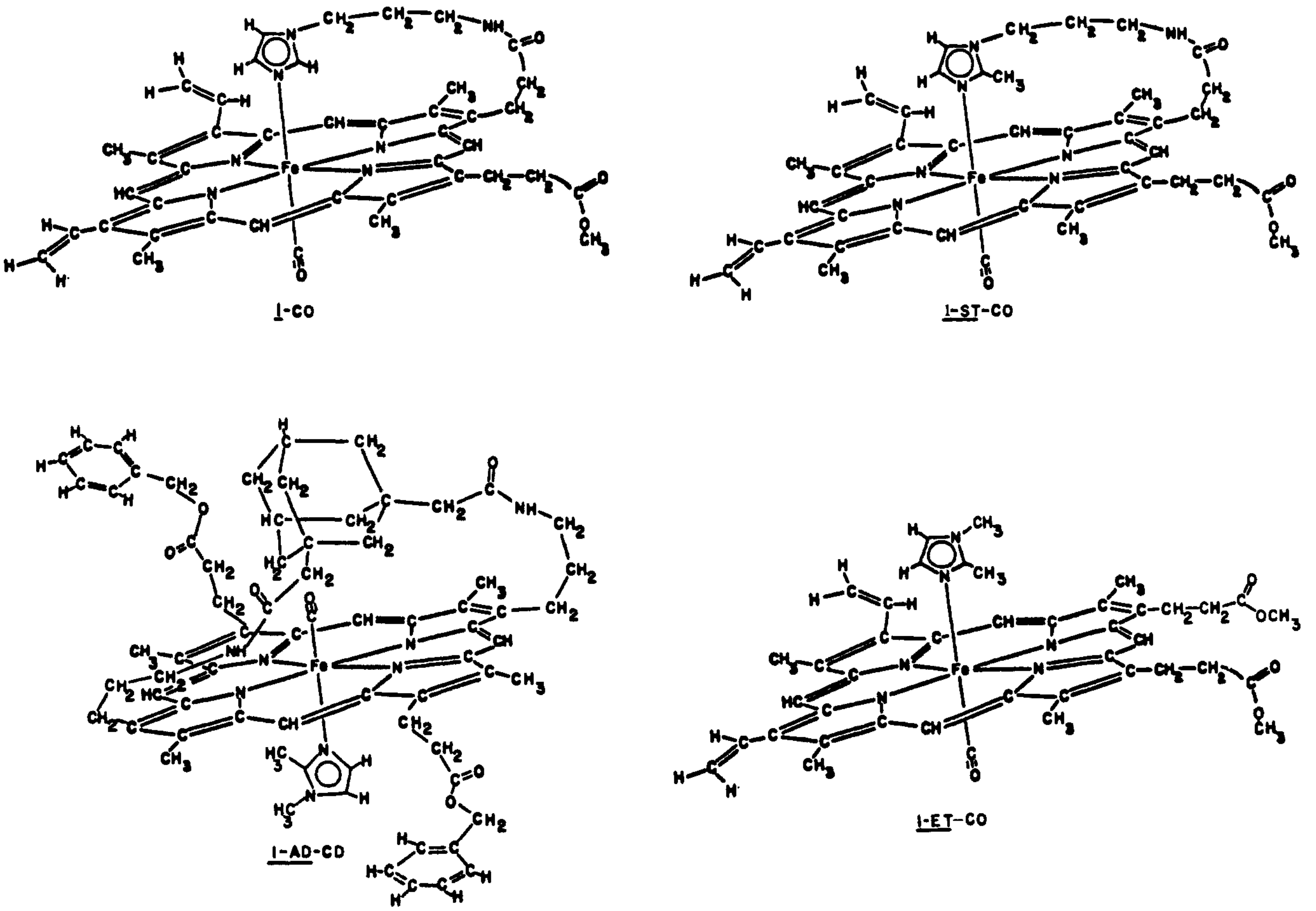


Figure 1. Structures of some of the synthetic compounds studied. The iron–imidazole bond in **1**–CO is unstrained while it is strained in **1-ET**–CO, **1-ST**–CO, and **1-AD**–CO.

hemes studied. It is prepared in the presence of an external base, 1,2-dimethylimidazole. Due to the possibility of binding two base molecules axially to the heme, this bulky disubstituted base is used. It introduces strain into the heme–imidazole bond, providing a model for T-state hemoglobin as well as preventing the binding of a second base to the complex. The second unchelated protoheme studied, **1-AD**, is one in which a bulky nonaromatic adamantine cyclophane group has been introduced distally to provide protection for the CO or O_2 binding site. Due to the possible interactions with water, this compound was prepared in anhydrous toluene and 1,2-dimethylimidazole was again used for the proximally bound external base.

Experiments on **1**–CO using benzene in place of CTAB were also done to examine the effects of solvent and environment on the photodissociation. None were found. The photointermediates arrived at the same time and had the same peak wavelengths, extinction coefficients, and band shape. Insofar as the dynamics observed in these experiments are independent of CO pressure and since there is no detectable geminate CO recombination, it is reasonable to expect effects on the photodissociation due to solvation to be minimal as diffusion has not yet occurred on the time scale studied.

To study the possibility of electronic effects on the photodissociation localized to the porphyrin ring, we examined **2**–CO, a compound similar to **1**–CO except for the substitution of ethyl groups in place of vinyl groups on the outside of the porphyrin ring. Earlier bimolecular kinetic studies[4a,e] had shown that this change in the electron distribution of the porphyrin had no effect on the reaction of CO with the heme. Our results also indicate that this perturbation has no apparent effect on the picosecond photodissociation.

Examination of the transient ΔA spectra reveals that the synthetic complexes photodissociate according to one of two pathways: (I) A → AS → B → C → D or (II) A → AS → X → B → C → D. AS, X, B, C, and D represent intermediates that have been experimentally observed and isolated in various time frames up to 50 ps. Assignment of these will be discussed shortly in terms of octahedral notation. The six-coordinate parent ground state ^{1}A is designated A.

Pathway I was observed for all the O_2 complexes studied, strained or unstrained, as well as for the unstrained CO complexes. This particular pathway is the same one observed in the photodissociation of the natural heme complexes[2] (HbCO, MbCO, HbO_2, and MbO_2) with the exception that there is no detectable geminate recombination to the limit of our experiment, 50 ps. Pathway II, observed for the strained CO complexes, reveals the presence of a fifth intermediate X found early in the dissociation that is either absent or undetectable in the natural or synthetic heme complexes following pathway I. The kinetics associated with the evolution of these intermediates will be discussed shortly. First, it is appropriate to examine the experimental ΔA difference spectra of several representative complexes, **1**–CO and **1**–ET–CO. A discussion of **1-ST**–CO and **1-ET**–O_2 is included for comparative purposes.

A. 1–CO. The actual experimental ΔA spectra for **1**–CO are shown in Figure 2 over delays ranging from −12 to 50 ps. For **1**–CO the spectroscopic parameters of the ground state and intermediates in terms of λ_{max} (nm), fwhm (nm), and ϵ_{max} (upper estimates in M^{-1} cm^{-1}) are, respectively, A (ground)[4b] 512, 11, 203K; AS 419, 15, 205K; B 430, 32, 75K; C 442, 24, 160K; and D 435, 20, 175K. The first event observed at the −12-ps delay is an indication of a positive absorbance change at the low-wavelength end of the spectrum. Even though this spectrum is noisy (±0.05 ΔA in the blue region), the increase in absorbance is apparent, and it furnishes evidence of the first intermediate, AS. AS has an absorption maximum at the same wavelength as the six-coordinate ground-state species, 419 nm, with a slightly higher extinction coefficient and a broader bandwidth. AS is not long lived, and by −8 ps the induced absorbance present at the low-wavelength end of the spectrum has been replaced by the

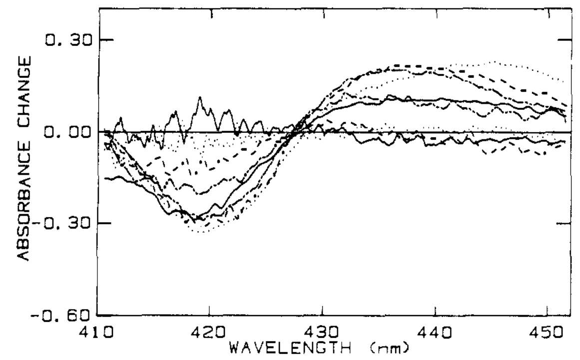


Figure 2. Experimental spectra for **1**–CO. Delays range from –12 to 50 ps: –12 to +4 ps (solid), –8 to +8 ps (dotted), –4 to +20 ps (dashed), and 0 to +50 ps (broken).

disappearance or bleaching of the ground-state spectrum. This transient is seen in all compounds, synthetic and natural, in which early time delays were obtained. The possibility exists that this induced absorption could be an artifact. However, since we observe no induced absorption at any delay setting under conditions of solvent only, our inclination is to discount this possibility. The presence of AS is in agreement with the Martin et al.[6] observation showing photoproduct as early as 350 fs after excitation.

The –4-ps transient spectrum reveals a form of the complex having a maximum positioned at a longer wavelength has grown in. After removal of the ground-state spectrum from the –4-ps difference spectrum, this second intermediate, B, is seen as a weak band with a λ_{max} at about 430 nm. B is replaced with a photoproduct that has a maximum at the far-red end of the spectrum by 8 ps. Isolation of this intermediate C from the 8-ps difference spectrum reveals a species that has a λ_{max} centered at 442 nm with an extinction coefficient of approximately three-quarters that of the ground state. This band at the red end of the spectrum gradually blue shifts and narrows, clearly revealing the presence of the final intermediate of the photodissociation process, D, which has an extinction coefficient that is similar to that for C with an absorption maximum approximately 7 nm lower, at 435 nm. D is highly developed by 50 ps, and with only slight differences, it is identical with the ground-state deoxy **1** species. This in fact is the case for all complexes studied; the five-coordinate deoxy ground state of the parent complex is fully developed in less than 100 ps, a result very much different from hemoglobin, where it takes greater than 20 ns for the iron to relax out of the plane of the porphyrin.

B. 1-ST–CO. Experiments done at the earliest delays of –12 and –8 ps show that this compound is dissociating similarly to **1**–CO. At first, there is an increased absorbance at the wavelength of the ground-state species. However, as the photodissociation proceeds, a noticeable difference becomes apparent in the transient spectra between this compound and the unstrained species. Although the second intermediate B can be seen to be appearing by 0 ps, the disappearance of the ground-state species does not progress as it did in the previous compounds. At a time of 0 ps the disappearance of the ground-state complex is only about 50% complete. This indicates either that there is a delay in the disappearance of the ground-state species that could, for example, be caused by a bottleneck in the path of the dissociation or that there is another intermediate present in this photodissociation of the strained compound that is not present in the unstrained compounds. Removing the ground state A and the intermediate B from the –4-ps difference spectra reveals a photointermediate in the same wavelength range as the ground-state species. This intermediate has a spectrum that is characterized by a double maximum and an extinction coefficient approximately one-half of that of the ground-state compound. The new intermediate appears in the reaction pathway after the formation of the species AS but before B. The pathway for dissociation then continues as it does in the unstrained compounds. X reacts to produce B and then C is observed, with the shifting and narrowing of the band characteristic of the final reaction product D. The intermediates, B, C, and D, are, within the accuracy of the experiment, sequentially equivalent to those produced in the photodissociation of **1**–CO, with differing dynamics and spectral parameters.

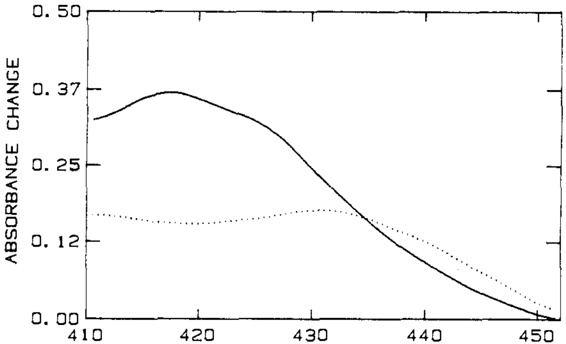


Figure 3. Ground-state spectra of the four-coordinate form of **1-ET** and **1-ET**–CO: six-coordinate (solid) and four-coordinate (dotted).

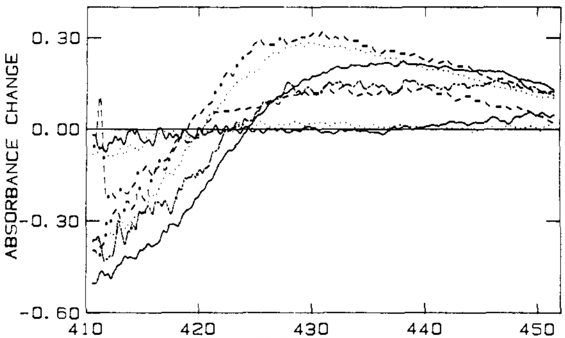


Figure 4. Experimental spectra for **1-ET**–CO. Delays range from –8 to 50 ps: –8 to +8 ps (solid), –4 to +20 ps (dotted), +4 ps (broken), and 0 to +50 ps (dashed).

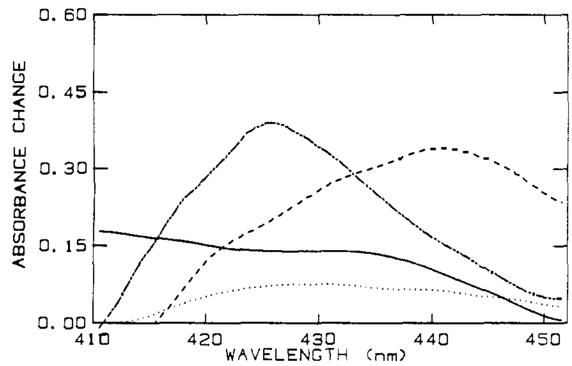


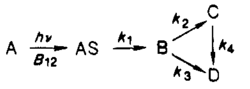
Figure 5. Spectra of the **1-ET**–CO intermediates: X (solid), B (dotted), C (dashed), and D (broken). Because of noisy conditions we were unable to collect data for delays of <–8 ps. As a result AS was not seen, though we believe it to be present in these earlier time forms.

C. 1-ET–CO. One of the advantages of examining this complex lies in the fact that the imidazole is incorporated as an external base that is not tethered to the porphyrin, allowing the ground-state spectrum of the four-coordinate species to be easily obtained. Figure 3 reveals that the spectrum of the four-coordinate ground state is characterized by a double-peaked band in approximately the same wavelength range as the six-coordinate ground state.

What can again be seen from workup of the transient absorption spectra of this strained complex is the early appearance of a new photoproduct X in the dissociative pathway that is not present in the unstrained CO complexes. The difference spectrum for the 0-ps delay shows only ~30% ground-state bleaching. Removing the remaining ground state and the intermediate B from this difference spectrum reveals the presence of X characterized by a double-peaked band. The transient absorption spectra and the spectra of the intermediates are shown in Figures 4 and 5. Because of background noise problems, we were unable to collect suitable data for this compound before −8 ps. As a result, AS has not been seen, though we believe it to be present in earlier time frames, as it is present for the other compounds studied. The spectroscopic parameters for **1-ET–CO**, λ_{max} (nm), fwhm (nm), and ϵ (upper estimates in M^{-1} cm^{-1}) are, respectively, A (ground state)[4d] 416, 28, 200K; X 380, 40, 120K; X 429, 25, 70K; B 430, 35, 25K; C 441, 32, 150K; and D 426, 78, 165K.

D. 1-ET–O_2. This complex is representative of the strained oxy heme compounds studied, and it gives clear evidence that these compounds photodissociate according to pathway I. Transient absorption data for this compound show no evidence of photoproduct X.

Kinetic Modeling. Kinetic modeling of the transient absorption spectra is necessary in order to obtain estimates of the lifetimes and rate constants associated with the evolution of the intermediates observed during the dissociation. A detailed description of the modeling may be found in our work on the picosecond photodissociation of the natural carboxy and oxy heme complexes.[2] The model is designed to treat the dissociation as a series of first- or second-order steps indicated in pathways I and II, ones that are consistent with the transient absorption data and with arguments based on bottlenecks encountered in the radiationless dissipation of energy in the manifold of ligand-field states, carboxy, oxy, and deoxy, available to the photodissociation. We have used simplified octahedral energy-level diagrams derived from extended Hückel calculations[5b,8] for this purpose. On the basis of direct and favorable comparisons between the results and analysis of our photodissociation work on the natural heme complexes and the results described above, we propose two kinetic schemes which adequately interpret our observations: mechanism 1 for the unstrained CO and strained or unstrained O_2 complexes and mechanism 2 for the strained CO complexes. Since geminate recombination was not observed for times of <50 ps, the rapid recombination step O_2 (or) $CO + C \rightarrow A$ (k_5) has been eliminated. Additionally, our experimental limit of 50 ps precluded examination of the slow O_2 (or) $CO + D \rightarrow A$ (k_6) recombination path from consideration.[2]



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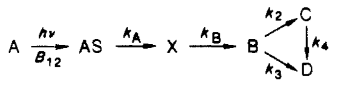

(2)

The analysis of the early <10-ps transient ΔA data is complicated by the fact that the pump and probe pulses each have 8-ps fwhm widths. Because of this, the pump pulse will excite different molecules at different times over a range of more than 8 ps. As the probe pulse passes through the sample it will see molecules at different stages of excitation and decay which in effect extends the range of the laser shot to over 16 ps and makes interpretation of the early time frame spectra difficult. This complication is resolved by analyzing the experimental absorbance change, ΔA (as a function of time delay Δt), using a Runge–Kutte–Nystrom numerical integration procedure that convolutes the pump and probe pulses together with the kinetic models for the photodissociation described above. The experimental parameters used in the modeling procedure include the λ_{max}, the ϵ_{max}, and the fwhm for the intermediates as well as these parameters plus the concentration for the six-coordinate ground-state complex. Initial guesses for the rate constants are also needed. These come from estimates of the intermediate lifetimes after workup of the transient ΔA data. For the calculation, all spectra are assumed to be Gaussian in shape. The rate constants are then adjusted to obtain the best computer fit to the experimental difference spectra for the entire set of probe–pump delays.

The calculation requires two sets of five differential equations to be solved simultaneously for mechanism 1 (two sets of six equations for mechanism 2). One set describes the interaction of the pump pulse with the sample and the resultant instantaneous concentrations of species A through D and the second set the apparent concentrations of these species as the probe pulse passes through the sample. Certain assumptions must be made in order to simplify the calculations. Amongst them, the light pulses are assumed to be an ensemble of photons whose cross section is Gaussian in shape, the excitation path is perpendicular to the sample cell, the volume swept out by the pump pulse, though in reality slightly conical in shape, is assumed to be cylindrical (the path of the interrogation pulse is considered to be cylindrical and coaxial with the pump pulse while in reality there is a 7° angle to the pump pulse, and further it is focused in the sample cell, resulting in an hour-glass-shaped volume), and finally the assumption is made that optical pumping caused by the probe pulse is negligible. The rate at which the ground state is depleted is expressed as a second-order rate equation, in terms of the laser pulse and the ground-state concentration, $-dA(t)/dt = B_{12}\rho(t)A(t)$. $\rho(t)$ is the photon density. B_{12} is the Einstein coefficient of absorption for the pump wavelength calculated as $B_{12} = \epsilon_{531}c \ln 10/N_0$, where c is the velocity of light and N_0 is Avogadro's number. Other details of the modeling may be found in ref 2.

There are a number of limitations in the modeling procedure that affect the accuracy of the curve fit between the modeled and the experimental difference spectra. As a result, the rates and lifetimes derived from the fit should be viewed as a means of achieving a better understanding of how the tertiary structure affects the dynamics or pathway of the photodissociation from a comparison of such data for complexes or classes of complexes, rather than as absolute rate data. The first limitation affecting the quality of the fit is the assumption of a Gaussian shape to describe the spectral bands of the intermediates and the six-coordinate ground state. A second limitation is that the fit of the computer-generated spectra in some cases was found to be best at time delays slightly different than those in the actual difference spectra. For example, difference spectra for time delays of 0, +4, and +8 ps in some instances were found to be best fit by computer-modeled spectra for time delays of +2, +6, and +10 ps. Such errors can be expected due to the experimental inaccuracy of the Duguay shutter-timing procedure. The relative time changes between the delay times, in this case 4 ps, are considered to be accurate. A third problem encountered is due to the group velocity dispersion or chirp in the pulse probe in the red end of the wavelength range studied.[2] The variations in velocity for various media in the laser apparatus were obtained, and the chirp was calculated to be 0.6 ps from the generation of the white light pulse in the continuum cell to the time the probe pulse interacts with the sample. For an intermediate such as AS which absorbs at the same wavelength as the ground state, no delay caused by chirp should be observed. For the intermediate C, however, whose λ_{max} is red shifted 20–30 nm from the ground state, the chirp would result in an additional delay in the arrival of the intermediate of approximately 0.5 ps. This type of delay was corrected for in the modeling procedure. One final limitation affecting the accuracy of the fit between the modeled and experimental difference spectra was the noise. This ranged from ±0.05 ΔA for the worst-case −12-ps delay at the blue end of the spectrum to better than ±0.02 ΔA for later delays.

The computer-modeled spectra for **1–CO** and **1-ET–CO** which illustrate the procedure are shown in Figures 6 and 7. For clarity, each figure contains curves generated for five of the highest delay settings, although curves for delays up to −20 ps were generated to establish the best overall set of rate constants. These figures may be compared to the experimental ones, as they are identically scaled. Once the experimental parameters λ_{max}, ϵ, and fwhm of the intermediates were established as discussed earlier, they remained invariant in the modeling procedure. We simply varied the rate constants to achieve the best overall fit for the set of delays. Because of inaccuracies due to the limitations discussed above

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(8) (a) Green, B. I.; Hochstrasser, R. M.; Weisman, R. B.; Eaton, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5255. (b) Chernoff, D. A.; Hochstrasser, R. M.; Steel, A. W. *Hemoglobin and Oxygen Binding*; Elsevier: Amsterdam, North Holland, 1982; pp 345–350. (c) Cornelius, P. A.; Hochstrasser, R. M.; Steele, A. W. *J. Mol. Biol.* **1983**, *163*, 119.

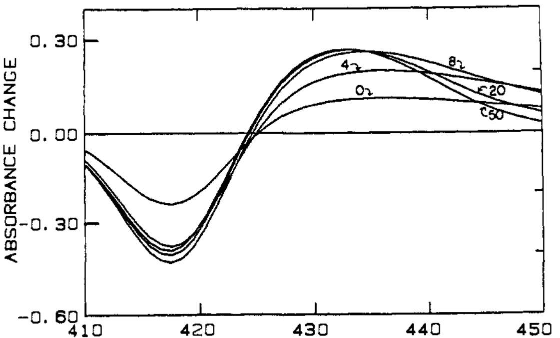

Figure 6. Modeled spectra for 1–CO at the delays indicated.

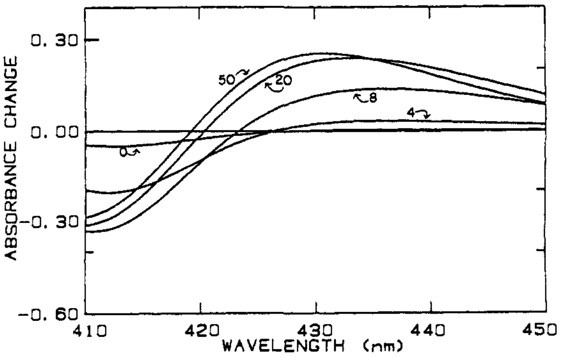

Figure 7. Modeled spectra for 1-ET–CO for the delays indicated.

Table I. Rate Constants for Complexes Dissociating According to Mechanism 1 (ps^{-1})

compd^a	k_2	k_3	k_4
1–CO	0.5	0.05	0.07
1–O_2	0.05	0.01	0.02
1-ET–O_2	0.05	0.02	0.02
1-AD–O_2	0.05	0.02	0.03
1-ST–O_2	0.5	0.02	0.03
2–CO	0.5	0.05	0.07

^{a}As the jitter in the pulse duration is nearly 1 ps, we have approximated k_1 as 4 ps^{-1} for each of the compounds studied to obtain a good fit to the experimental data for the earliest delays.

and our choice of not adding to the complexity of the modeling by varying the spectral parameters which was not justified, we did not expect to generate exact fits. Of primary importance in determining the best set of rate constants k_1 or k_A through k_4 was the quality of the fit in terms of the overall shape of the progressively delayed spectra, whether, for instance, the photoproduct spectra narrowed and blue shifted with successive delays. In some cases for a particular delay the absolute error in ΔA was as high as 0.06 when comparison is made to the smoothed experimental spectrum. However, because of the limitation of the model, we considered this to be an acceptable fit. With these considerations, we report the rate constants for the various compounds studied to one significant figure in Tables I and II and the lifetimes of the intermediates in Table III. Since the jitter in the pulse duration is nearly 1 ps and because the noise in the −12-ps delay experiment is severe, we can only approximately establish k_A or k_1 as 4 ps^{-1} ($\tau_{AS} \sim 0.25$ ps).

Discussion

With reference to the octahedral energy-level diagrams for oxy, carboxy, and deoxy hemoglobin derived from extended Hückel calculations[6b,9] and our earlier work on the natural heme complexes, the results of this study can be discussed. Assignments discussed in this section are based on transient $\pi\pi^*$ absorbance changes polarized in the plane of the porphyrin. Although they are sensitive to the localized heme geometry in terms of whether it is four, five, or six coordinate, they contain only small metal d orbital contributions. Consequently, assignments which are discussed in terms of metal d–d transitions should be viewed as tentative until other time-resolved picosecond methods of suitable resolution are developed and applied to these systems. These methods would necessarily need to directly interrogate metal d–d transitions or specific normal modes localized to the heme, namely, CD, MCD, or RR.

Table II. Rate Constants for Complexes Dissociating According to Mechanism 2 Showing the Pseudo-Four-Coordinate Intermediate X (ps^{-1})

compd^a	k_B	k_2	k_3	k_4
1-ST–CO	0.2	0.02	0.03	0.03
1-ET–CO	0.2	0.08	0.01	0.03
1-AD–CO	0.1	0.04	0.02	0.05

^{a}As the jitter in the pulse duration is nearly 1 ps, we have approximated k_A as 4 ps^{-1} for each of the compounds studied to obtain a good fit to the experimental data for the earliest delays.

Table III. Lifetimes of Intermediates (ps)

compd^a	τ_X	τ_B	$\tau_C{}^b$
1–CO		2	15
2–CO		2	15
1-ST–CO	5	20	33
1-ET–CO	5	11	33
1-AD–CO	10	17	20
1–O_2		16	50
1-ST–O_2		2	33
1-ET–O_2		14	50
1-AD–O_2		2	33

^{a}As the jitter in the pulse duration is nearly 1 ps, we have estimated the lifetime of AS to be 0.25 ps for all compounds studied to obtain a good fit to the experimental data. ^{b}No geminate recombination (C → A) present.

A. Intermediates for the CO Complexes. The first intermediate seen in very early time frames, AS, is manifested by an induced absorption in the area of the peak wavelength of the ground state $^1A_1$. Referring to the previous section, we do not believe this transient to be an artifact since it is seen in all the compounds for which we have early-delay data; it is not seen when solvent is used in place of sample, and its observation is in agreement with the experiments of Martin et al.[6] indicating that photoproducts appear as early as 350 fs after excitation. The presence or absence of AS is not critical to the subsequent kinetic analysis of the photodissociation since its lifetime, <0.5 ps, is at least a factor of 5 shorter than the lifetime of B. Proceeding with the assumption that AS is real, then rapid vibronic relaxation would follow the absorption of a 531-nm photon in the Q band to the vibrationless origin of the ^1Q state. It is possible that this relaxation and the subsequent rapid relaxation into $^1T_1$ directly below ^1Q could be manifested through an $S_N \leftarrow S_1$ absorption process originating from ^1Q. It is also possible that the induced absorption could be a result of slight geometry changes in the heme associated with $^1T_1$ acting as a bottleneck in terms of the subpicosecond internal conversion from ^1Q itself. We are inclined to assign AS to an intermediate from $^1T_1$ as this is the lowest excited singlet of the six-coordinate complex. This assumes that $^1T_1$ is not the photoactive state that promotes the photodissociation as proposed by Waleh and Loew.[5c–e]

Once relaxation to the lowest excited singlet has occurred, the next intermediate expected would be due to the bottleneck in the six-coordinate manifold caused by intersystem crossing into the $^3T_1$ and $^5T_2$ states that lie below $^1T_1$. $^3T_1$ and $^5T_2$ are very close in energy, and it is likely that the radiationless transition from the singlet $^1T_1$ state would result in the population of both of these states with interconversion between the two. We suggest that photoproduct B is a transient species composed of a mixture of these six-coordinate states characterized by a low extinction coefficient and a maximum wavelength found about midway between $^1A_1$ and the λ_{max} of the next intermediate C. Intersystem

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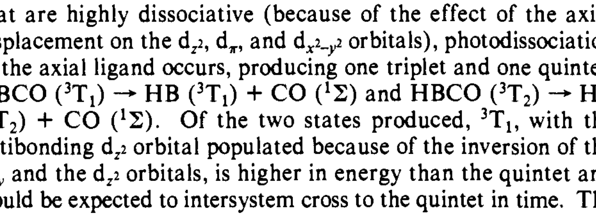
[9] (a) Gelin, B. R.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 801. (b) Baldwin, J.; Chothia, C. *J. Mol. Biol.* **1979**, *129*, 175.

crossing from $^1T_1$ to $^3T_1$ in the six-coordinate manifold does not necessarily lead to a configuration that is dissociative, since the d_{z^2} orbital is not occupied: $d^4_\pi d^1_{x^2-y^2} d^1_{xy} d^0_{z^2}$. However, conversion into the $^5T_2$ state may, in the six-coordinate manifold, result in an unstable dissociative configuration, as the parent quintet state originates via $d_{\pi,x^2-y^2} \rightarrow d_{z^2}, d_{xy}$ orbital promotions giving rise to the occupancy $d^3_\pi d^1_{x^2-y^2} d^1_{xy} d^1_{z^2}$. As Waleh and Loew[6c–e] have shown, both $d_\pi \rightarrow d_{z^2}$ and $d_{x^2-y^2} \rightarrow d_{z^2}$ decrease in energy as a function of increased Fe–CO bond distance promoting the dissociation process. Unfortunately, the combined effect of the imidazole–iron–CO (or O_2) stretch on these transitions has not yet been reported. We may assume, though, that the $^5T_2$ state is photoactive, and population of it could result in the tetragonal displacement of both axial ligands. These arguments would be applicable to both the CO and O_2 ligands and the imidazole. Thus we consider the intermediate B to be an unresolved mixture of the $^5T_2$ and $^3T_1$ states with the possibility of interconversion between them. When the potential surfaces of these states cross over into the corresponding ones in the five-coordinate manifold that are highly dissociative (because of the effect of the axial displacement on the d_{z^2}, d_π, and $d_{x^2-y^2}$ orbitals), photodissociation of the axial ligand occurs, producing one triplet and one quintet: HBCO ($^3T_1$) → HB ($^3T_1$) + CO ($^1\Sigma$) and HBCO ($^3T_2$) → HB ($^5T_2$) + CO ($^1\Sigma$). Of the two states produced, $^3T_1$, with the antibonding d_{z^2} orbital populated because of the inversion of the d_{xy} and the d_{z^2} orbitals, is higher in energy than the quintet and would be expected to intersystem cross to the quintet in time. The evolution of the intermediates shows the production of a species at the red end of the spectrum which progressively narrows and whose maximum wavelength shifts to a lower λ. Intermediate C has been seen in the photodissociation of both the CO and O_2 forms of hemoglobin.[2] This shifting of the wavelength maximum can be attributed to the production and subsequent reaction of an intermediate with a relatively high λ_{max}. We assign C to $^3T_1$.

C can then undergo further intersystem crossing to the $^5T_2$ state, D. The final intermediate produced in the photodissociation of the synthetic hemes, D, has been shown to be, within experimental error, equivalent to the ground deoxy state. D correlates with the production of the quintet state, both directly from B and through further reaction of the deoxy state C. Therefore, we assign D to $^5T_2$ and suggest that the equivalence of D to the ground deoxy state supports the fact that the iron has fully relaxed out of the heme plane. Thus, for the unstrained hemes, the photodissociative pathway is entirely consistent with that for hemoglobin,[2] where the rate constants and lifetimes are listed in Tables II and III.



For the CO forms of the strained compounds studied, the photodissociative pathway is noticeably different in the sequential appearance of an intermediate not seen in the unstrained synthetic complexes or in the natural heme complexes. After relaxation to the lowest excited singlet $^1T_1$, the strained compounds show evidence of an intermediate X before intersystem crossing to B. In all strained compounds tested, this photoproduct is characterized by a spectrum having a double maximum in the region of the six-coordinate ground-state species. Because of similarities in its spectral characteristics to the corresponding four-coordinate (axially unligated) band found in the ground-state species, we are inclined to suggest that this intermediate is indicative of a pseudo-four-coordinate species. The similarities in spectral band shape are apparent in Figures 6 and 7. Since AS and B are spectrally very similar to the corresponding intermediates in both the unstrained and the natural hemes and since these intermediates are both considered to be six-coordinate species, it is highly unlikely that X is a true four-coordinate complex. Instead it is more probable that it is a six-coordinate species in which the Fe–CO and Fe–imidazole bonds are stretched and weakened to the stage where the complex behaves more like it is four coordinate than six coordinate. Our experiment follows the evolution of these intermediates by monitoring the $\pi\pi^*$ porphyrin Soret absorption changes as a function of time. Since such changes in the Soret wavelength maximum, intensity, and band shape are known to be extremely sensitive to the state of coordination of the heme complex, this could account for the small differences between the spectrum of the intermediate X and that of the ground state of the four-coordinate species.

It is difficult, if not impossible, to justify a theoretical mechanism for the formation of this pseudo-four-coordinate species, X, without first a great deal more theoretical work, which might involve extensions of existing investigations or new approaches that would examine the effect of geometry on the dissociation of both axial ligands, and second new time-resolved spectroscopic investigations of the photodissociation by monitoring, directly, various critical d–d transitions or d–ligand charge-transfer transitions or from direct structural studies employing resonance Raman technique(s). However, we might envision the formation of X by invoking the Waleh–Loew[6c–e] $^1T_1$ ($d_\pi \rightarrow d_{z^2}$) suggestion that has already been discussed. The essential difference here is that there is considerable strain on the Fe–imidazole bond, resulting in corresponding and favorable adjustments to the disposition of the $d_\pi \rightarrow d_{x^2-y^2}$ transition energies, making the $^1T_1$ route competitively dominant over that of the bottleneck mechanism. Thus it can be supposed that rapid conversion from ^1Q into one of the $^1T_1$ states below it would result in a pseudo-four-coordinate complex where the axial bonds to the iron have weakened considerably. It is likely that this pseudo geometry would be followed by movement of the iron relative to the plane of the porphyrin to release this strain and return the complex to the six-coordinate species B. Consequently, the lifetime of B would necessarily be lengthened, as verified in Table III. The dissociation then proceeds according to the mechanism discussed earlier.

B. Intermediates for the O_2 Complexes. The results obtained on the oxygen forms of the compounds studied revealed a similar set of intermediates, AS, B, C, and D, though the spectral parameters and rates associated with formation of the intermediates are in many cases noticeably different from those of the CO forms. As C and D are postdissociative intermediates, it would be expected that the spectral characteristics of these intermediates should be fairly similar for either the CO or the O_2 forms of the parent compounds, and they are. For example, the interconversion rates k_4 for C → D are very similar. Assigning the intermediate B to the same states proposed for the CO complexes cannot be done, as the mechanism for photodissociation is undoubtedly different. The process is complicated by the fact that there are low-lying singlet and triplet charge-transfer states present in the six-coordinate oxygen species that open up several competitive routes leading to photodissociation. Possibilities for these competitive paths have been discussed by Waleh and Loew,[5c–e] who conclude that the higher energy channel, which is not accessible by the Q band pump, is $d_\pi \rightarrow d_{z^2}$ in nature. In the octahedral scheme, this possibility competes with the $d_{\pi x^2-y^2} \rightarrow d_{z^2 xy}$ promotion associated with the quintet state. They suggest that competitive decays to the lower charge-transfer states from this transition and to the ground state from these low-lying charge-transfer (CT) states account for the low quantum yield of O_2 photodissociation. It is also worth noting that these low-lying CT states are not readily accessible from the $\pi \rightarrow \pi^*$ porphyrin singlet due to the small value of the Franck–Condon spatial overlap between the two states.

Taking these facts into consideration, AS can be assigned to the lowest excited singlet ^1Q. The quintet $^5T_2$ and the triplet $^3T_1$ states in the six-coordinate manifold are much farther apart in the oxygen complexes than they are in the CO form, with the $^5T_2$ state lying very close in energy to the singlet ^1Q state. Because of this, the intermediate B, considered a mixture of the two states, would be expected in the oxygen compounds to consist mainly of the triplet $^3T_1$ state mixed with the CT states. Dissociation of the ligand will again produce the triplet $^3T_1$ and the quintet $^5T_2$

states of the deoxy heme. The $^3T_1$ deoxy heme will then decay into the ground deoxy state, $^5T_2$. A comparison of the final intermediate D with the intermediate D for the carbon monoxide experiments shows that, within experimental error, these states are identical, as would be expected if the species D represented the ground deoxy state of the heme.

Studies on the oxygen forms of the strained compounds show no evidence for the intermediate X. One possibility is that the reaction X → B is extremely fast, as might be expected for the essentially geminate recombination of $^3O_2$ back to the heme due to favorable spin-allowed pathways (see below). Similar argu-

$$\mathrm{HBO_2\ (^5T_2) \rightarrow HB\ (^3T_1)\cdots O_2\ (^3\Sigma_g^-)}$$

$$\mathrm{HBO_2\ (^3T_1) \rightarrow HB\ (^5T_2)\cdots O_2\ (^3\Sigma_g^-)}$$

ments were discussed by Cornelius et al.[8c] in regard to the ultrafast picosecond relaxation found in nitrosylhemoglobin. Another possibility is that the direct dissociation of the oxygen is more sensitive to strain than in the carbon monoxide complexes and is simply more efficient and competitively dominant over the pseudo-four-coordinate pathway.

C. Geminate Recombination. Both the experimental and the modeling results discussed earlier indicate that the short-term O_2 + C → A geminate recombination pathway is inoperative for any of the synthetic compounds studied. If there is any recombination at all, the estimate for the return is less than 5% by 50 ps. The CO forms of these compounds show no evidence of short-term geminate recombination as well. In the modeling studies the short-term recombinant pathway was nevertheless included for completeness since return to the ground state with lifetimes of less than 20 ps could effectively compete for C. Not surprisingly, the results contrast sharply with the short-term geminate recombination pathway found in hemoglobin and myoglobin.[2] The absence of fast geminate recombination in the synthetic compounds is most likely due to the absence of the protein surrounding the heme pocket in terms of the role it plays in trapping the oxygen near the heme.

D. Kinetic Modeling. For all the synthetic heme complexes studied, the lifetime of the first intermediate AS was on the order of 250 fs in agreement with the femtosecond study of Martin et al.[6] As the jitter in the pulse duration is on the order of 1 ps, the AS lifetime is very approximate and chosen to obtain the best fit to the experimental spectra.

The lifetimes for the intermediate B, the species considered to be a mixture of the lowest excited triplet and quintet states of the six-coordinate species, vary widely between the compounds. This lifetime, $\tau_B = 1/(k_2 + k_3)$, ranges from $\simeq 2$ ps to as high as 20 ps for the various complexes studied, with an average lifetime of 9.5 ps for this intermediate. One fact that should be noted is the lifetime of B in the carbon monoxide forms of the compounds studied. For the two unstrained chelate hemes **1**–CO and **2**–CO the lifetime for this intermediate is very short, on the order of only 2 ps, indicating a fast dissociation of the axial ligand CO from a photoactive state. However, in the remaining CO complexes the situation is much different. The three other carbon monoxide complexes studied as strained compounds that photodissociate through X, a photoproduct that we have assigned as a pseudo-four-coordinate intermediate. In these complexes the lifetime for B is found to be 5–10 times longer than in the unstrained species. X has been postulated to be a species in which the axial bonds of the iron to both the CO and imidazole are considerably weakened. This geometry would likely be followed by a movement of the iron to release strain on the complex and return it to the six-coordinate species. As mentioned earlier, this process should result in a lengthening of the lifetime of B, which is indeed what has been found in these cases.

The lifetime of intermediate C for these complexes should be found to be independent of the ligand present on the parent species, since C has been assigned to a triplet deoxy state in which the axial ligand has been dissociated. As can be seen in Table III, in most cases lifetimes τ_C are found to compare favorably between the carbon monoxide and oxygen forms of the synthetic complexes. In all cases the rate constants used had an accuracy of only one significant figure, resulting in an accuracy no better for the lifetimes of these states. One noticeable discrepancy in τ_C occurs between the chelated protoheme **1**–CO and the oxygen form of this compound, **1**–O_2.

Conclusions

Using standard pump–probe picosecond methods, we have examined the picosecond photodissociation of the O_2 and CO forms of a number of natural[2,3] and synthetic heme complexes by following $\pi\pi^*$ Soret absorption changes localized to the plane of the porphyrin. Keeping in mind that the absorption characteristics of this band are very sensitive to the coordination and geometry of the heme, our collective results for the natural and synthetic complexes clearly reveal the importance of heme geometry and strain on the dynamics and mechanisms of the dissociation. We have interpreted our results on the synthetic and natural heme complexes as occurring through a series of sequential steps that are evident in the transient photoproducts which we have isolated and characterized spectroscopically and kinetically. Of the four intermediates observed in the unstrained and natural heme complexes, we have assigned the first two as originating from excited predissociative states found in the six-coordinate manifold and the last two as postdissociative states found in the five-coordinate manifold. This interpretation of the photodissociation of the CO complexes is consistent with a series of bottlenecks encountered during the deactivation and dissociation processes. The bottleneck possibility was first discussed by Green and Hochstrasser.[8a] However, this work, along with our earlier work,[2] is the first to discuss the AS and B intermediates, as well as provide evidence for a pseudo-four-coordinate intermediate found early in the dissociation of certain synthetic complexes having strained imidazole–Fe linkages.

The differences that we see in the picosecond photodissociation between the natural and synthetic complexes may be linked to important structural differences in the heme pocket or constraints on the teritary geometry of the heme imposed by the protein. In particular we would like to comment on the proximal imidazole. Recent theoretical,[9a] X-ray structural,[9b] and resonance Raman work[3] all suggest that the affinity of the sixth axial ligand for the heme is critically dependent on the teritary heme structure that originates from the linkage of the imidazole to the heme. In the natural complexes, this imidazole (His F8) and the F helix to which it is bound undergo major structural movements; these changes for the α and β subunits include a 1-Å translation of the F helix across the porphyrin face and a movement of the imidazole from a position that is asymmetric with respect to the porphyrin nitrogens to one that is more symmetric. In this regard, Table III shows some trends. Preliminary results in our laboratory indicate that intermediate C in the α Hb complex has a much longer lifetime than the other natural complexes studied, possibly because quaternary constraint(s) present in the other natural complexes studied are absent or at least relaxed in the α complex. Similarly, we find that all the synthetic complexes are characterized by relatively long C lifetimes where such constraint is absent. It appears that imidazole strain is most effective in lengthening the lifetime of B in the CO forms of the synthetic complexes studied.

The results presented in this work coupled with our earlier work[2] suggest that the proposal offered by Waleh and Loew with regard to dissociation is not necessarily correct for the unstrained synthetic and natural compounds studied.[5c–e] Their results suggest that population of the $^1T_1$ state ($d^2_{x^2-y^2}d^3_\pi d^1_{z^2}$) found just below the ^1Q state should lead to prompt dissociation though the singlet $d_\pi \rightarrow d_{z^2}$ pathway. If this pathway dominates, then pumping the ^1Q band would be followed by rapid radiationless singlet interconversion to $^1T_1$, leading to a fast, possibly subpicosecond, dissociation. Possibly, with sufficient strain in the Fe–imidazole bond, favorable adjustments to the $d_\pi \rightarrow d_{z^2}$ and $d_{x^2-y^2}$ transitional energies occur, allowing the $^1T_1$ route to competitively dominate over the bottleneck mechanism as suggested by our evidence that supports a pseudo-four-coordinate complex. Otherwise, our results on the synthetic and natural complexes show that the photodissociation initiated by pumping the Q band, as judged by the lifetime of B, takes some picoseconds to occur and depends on the heme geometry. To the extent that the photodissociation has its beginnings in the subpicosecond regime, we are in agreement with the femtosecond report of Martin et al.[6] However, in their study on CO complexes of Hb and Mb a deoxy-like species appeared with a time constant of 350 fs which had spectral characteristics that did not change for 100 ps. At the moment, we cannot explain the difference between our results and this femtosecond observation in regard to the evaluation of band shape in the transient difference spectra. Earlier picosecond work showed evidence and discussed the development of the deoxy photoproduct band shape changes that we have identified with intermediates C and D.[8] As the ligand-field level system is complex, the possibility exists that at the 307-nm pump wavelength of the femtosecond experiment a fast photodissociative channel is available that is not otherwise open with the Nd^{3+} 353- or 531-nm pump wavelengths.

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First Observation of 5-Coordinate Aluminum by MAS ^{27}Al NMR in Well-Characterized Solids[†]

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Abstract: The first magic angle spinning ^{27}Al NMR spectra of 5-coordinate aluminum in well-characterized solids are reported. Two solids containing 5-coordinate aluminum, barium aluminum glycolate and the mineral andalusite, were examined. The signal is upfield of that for tetrahedral aluminum and downfield of that for octahedral aluminum. All of the aluminum in barium aluminum glycolate is in highly distorted, AlO_5 trigonal bipyramids. These AlO_5 groups exhibit a complex line shape at δ 37 at 11.7 T. Andalusite has highly distorted AlO_5 and AlO_6 species. Even at 11.7 T, the trigonal bipyramidal AlO_5 species exhibits a substantial second-order quadrupole-induced broadening and upfield shift of the centerband ($e^2qQ/h = 5.9$ MHz and $\eta = 0.70$), whose center of gravity is at δ 21 (δ 35 after correction for second-order quadrupole effects). The signal for the AlO_6 species is extremely complex and shifted far upfield. The advantages of using MAS and VAS in studying the NMR of nonintegral spin quadrupolar nuclei in solids that exhibit complex spectra are discussed.

In the last several years, many papers have appeared on MAS ^{27}Al NMR studies of zeolites, clays, minerals, etc.[1-3] Consequently, much more is now known about the tetrahedral and octahedral aluminum in these species. In contrast, relatively few 5-coordinate aluminum species are known: the minerals andalusite, grandidierite, yoderite, augelite, and senegalite,[4] as synthesized $AlPO_4$-12,[5] as synthesized $AlPO_4$-21,[4,6] and several organic complexes.[7-13] We are unaware of any magic angle or variable angle spinning ^{27}Al NMR work that has conclusively shown the presence of 5-coordinate aluminum in a solid. However, there are at least seven reports[14-20] involving ^{27}Al NMR spectra of 5-coordinate aluminum species in liquid solutions. Where data are available,[14,15,17-20] 5-coordination results in a substantial upfield shift relative to 4-coordination. This paper reports the first examples of magic angle and off angle spinning ^{27}Al NMR spectra of species containing 5-coordinate aluminum.[21] The complex barium aluminum glycolate[13] and the mineral andalusite were chosen for study to avoid any complications that might occur in assigning the signal for 5-coordinate aluminum if atoms such as phosphorus,[24-28] nitrogen,[29,30] or boron[31] were also part of the framework. The spectra of barium aluminum glycolate and andalusite show that the signal for 5-coordinate aluminum is upfield of the signal for tetrahedral aluminum but downfield of the signal for octahedral aluminum.

Experimental Section

1. Synthesis of Barium Aluminum Glycolate ($BaAlO_8C_{6.75}H_{14.75}$).[13] A modification of the procedure of ref 13 was used.

Barium hydroxide octahydrate (31.5 g, 0.10 mol) was partially dissolved in distilled water (16.2 g, 0.90 mol), and then aluminum isopropoxide powder (40.8 g, 0.20 mol) was added to ethylene glycol (248 g, 4.0 mol). The barium hydroxide suspension was added to the alu-

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[†] However, see Note Added in Proof. Except for this note, the manuscript reflects our knowledge when the manuscript was initially submitted (February 1986).

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