Communications to the Editor

Resonance Raman Spectroscopy in the Picosecond Time Scale: The Carboxyhemoglobin Photointermediate[1]

Sir:

Time-resolved resonance Raman (RR) detection of photochemical and photobiological intermediates has been demonstrated on the milli-,[2] micro-,[3] and nanosecond[4,5] time domains during the past few years.[6] With picosecond pulses, Raman spectra of stable species, but not of short-lived transients, have been obtained.[7] In this communication, we report the first observations of the RR spectrum of a photointermediate obtained within picoseconds of its formation.

The photointermediate studied is that of carboxyhemoglobin (COHb) that was previously detected by picosecond *optical* methods.[8-10] Broad optical absorption spectra, however, do not yield so detailed structural information as vibrational spectra; thus, several attempts have been made to identify this intermediate by transient RR methods. Nanosecond RR spectra have been found to be similar to that of the deoxyhemoglobin[5] (deoxyHb) and have been interpreted in terms of relaxation of the iron-porphyrin system within several nanoseconds after dissociation. In an attempt to examine the structure of the picosecond iron porphyrin intermediate,[6-10] therefore, we developed a picosecond RR technique.

A flow system, similar to the one used to determine nanosecond RR spectra of the photointermediates of bacteriorhodopsin,[4b] was employed in the present work. A train of picosecond pulses was focused on a jet stream of nonrecirculating aqueous solution of COHb. The residence time of the sample in the laser beam is shorter than the time between pulses. Scattered photons from both unphotolyzed COHb and photointermediate formed during the $\sim$30-ps pulse were collected at 90° and analyzed with a 0.5-m Spex 1870 monochromator equipped with a cooled image-intensified Vidicon (PAR SIT 1205A/D, 1207). The laser system[11] consisted of an extended Spectra-Physics 375 dye cavity fitted with a cavity-dumper that was synchronously pumped by a mode-locked Spectra-Physics 171 argon-ion laser operated at 2-W average power at 515 nm. Mode-locking was achieved with a Spectra-Physics Model 466 mode-locker/cavity-dumper driven by a Programmed Test Sources PTS-160 frequency synthesizer. The output of the Rhodamine 560 (Exciton) dye laser was tuned to 576 nm and narrowed to 0.05-nm line width with Spectra-Physics standard wedge and etalon. At a 0.84-MHz dumping rate, the average output power of the dye laser was $\sim$8 mW, equivalent to $\sim$10 nJ/pulse. The duration (full width/half height) of the output pulses obtained under these conditions has been demonstrated to be $<$30 ps both by two-photon mixing autocorrelation observations and direct observation with commercial streak cameras. Pulses of $\sim$20-ns duration were obtained by CW pumping the cavity-dumped dye laser.

When the laser was diffusely focused, Raman spectrum of unphotolyzed COHb was obtained which agrees with that previously reported.[12] Tight focusing, however, produced three bands between 1540 and 1620 cm^{-1} that are distinct from bands of COHb or deoxyHb.[13] These three bands are attributable to an intermediate in COHb photolysis which develops within 30 ps of the excitation.

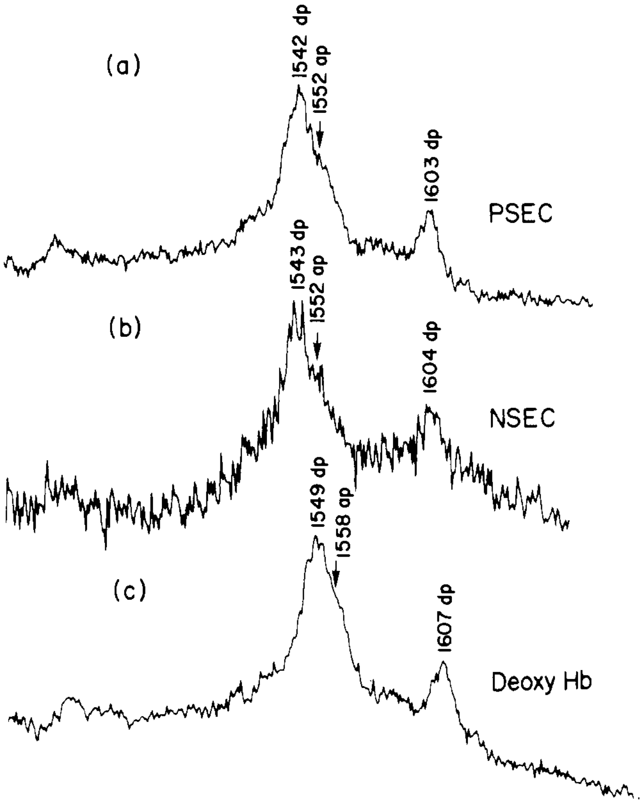

Figure 1. (a) Resonance Raman spectrum of the phototransient of COHb obtained by computer subtraction of partially photolyzed and nonphotolyzed flowing COHb using 10-nJ laser pulses of $\sim$30-ps duration at 0.8 MHz and excitation at 5760 Å. (b) Identical with (a) except that the pulse duration was increased to 20 ns. (c) Steady-state resonance Raman spectrum of deoxyHb in a melting point capillary using 25 mW of CW laser excitation at 5752 Å.

The spectrum of the photointermediate, obtained by computer subtraction of the COHb spectrum, is shown in Figure 1a. It is similar to the CW spectrum of deoxyHb (Figure 1c), but the frequencies are 4–7 cm^{-1} lower. The same shifts have been observed with 5–10-ns YAG laser pulses by Lyons et al.[5c] and confirmed by Woodruff.[14] We have determined the polarizations of the bands at 1603, 1552, and 1542 cm^{-1} and found them to be depolarized, anomalously polarized, and depolarized, respectively. This identifies them as structure-sensitive porphyrin bands V, IV, and III, respectively.[15] Bands IV and V correlate with porphyrin core size.[15,16] Their relatively low values in deoxyHb, 1607 and 1558 cm^{-1}, are associated with an expanded core of the high-spin five-coordinate Fe^{II} complex.[18] The still lower values of the photointermediate indicate an even more expanded core as suggested by Lyons et al.[5c] which would be expected for a high-spin Fe^{II} heme with the iron atom in the porphyrin plane. The high-spin six-coordinate bistetrahydrofuran adduct of iron(II) tetraphenylporphine (Fe^{II}TPP)[19] provides an example for comparison. The core size (C_t–N) of this complex where Fe^{II} is in the porphyrin plane is 2.057 Å. This is 0.013 Å greater than the size of the core of the five-coordinate high-spin 2-methylimidazole adduct of Fe^{II}TPP.[18] The 0.013-Å expansion is expected to lower bands IV and V by 7.2 and 5.5 cm^{-1}, respectively, on the basis of the established core size correlations.[17] This is within experimental error of the observed 6- and 4-cm^{-1} lowerings of the frequencies of these bands. Indeed, we find[20] that bistetrahydrofuran iron(II) octaethylporphyrin shows the same

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frequencies as those for the photointermediate for bands IV and V. We thus infer that photolysis of COHb leaves Fe^{II} transiently in the heme plane but changes the spin state from $S = 0$ (in the COHb ground state) to $S = 2$ within 30 ps. This must occur in an excited state, since the thermal interconversion rate between $S = 0$ and $S = 2$ states in Fe^{II} complexes lies in the 10^7–10^8-s^{-1} range.[21]

The 576-nm pulses excite the lowest porphyrin singlet π–π^* (Q) state. Although this or the corresponding π–π^* triplet state might be directly responsible for CO photodissociation,[22] the energy also could funnel into a lower-lying ligand field state, as suggested by Shank et al.[8] Eaton et al.[23] place the $^1T_1$ state of COHb just below the Q state; the lowest energy state is estimated to be $^3T_1$ but $^5T_1$ is nearby.[10] The latter is the most plausible parent state for a high-spin photoproduct. "CO photodissociation" may leave the iron porphyrin structure in spin and geometry similar to the "dissociative" excited state.[24] (The large Franck–Condon factor for such a channel would imply a fast dissociation rate.) The broadened deoxyHb-like absorption spectrum detected by Greene et al.[10] within 8 ps of photolysis may be associated with the in-plane, high-spin photointermediate suggested by the Raman spectrum.

The broadened absorption is found to last 680 ps following photolysis,[10] and the Raman frequency lowering relative to deoxyHb persists to 10 ns.[5c] The middle spectrum of Figure 1 shows that the frequencies are the same when the photolysis pulse is widened even to 20 ns. The agreement between this spectrum and that obtained with the YAG laser pulses[5c] is important since the pulse energy of the latter (20 mJ) is much higher than in our experiment (6 nJ) and might have produced multiphoton effects. Further RR experiments in a time scale longer than 20 ns are needed to determine the time required for the iron atom and its proximal imidazole ligand to move away from the heme plane to its characteristic position in deoxyHb.[25]

References and Notes

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Can Cyclopropyne Really Be Made?

Sir:

Monocyclic, otherwise saturated acetylenes, are of considerable current interest in organic chemistry. For example, Anet and Rawdah[1] recently examined the major conformational features of cyclododecyne (twelve carbon atoms, one triple bond) using DNMR. A question of long-standing and fundamental interest concerns the size of the smallest possible, otherwise saturated ring containing a triple bond. The smallest such ring for which a complete structural determination is available is apparently cyclooctyne. However, Chapman[3] has recently synthesized the much smaller cyclopentyne and has speculated that both cyclobutyne and cyclopropyne should exist.

We were uncertain as to whether the triple-bond cyclopropyne species is a relative minimum on the C_3H_2 potential surface. Minimum basis set SCF studies by Pople and coworkers[4] suggest that the singlet lies considerably above the vinylidene $CH_2{=}C{=}C{:}$. Further, preliminary theoretical work here raised the possibility that the singlet isomerization to propadienylidene (eq 1) might proceed with no barrier at all. For these reasons it was decided to pursue this possibility in theoretical detail and also examine triplet cyclopropyne. The results should provide definitive answers concerning the ultimate limit of stability of cyclic alkynes.

(1)

The first step in the present research was to determine the equilibrium geometries of singlet and triplet ($^3B_2$) cyclopropyne within the constraint of C_{2v} symmetry. For this purpose a standard double zeta (DZ) basis set,[5] labeled C(9s 5p/4s 2p), H(4s/2s), was chosen. From the fact that Hehre et al.[4] used complex molecular orbitals to describe singlet cyclopropyne, this species is expected to have considerable diradical character. Therefore a two-configuration self-consistent-field (TCSCF) procedure[6] was adopted:

$$\psi = c_1 2b_1^2 6a_1^2 + c_2 2b_1^2 3b_2^2 \qquad (2)$$

At the predicted equilibrium geometry for the $^1A_1$ state, the two mixing coefficients are $c_1 = 0.933$ and $c_2 = -0.359$. For the $^3B_2$ state a single configuration description—$2b_1^2 6a_1 3b_2$—is appropriate. Constrained C_{2v} equilibrium structures were determined using recently developed gradient procedures[7] for open-shell and multiconfiguration SCF wave functions. The predicted structures are seen in Figure 1.

The location of C_{2v} minima for singlet and triplet cyclo-