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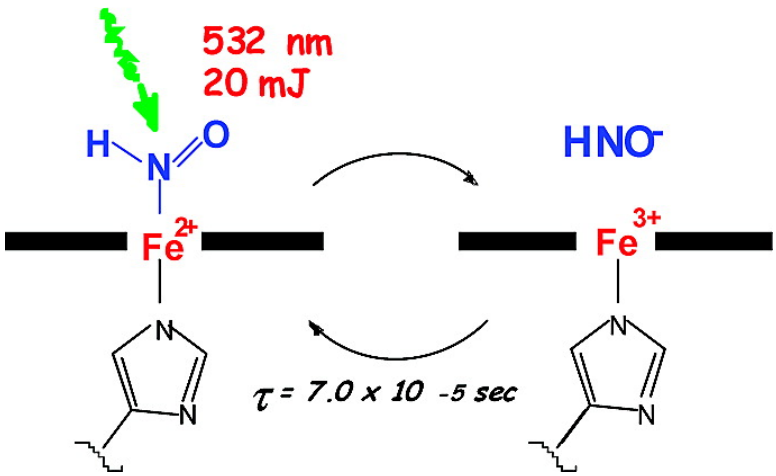

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Photolysis of the HNO Adduct of Myoglobin: Transient Generation of the Aminoxyl Radical

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Nitroxyl or nitrosyl hydride, HNO, is the one-electron reduced form of nitric oxide in aqueous solution.[1] We have demonstrated that deoxy myoglobin (Mb) traps free HNO to form a stable adduct, HNO-Mb,[2] which has been characterized by ^{1}HNMR,[3,4] resonance Raman, and XAS.[5] There remain ambiguities in bonding description of this species[6] as well as in its possible involvement in the observed activities of HNO-releasing compounds on cardiovascular disease.[7] HNO-Mb is very stable, with a half-life of greater than 6 months at 4 °C in the absence of light and air.[8] Prolonged laser excitation at 413 nm during resonance Raman experiments resulted in the appearance of an absorption band at 1373 cm^{-1} attributable to metMb.[5] Likewise, its thermal decomposition to metMb at 37 °C doubles in rate under ambient light (see Supporting Information (SI)).

To understand this behavior, photolysis of HNO-Mb was investigated using nanosecond-pulsed transient absorbance experiments, a method widely used to obtain rates of geminate recombination, escape, and bimolecular rebinding of heme-bound diatoms like O_2, NO, and CO in proteins.[9] For example, the photodissociation of NO-Mb has been extensively investigated: the photoliberated NO rebinds to the ferrous heme very efficiently with the quantum yield for NO release at less than 0.001 and geminate recombination rate constant on the order of 100 picoseconds.[10]

For HNO-Mb, initial transient absorbances traces obtained from excitation at 532 nm showed distinctive absorbance changes on two timescales, a dominant millisecond transient, identical in rate to that observed for photolysis of NO-Mb under similar conditions,[11] and a microsecond transient we attributed to HNO-Mb, Figure 1. NO-Mb impurities are difficult to avoid during HNO-Mb generation, and can range from 30 to 50% in the original synthetic methods. Reduction of NO-Mb by the cation radical of 4,4′-dimethyl-1,1′-trimethylene-2,2′dipyridinium, DTDP, yielded HNO-Mb samples with less than 10% NO-Mb impurity (SI).[12] Traces obtained for photolysis of such samples, Figure 1, showed much larger microsecond transients and were used in further experiments. The photolysis products were identified by comparison of transient spectra (constructed from single wavelength transient measurements 0.1 ms after the laser pulse, from 390 to 440 nm) with difference spectra obtained by subtraction of spectra for equimolar concentrations of HNO-Mb, deoxyMb, and metMb, (SI). As shown in Figure 2, the transient spectrum best matched that predicted for generation of metMb, implicating that its geminate partner is the aminoxyl radical anion, HNO^-. A simple three state model for this reactivity is given in eq 1; photolysis of HNO-Mb creates a geminate pair, which then recombines in a unimolecular process, or dissociates to generate the observed metMb photoproduct.

$$[\text{HNO-Mb}] \rightleftarrows [\text{HNO}^-/\text{metMb}] \rightleftarrows \text{HNO}^- + \text{metMb} \quad (1)$$

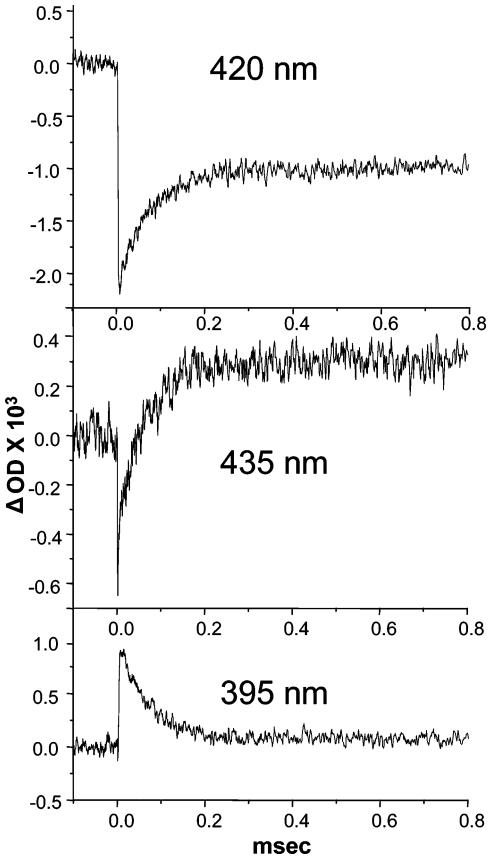

Figure 1. Transient absorbance traces at of 4 uM MbHNO after excitation at 532 nm. Each trace represents the average of 500 shots.

To investigate the recombination, kinetic traces at 395 nm were selected as this wavelength is an isosbestic absorbance for NO-Mb and deoxyMb, Figure 3. Transient absorbances at this wavelength were fit to a single-exponential decay, obtaining τ of 7.0 ($\pm$ 0.5) $\times$ 10^{-5} sec assignable to the recombination. As seen in Figure 3, there is residual absorbance at 395 nm after the microsecond recombination, indicative of escape of HNO^- into solution, ranging from 5 to 15% in different experiments. During bulk photolysis of HNO-Mb at pH 7, both deoxyMb and metMb are observed (SI). Analysis of the reaction mixture after extensive photolysis showed the presence of H_2NOH (~10% by N-content) and N_2O, suggesting multiple secondary reactions of the released HNO^- (SI). Literature suggests that aminoxyl radicals couple to form dinitrogen,[13] but analysis of the headgas after photolysis of ^{15}N-labeled HNO-Mb showed only the presence of $^{15}N_2O$ (SI).

There has been much discussion over the nature of bonding in HNO adducts of ferrous hemes,[6] such as in the putative catalytic intermediate in nitric oxide reductase P450nor.[14] That observed microsecond recombination is similar to that seen for NO-metMb,[15]

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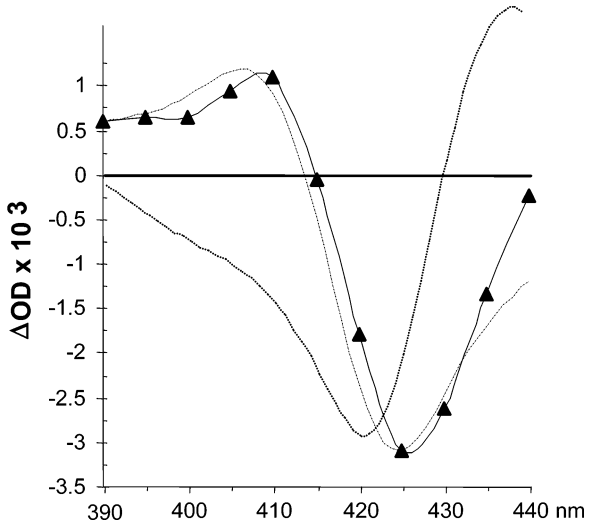

Figure 2. Transient absorbance changes from photolysis of 4.5 uM MbHNO after 0.05 ms (triangles, solid line) overlapped with normalized difference spectra of metMb and MbHNO (dot dashed line) and deoxyMb and MbHNO (dashed line).

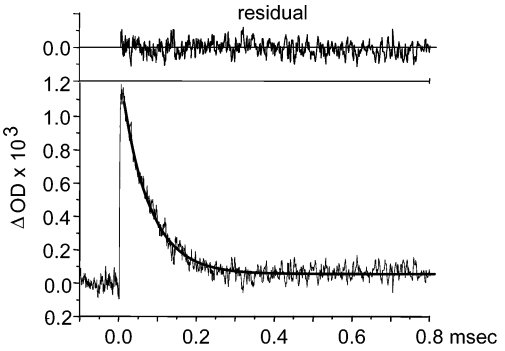

Figure 3. Fit of transient absorbance trace at 395 nm of 4 uM MbHNO after excitation at 532 nm, $\tau = 70 \pm 0.5$ μs, with ca. 8% of difference remaining at 0.8 ms.

and the production of metMb suggests ferric character to the adduct, that is, (HNO^{-})-Fe^{III}Mb, analogous to that of the isoelectronic species, oxyMb as (O_2^{-})-Fe^{III}Mb.[16] It is possible that the observed transients are secondary to true geminate recombination, perhaps owing to a spin change at the metal[17] or protonation of the aminoxyl radical anion. Indeed, the p*K*a value of H_2NO was recently reported as 12.6,[13] suggesting that the aminoxyl radical is protonated under the conditions described. No change was found in the observed recombination rate at 395 nm for photolysis of HNO-Mb performed in the range of pH 7 to 10, (SI). Recent calculated reduction potentials suggest that the proton-coupled oxidation HNO/Fe^{II} to H_2NO/Fe^{III} is enthalpically favorable.[13,18] Further characterizations of the photolysis of HNO-heme protein adducts are underway.

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Supporting Information Available: Experimental details, including synthesis of labeled HNO-Mb, determination of NO-Mb contamination, and photoproduct analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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