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Photoexcitation dynamics of nitric oxide bound ferric myoglobin probed by femtosecond IR spectroscopy

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Abstract. Time-resolved vibrational spectra show that photolysis quantum yield of NO bound ferric myoglobin is smaller than 0.86, the deligated NO geminately rebinds with subnanosecond time scale, and the rebinding kinetics depends on protein conformation.

1 Introduction

Nitric oxide (NO) has been found to perform a number of physiological functions such as smooth muscle relaxation, platelet inhibition, neurotransmission, immune regulation, and penile erection. Much NO's biological function comes from its interactions with heme proteins, such as cytochrome c oxidase, myoglobin (Mb), hemoglobin, NO synthase enzymes. Recently, it has been revealed that MbO_2 plays a role as an NO scavenger, regulating NO level in the cell.[1-3] During the NO scavenging process, ferric Mb (Mb^{III}) is yielded. Because Mb^{III} can bind to NO, it reacts with another NO molecule to yield ferrous MbNO. Other neutral ligand such as CO and O_2 binds only to ferrous Mb and anions can bind to ferric heme but NO can bind both ferrous and ferric heme.

Rebinding dynamics of NO to ferrous Mb after photolysis of MbNO was investigated to probe how protein dynamics is related to its structure and function.[4] Most of photodissociated NO geminately rebinds nonexponentially in 1 ns. Vibrational bands of NO in MbNO showed two bands and kinetics of the rebinding of both bands are the same, suggesting that the dynamics of geminate rebinding to ferrous Mb is conformation independent. Here we extend the previous work and comparatively investigated rebinding dynamics of NO to ferric Mb with ferrous one.

2 Experimental methods

To prepare 12 mM of NO bound Mb^{III} ($Mb^{III}NO$), desired amount of lyophilized horse skeleton Mb^{III} was dissolved in D_2O buffered with 0.4 M potassium phosphate (pD 6.3). The solution was deoxygenated extensively by purging with N_2 gas for more than 20 min. The NO/N_2 mixture gas was bubbled through 2M NaOH solution to eliminated NO_2 produced by oxidation of NO, and then introduced to the deoxygenated Mb^{III} solution for short time to ligate only about 50% of the protein with NO.

Home-built two optical parametric amplifiers (OPA) were used to generate a visible pulse and a mid-IR pulse.[4, 5] Frequency doubling of a signal pulse of one OPA generated a visible pulse at 575 nm with 3 μJ of energy and difference frequency mixing of the signal and idler pulses of the other OPA generated a mid IR pulse with ca. 1 μJ of energy. Mid-IR pulse with a typical spectral

bandwidth of 180 cm^{-1} was collected by a curved mirror, filtered by a long pass filter and separated into pump and probe pulse by a wedged BaF_2-window. Both IR pump and probe pulses generated in one OPA were used for IR pump-IR probe spectroscopy while blocking the visible pulse from other OPA. For visible pump-mid IR probe spectroscopy, the IR pump pulse was blocked and the IR probe pulse was used with a visible pump pulse. A 64-elements N_2(l)-cooled HgCdTe array detector, mounted in the focal plane of a monochromator, was used to detect the broadband transmitted mid-IR probe pulse.

3 Results and Discussion

Vibrational spectrum of NO stretching mode of $Mb^{III}NO$ in D_2O solution at room temperature can be decomposed into two bands, the major one at 1921 cm^{-1} (97.7%) with a full width at half maximum (FWHM) of 9 cm^{-1} and the minor one at 1902 cm^{-1} (2.3%) with a FWHM of 11 cm^{-1}. These bands, denoted to A_0 (the minor band) and A_1 (the major band),[6, 7] have been suggested to arise from the presence of discrete bound-state conformations. It was proposed that the band A_1 results from an interaction between the bound NO and the lone-pair electron on a deprotonated His-64-Nε and the band A_0 from conformation where a His-64 side chain does not interact with NO [7, 8]. At cryogenic temperature A_1 band was found to be at 1927 cm^{-1} for both sperm whale and horse Mbs.[8] Clearly the position of A_1 band is more sensitive to the interaction between the His-64 and bound NO than species variation of the protein and the extent of the interaction at cryogenic temperature may be stronger than at room temperature.

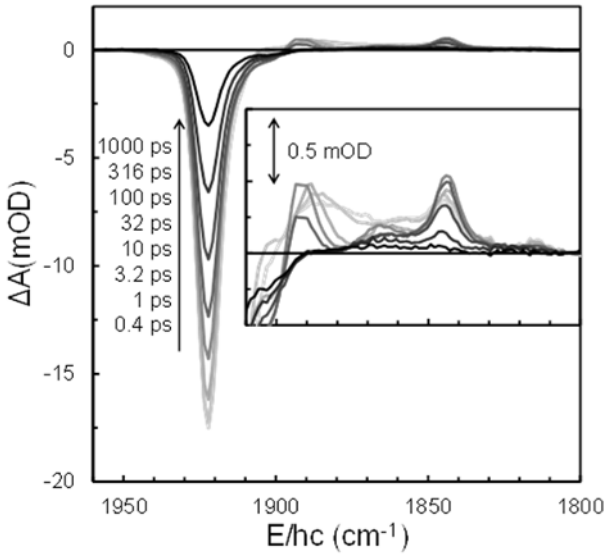

Fig. 1. Time-resolved vibrational spectra after photoexcitation of $Mb^{III}NO$ in D_2O solution with a 575 nm pulse at 294 K. Inset is the expanded view to show small but significant new absorption features in lower energy side of the main band.

Figure 1 shows femtosecond vibrational spectra in the region of NO stretching mode after photoexcitation of $Mb^{III}NO$ in D_2O solution at 294 K with a 575-nm pulse. The negative-going feature (bleach), appearing faster than the time-resolution of the instrument, is similar to the inverted equilibrium spectrum of NO stretching mode in $Mb^{III}NO$. It arises from the loss of $Mb^{III}NO$ in the ground NO vibrational state of the ground electronic state of the heme. The spectra show small but significant new absorption features at lower energy side of the main band, in the region of 1920 – 1880 cm^{-1}, that can be modeled with two evolving absorption bands. Two bands shift toward higher

energy and narrow with a time constant of 2 ps, indicating that both bands evolve with thermal relaxation of the heated heme. One band settled at the position of 1893 cm^{-1} within 2 ps and its amplitude decays with 24 ps. Both the position of the band and the relaxation time suggest that it arises from the vibrationally excited NO in $Mb^{III}NO$ resulting from rapid electronic and thermal relaxation of the photoexcited $Fe^{III}NO$ without photodissociation of the NO ligand from the heme. The other band, settling at the position of 1917 cm^{-1} within 2 ps, can be further decomposed into two evolving bands: one relaxing into equilibrium spectrum with 2 ps time constant and the other growing with early geminate rebinding of the dissociated NO, settling at 1917 cm^{-1} and decaying with 7 ps time constant. We assigned the band at 1917 cm^{-1} to ultrafast rebound $Mb^{III}NO$ with unrelaxed conformation. Absorption features assigned to protein undergoing thermal relaxation without ligand dissociation add up to 14% of the total bleach, implying that photolysis quantum yield of $Mb^{III}NO$ is smaller than 0.86. The photodissociated NO undergoes fast geminate recombination with subnanosecond time scale and the rebinding kinetics depends on the conformation of the protein. Geminate rebinding of NO to Mb with major conformation shows highly nonexponential kinetics that was described by the stretched exponential function, $0.77 \exp(-(t/330\ \text{ps})^{0.58} + 0.09)$. The NO rebinding to Mb with minor conformation is slower than that with the major conformation and exponential, which is $\exp(-t/1.8\ \text{ns})$. There were other new absorption features appearing at 1860 - 1840 cm^{-1}. They are located at even lower energy side of NO band in the gas phase and thus assigned to the stretching mode of NO photodissociated from $Mb^{III}NO$ that resides interior of the protein. They decay as NO geminately rebinds and escapes to solvent. As demonstrated, time-resolved vibrational spectra with high sensitivity reveal a rich photophysical and photochemical processes of photoexcited $Mb^{III}NO$.

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References

1. M. Brunori, Trends Biochem. Sci. **26**, 209 (2001).
2. U. Flogel, M. W. Merx, A. Godecke, U. K. M. Decking, and J. Schrader, Proc. Natl. Acad. Sci. U. S. A. **98**, 735 (2001).
3. D. J. Garry, A. Meeson, Z. Yan, and R. S. Williams, Cell. Mol. Life Sci. **57**, 896 (2000).
4. S. Kim, G. Jin, and M. Lim, J. Phys. Chem. B **108**, 20366 (2004).
5. S. Kim, G. Jin, and M. Lim, Bull. Korean Chem. Soc. **24**, 1470 (2003).
6. K. Nienhaus, P. Deng, J. M. Kriegl, and G. U. Nienhaus, Biochemistry **42**, 9647 (2003).
7. K. Nienhaus, P. Palladino, and G. U. Nienhaus, Biochemistry **47**, 935 (2008).
8. L. M. Miller, A. J. Pedraza, and M. R. Chance, Biochemistry **36**, 12199 (1997).