Femtosecond Vibrational Spectroscopic Study on Photoexcitation Dynamics of DNO-bound Myoglobin

Taegon Lee, Seongchul Park, and Manho Lim*
Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 609-735 Korea
Author e-mail address:mhlim@pusan.ac.kr

Abstract: Time-resolved vibrational spectra of DNO-bound myoglobin showed instantaneous bleach that decays on a picosecond time scale, suggesting that most of the photoexcited MbDNO undergoes picosecond geminate rebinding of DNO to Mb after its immediate deligation.
OCIS codes: (300.6340) General; (350.5130) General [8-pt. type. For codes, see www.opticsinfobase.org/submit/ocis.]

1. Introduction

Nitric oxide (NO) plays various important physiological roles. Recently, nitroxyl (HNO), a reduced form of NO, has been proposed to be an immediate precursor of NO and an intermediate in the denitrification process [1]. HNO was also found to play various biologically important roles [2]. In particular, the reactivity of HNO with various hemes was proposed to be physiologically important [1].

Although HNO exists as a dimer in aqueous solution that decomposes to N_2O and H_2O rapidly, it reacts with the ferrous atom of myoglobin (Mb) to form a very stable HNO-bound ferrous Mb (MbHNO). Thus MbHNO has been intensively studied using various spectroscopic method [3], which elucidated the structures of HNO moiety and the porphyrin ring in MbHNO reasonably well. However, the binding dynamics of HNO to heme proteins has hardly been investigated. Only a recent experiment showed that HNO with Mb was generated when it was excited by a visible pulse and about 90% of the photodeligated ligand geminately rebinds in 70 μs. The reported geminate rebinding (GR) rate of HNO to Mb is remarkably slow compared with the picosecond GR of NO to Mb [4]. Here, we report femtosecond vibrational spectra of photoexcited MbDNO in D_2O solution at 294 K with a 575-nm pulse that evolves on the picosecond time scale. The picosecond evolution of the bands was used to estimate the GR kinetics of DNO to Mb.

2. Experimental methods

Femtosecond vibrational spectrometer was described elsewhere [4]. Briefly, a 575 nm visible pump pulse with 3 μJ of energy and a 1280 ~ 1450 cm^{-1} mid-IR probe pulse were generated by two optical parametric amplifiers, pumped by a Ti:S amplifier at a repetition rate of 1 kHz. The probe pulse was dispersed by the 120 l/mm grating of a 320 mm monochromator and detected with a 64-element-N_2(l)-cooled HgCdTe array detector. Instrument response function was about 0.3 ps.

A 15 mM metMb solution was prepared using 200 mM carbonate buffer in D_2O solution (pD = 9.6). The solution was bubbled with N_2 gas to remove oxygen. After preparing $NaNO_2$ in pD 9.6 buffer and $NaBH_4$ in 1 N NaOD, 20 equivalents of $NaNO_2$ and 60 equivalents of $NaBH_4$ were added to the metMb solution in sequence under N_2 to produce MbDNO [3]. Once MbDNO was generated, the solution was immediately diluted into 200 mM phosphate buffer with pD = 7.4 and concentrated using an Amicon Ultra-15 filter. The concentrated sample was loaded into a sample cell with a 30 μm-thick Teflon spacer. Temperature of the sample cell was maintained at 294 ± 1.

3. Results and Discussion

Protein samples have strong absorption bands in our spectral range of interest (amide II at 1450~1580 cm^{-1} and amide III at 1200~1350 cm^{-1}). Therefore, the N–O stretching (ν_{N-O}) mode of MbDNO is expected to be buried between two strong amide bands. To effectively assign the vibrational absorption bands related to the ν_{N-O} mode, time-resolved spectra of both MbDNO and Mb$D^{15}NO$ were collected. To remove conformational band not related to the NO band, time-resolved spectra of MbCO were also obtained and subtracted from transient spectra of MbDNO.

Figure 1 shows the treated spectra of MbDNO and Mb$D^{15}NO$. The treated spectra show features that are minimally affected by thermal and conformational relaxation of the photoexcited protein except the spectral features related to the N atom. When the two treated spectra of MbDNO and Mb$D^{15}NO$ are compared, most of the features shift, except an absorption band near 1338 cm^{-1}. The non-shifting band at 1338-cm^{-1} likely arises from the band change of the histidine residues, which is not fully compensated by subtracting the MbCO spectrum. Clearly, DNO-

related bands are negative features (bleaches) near 1399, 1385, and 1368 cm^{-1} in the treated spectra of MbDNO and a broad bleach near 1353 cm^{-1} in the treated spectra of $MbD^{15}NO$.

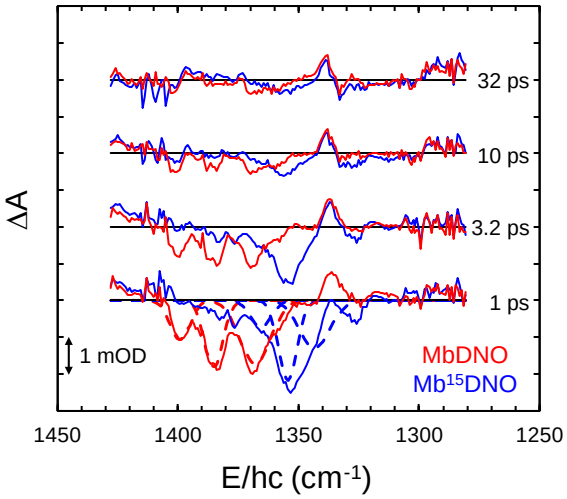

Figure 1. Representative treated spectra of MbDNO (red) and $MbD^{15}O$ (blue) (see text).

The bleach in the treated spectra, showing instantaneous bleach that decays on a picosecond time scale, was described by three vibrational bands, each modeled with a Gaussian function. The bleach in the treated spectra of MbDNO was composed of three bands at 1399, 1385, and 1368 cm^{-1} with relative intensities of 0.7, 1, and 1.6, respectively. In general, when the spectrum of an isotope-labeled molecule is compared with that of the unlabeled molecule, the vibrational band related to the isotope-labeled atom shifts according to the reduced mass change of the isotope. Thus, the bleach in the treated spectra of $MbD^{15}NO$ was also described by three bands. The fitted bands for $MbD^{15}NO$ peaked at 1366, 1354, and 1343 cm^{-1} with relative intensities of 0.4, 1, and 0.8, respectively. The decay kinetics of three bands turned out to be about the same and thus, we treated the decay kinetics of three bands globally in the final fitting. The decay kinetics was well described by $0.92 \exp(-t/4\ \text{ps}) + 0.08$.

The fitted band positions imply that the bands at 1399, 1385, and 1368 cm^{-1} shift by 33, 31, and 25 cm^{-1} upon the ^{15}N isotope substitution in MbDNO. The band at 1385 cm^{-1} that shifts to 1354 cm^{-1} upon ^{15}N labeling is consistent with the ν_{N-O} mode of HNO in MbHNO observed by Raman spectroscopy [5]. The position of the ν_{N-O} mode in MbDNO was calculated to be almost the same as that of MbHNO [6]. The remaining two bands at 1399 and 1368 cm^{-1} have not been observed before but they are clearly related to the vibrational band involving the N atom in MbDNO.

Based on the transient spectra of $MbD^{15}NO$ as well as vibrational wavenumber calculation using the *ab initio* calculation of the vibrational frequency on a DNO-bound model heme, three bands were attributed to Fermi interaction between the strong ν_{N-O} mode and weak overtone and combination modes involving the N atom. The picosecond decay of the bleach likely arises from either photodeligation of DNO from MbDNO and ps GR of DNO to Mb or rapid thermal relaxation subsequent to rapid electronic relaxation of the photoexcited MbDNO, without DNO deligation. The same appearance and decay in the conformational bands near 1410 cm^{-1} in MbCO and MbDNO indicates that there is an instantaneous photodeligation of DNO and, thus, the ps decay in the ν_{N-O} band in MbDNO likely arises from GR of DNO to Mb. GR of DNO to Mb appears to be the fastest rebinding in heme proteins, which can be attributed to the reactivity and the bonding structure of the ligand.

4. References

[1] B. A. Averill, "Dissimilatory nitrite and nitric oxide reductases," *Chem. Rev.* **96**, 2951-2964 (1996)
[2] K. M. Miranda, "The chemistry of nitroxyl (HNO) and implications in biology," *Coord. Chem. Rev.* **249**, 433-455 (2005).
[3] M. R. Kumar, J. M. Fukuto, K. M. Miranda, P. J. Farmer, "Reactions of HNO with heme proteins: New routes to HNO-heme complexes and insight into physiological effects," *Inorg. Chem.* **49**, (14), 6283-6292 (2010).
[4] S. Kim, M. Lim, "Protein conformation-controlled rebinding barrier of NO and its binding trajectories in myoglobin and hemoglobin at room temperature," *J. Phys. Chem. B* **116**, 5819-5830 (2012).
[5] C. E. Immoos, F. Sulc, P. J. Farmer, K. Czarnecki, D. F. Bocian, A. Levina, J. B. Aitken, R. S. Armstrong, P. A. Lay, "Bonding in HNO-myoglobin as characterized by X-ray absorption and resonance Raman spectroscopies," *J. Am. Chem. Soc.***127**, 814-815 (2005).
[6] D. P. Linder, K. R. Rodgers, "Structural, electronic, and vibrational characterization of Fe-HNO porphyrinates by density functional theory," *Inorg. Chem.* **44**, 8259-8264 (2005).