

Photodissociation dynamics of nitric oxide from roussin's red ester probed by time-resolved infrared spectroscopy

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Abstract. To explore nitric oxide (NO)-donating capability of Roussin's red ester $[\text{Fe}_2(\mu\text{-RS})_2(\text{NO})_4]$ (RRE), photodissociation of NO from RRE and its subsequent reaction dynamics were probed by monitoring the N–O stretching mode after excitation with a 400-nm photon. As a result of the experiment, the NO generation quantum yield was obtained.

1 Introduction

Nitric oxide (NO) is an important cell signalling molecule involved in many physiological and pathological processes and is a potent vasodilator with a short half-life in the blood [1,2]. Vasodilation through NO can relieve pain caused by angina by reducing the load on the heart. NO drugs can lower arterial pressure and left ventricular pressure by expanding the arteries. This vasodilatation does not reduce the blood volume of the heart pump, but it reduces the force that the heart muscle must exert to pump the same amount of blood [2]. The NO function has motivated interest in developing stable, nontoxic, and efficient NO donors for medicinal application.

Roussin's red ester (RRE), $[\text{Fe}_2(\mu\text{-RS})_2(\text{NO})_4]$ was discovered for a long time ago and recent interest in NO donor has increased study of RRE as a NO donor in biology and medicine. RRE has been an important NO donor because it has a relatively low toxicity and good stability as well as its synthesis is simple. Photoexcitation of RRE produces NO that can be utilized as the aforementioned medical purposes. Various thiol molecules were used to synthesize RRE. Stability, quantum yield for NO generation, and solubility of RRE are related to the thiol molecule used [3]. Thiol with a polar group is advantageous for biological usage which requires water solubility. Since cysteine (CysSH) is a water soluble natural amino acid, it was used in synthesizing RRE for a biological application. CysSH-containing RRE, $[\text{Fe}_2(\mu\text{-CysS})_2(\text{NO})_4]$ (**Cys-RRE**) is thermally stable for about a day at room temperature. Here, photodissociation dynamics of NO from **Cys-RRE** in water was investigated by probing the N–O stretching mode. We were able to identify reaction intermediates in the photolysis and establish subsequent reactions of the intermediates as well as photodissociated NO with nascent photoproducts. These observations can be utilized to identify optimal thiol molecule used in synthesis of RRE for better stability and higher NO quantum yield, which can serve getting requirement for a better NO donor.

2 Experimental methods

Picosecond experiment (0.3 – 1000 ps) was performed using a femtosecond IR spectrometer described elsewhere [4]. Briefly it is based on a home-built optical parametric amplifier (OPA) pumped by a commercial Ti:sapphire amplifier (110 fs, 1 mJ, and 800 nm). The 400-nm pump pulse was produced by frequency doubling of the 800-nm pulse. The near-IR outputs of the OPA were difference-frequency mixed in a 1-mm-thick AgGaS₂ crystal to generate 110-fs tunable mid-IR probe pulse. Up to 1 ns, optical delay was adjusted by a translational stage but, for the pump-probe delay longer than a few nanoseconds, usage of the translational stage becomes impractical. Thus, the fs pump pulse was replaced with a commercial ns laser based on an optical parametric oscillator that generates 2.5-ns visible or UV pulse. The ns pump pulse was synchronized with the fs probe pulse by an electronic digital delay generator. The probe pulse at 1775 cm⁻¹ with 160 cm⁻¹ of spectral width was sent through the sample, dispersed in a 320 mm monochromator, and then probed with a 1×64-pixel HgCdTe array detector, resulting in the spectral resolution of 2.5 cm⁻¹.

FeSO₄, CysSH, and NO gas (98%) were purchased from Sigma-Aldrich Co and used as received. For the synthesis of 3 mM **Cys-RRE**, 6 mM FeSO₄ and 12 mM CysSH were mixed in 100 mM phosphate buffer in D₂O solution (pD = 7), the solution was bubbled with N₂ gas to remove dissolved O₂, and then, excessive amount of NO gas was injected to the buffer solution [5]. To obtain maximum transient absorption, 3 mM **Cys-RRE** was loaded in a 100-μm-pathlength sample cell with two 2-mm-thick CaF₂ windows. Sample was flowed to ensure each pump laser pulse to excite a fresh volume of the sample. Reduction in sample concentration due to the dissociation reaction was kept below 5% by using large volume of sample. Circulating coolant through the sample mount block maintained sample temperature at 293±1 K during the measurement.

3 Results and discussion

Time-resolved IR spectra of **Cys-RRE** in D₂O, shown in figure 1(a), were obtained in the spectral region of N–O stretching mode in the pump-probe delay time spanning from 0.3 ps to 10 μs after photolysis with a 400-nm photon. The negative going bands at 1791 and 1763 cm⁻¹, corresponding to the antisymmetric NO stretching mode of **Cys-RRE** and arising from the loss of the ground-state population of **Cys-RRE**, appear immediately after photolysis, suggesting that the photolysis of NO occurs < 0.3 ps. After the photolysis of the RRE, 34% of the bleach signal recovers with a time constant of 2.4 ps. The fast recovery of the bleach arises from the relaxation of the thermally excited ground electronic state produced by fast relaxation of the electronically excited state. Thermal relaxation was characterized by narrowing and shift toward the ground state absorption band of red-shifted broad absorption band. DFT calculations were carried out to assign three new absorption bands at 1801, 1795, and 1725 cm⁻¹, appearing immediately after photolysis. Whereas band at 1801 cm⁻¹ decays with a time constant of 14 μs, two bands at 1795 and 1725 cm⁻¹ decay with a time constant of 5.5 ns. Two bands at 1795 and 1725 cm⁻¹, initially red-shifted and broad, establish with a time constant of 8.2 ps. Based on DFT calculations on the frequency of the N–O stretching mode for various expected reaction intermediates after photolysis of **Cys-RRE**, band at 1801 cm⁻¹ was assigned to **Cys-RRE** deficient in one NO molecule, [Fe₂(μ-CysS)₂(NO)₃] radical (**A**) and bands at 1795 and 1725 cm⁻¹ to an isomer of **Cys-RRE** having S-bound NO, [Fe₂(μ-CysS)(μ-CysSNO)(NO)₃] (**B**). The isomer **B**, 49 kcal/mol higher in energy than **Cys-RRE**, is likely formed due to strong interaction of the dissociated NO with the S atom of the **A** after photodissociation. Establishment of two

bands with a time constant of 8.2 ps can be attributed to the formation process of the isomer from the radical **A** and the interacting NO.

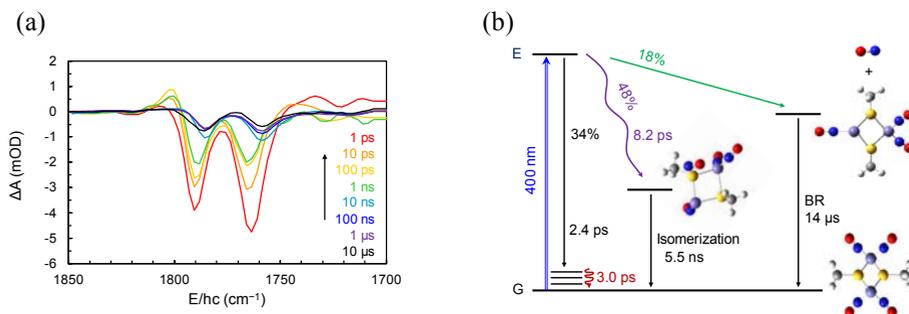


Fig. 1. (a) Representative time-resolved IR spectra of **Cys-RRE** in D_2O at 293 K after excitation at 400 nm. (b) A kinetic scheme obtained by fitting the time-resolved spectra globally. The long wavy purple line represents formation process of the isomer **B**. In the molecular model, **CysS** was depicted by CH_3S for the sake of simplicity.

As shown in figure 1(b), a kinetic scheme was obtained by globally fitting whole time-resolved spectra using the assigned basis spectra. According to the kinetic scheme, 66% of the photoexcited **Cys-RRE** releases one NO molecule and 34% of them decays without photodeligation. However, 73% of the dissociated NO strongly interacts with S atom of the partner radical **A** producing the isomer **B** that isomerizes back to **Cys-RRE** with a time constant of 5.5 ns, and the remaining 27% of them stay as free NO and the radical **A**, which bimolecularly react with diffusion-limited reaction rate. Clearly majority of the photodissociated NO strongly interacts with S atom of **A**, which leads to isomerization back to **Cys-RRE**, resulting in decrease of NO quantum yield. Bulky molecular group at α carbon of a thiol may decrease the interaction strength between free NO and S atom of the radical, reducing formation of the isomer, and thus increase the formation of free NO. We have synthesized RRE using N-acetylpenicillamine (**NAP-RRE**) which has three methyl groups at α carbon. **NAP-RRE** has showed higher thermal stability than **Cys-RRE** and produced more NO gas when exposed to UV lamp. Photodissociation of NO from **NAP-RRE** will be carried out and compared with that from **Cys-RRE** to prove the mechanistic reason for the higher quantum yield for NO generation.

References

1. E. E. van Faassen, S. Bahrami, M. Feelisch, N. Hogg, M. Kelm, D. B. Kim-Shapiro, A. V. Kozlov, H. Li, J. O. Lundberg, R. Mason, H. Nohl, T. Rassaf, A. Samouilov, A. Slama-Schwok, S. Shiva, A. F. Vanin, E. Weitzberg, J. Zweier, and M. T. Gladwin *Med. Res. Rev.* **29**, 683 (2009).
2. J. Abrams, *Am. J. Cardiol.* **77**, 31C (1996).
3. C. L. Conrado, S. Weckler, C. Egler, D. Magde, and P. C. Ford, *Inorg. Chem.* **43**, 5543 (2004)
4. S. Kim, M. Lim, *J. Phys. Chem. B* **116**, 5819 (2012).
5. J. C. M. Pereira, A.V. Iretskii, R. Han, and P. C. Ford, *J. Am. Chem. Soc.* **137**, 328 (2015)