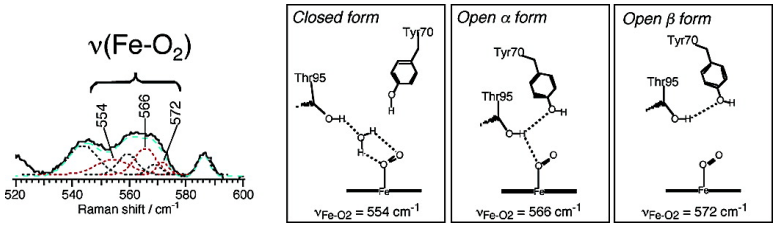
Communication

Oxygen-Sensing Mechanism of HemAT from *Bacillus subtilis*: A Resonance Raman Spectroscopic Study

Takehiro Ohta, Hideaki Yoshimura, Shiro Yoshioka, Shigetoshi Aono, and Teizo Kitagawa

J. Am. Chem. Soc., **2004**, 126 (46), 15000-15001• DOI: 10.1021/ja046896f • Publication Date (Web): 28 October 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 3 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/28/2004

Oxygen-Sensing Mechanism of HemAT from *Bacillus subtilis*: A Resonance Raman Spectroscopic Study

Takehiro Ohta, Hideaki Yoshimura, Shiro Yoshioka, Shigetoshi Aono, and Teizo Kitagawa*

Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Okazaki, Aichi 444-8787, Japan

Received May 26, 2004; E-mail: teizo@ims.ac.jp

Specific sensing of gas molecules such as O_2, NO, and CO is a novel function of hemoproteins,[1,2] while hemoproteins carry out a wide variety of functions such as oxygen storage/transport, electron transfer, and catalysis as enzymes. HemAT-*Bs* is a heme-based signal transducer protein responsible for aerotaxis of *Bacillus subtilis*, which detects oxygen and transmits the signal to regulatory proteins that control the direction of flagella rotation.[3] CO and NO are also caught at the same position as O_2, but the signals would be differentiated. Binding of oxygen to the sensor domain of this protein is supposed to alter the protein conformation in the vicinity of heme, which is propagated to the signaling domain through the linker region in a way different from the other case in binding of other gases. For the cooperative oxygen binding of hemoglobin (Hb), a proximal allosteric pathway is emphasized, because the strain on the Fe−His bond determines the T and R states.[4] If such a proximal pathway is the only way to convey the information of ligand binding, O_2 may not be distinguished from CO, because both molecules form similar six-coordinate (6c) heme complexes in terms of the proximal geometry. In the case of a physiological NO receptor hemoprotein, soluble guanylate cyclase (sGC), an idea of proximal signaling works with formation of 5c NO−heme adduct.[5]

Specific sensing of O_2, CO, and NO might have been required for aerophilic bacteria in the early times of the earth, when CO and NO were more abundant than O_2.[6] In support of this idea, our previous resonance Raman (RR) study of the oxygen-bound form of HemAT-*Bs* has demonstrated that the Fe−O_2 stretching (ν_{Fe-O_2}) frequency (560 cm^{-1}) is noticeably lower than those of general oxygen-bound hemoproteins[7] but similar to the frequencies observed for invertebrate, plant, and bacterial Hbs,[8] suggesting that the bound oxygen is incorporated into a unique hydrogen bonding network in the distal environment. Recently, X-ray structures have been determined for the unligated and cyanide-ligated forms of the HemAT-*Bs* sensor domain.[9] The sensor domain contains a unique distal heme pocket surrounded by Tyr70, Thr95, and a water molecule. Since Tyr70 shows distinct conformational changes in one subunit when the ligands are removed, the symmetry breaking of HemAT-*Bs* was proposed to play an important role in initiating the chemotaxis signaling transduction cascade.[9] Here we present RR evidence for structural linkage between the distal heme pocket and the signaling domain by using the linker-lacking protein as well as the wild-type (WT) and the Y70F and T95A mutants of full-length HemAT-*Bs*.

The newly observed spectra[10] together with the results from the band fitting analyses are depicted in Figure 1. We demonstrated previously that the band around 560 cm^{-1} of O_2-bound HemAT-*Bs* had oxygen isotope sensitivity.[7] The band-fitting analyses located the center of oxygen isotope sensitive Gaussian bands at 554, 566, and 572 cm^{-1}, which were shifted to 537, 547, and 554 cm^{-1}, respectively, upon $^{18}O_2$ substitution, for the WT form as delineated by dark red dotted lines in Figure 1A (a and b). The isotope shifts of 17−19 cm^{-1} are in good agreement with a calculated shift value

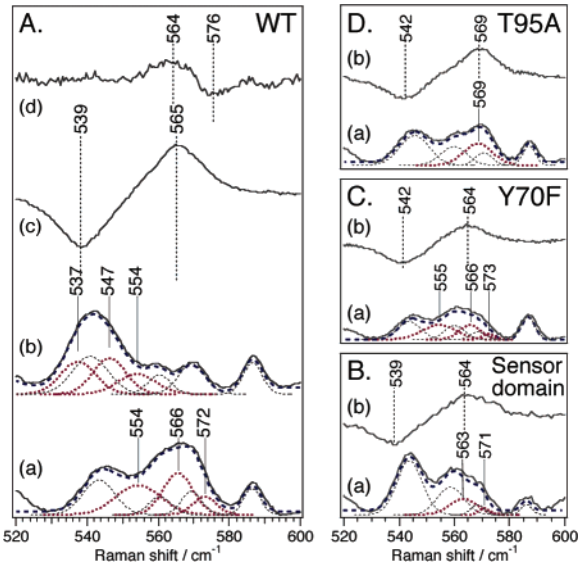


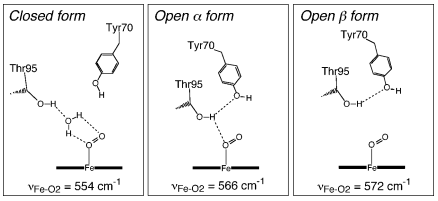
Figure 1. Resonance Raman spectra of O_2-bound WT and mutants HemAT-*Bs*.[10] (A) $^{16}O_2$- and $^{18}O_2$-bound WT HemAT-*Bs* in H_2O are shown by traces a and b, respectively. Trace c is the $^{16}O_2 - {}^{18}O_2$ difference spectrum, and trace d is the difference spectrum between the $^{16}O_2$-bound forms in H_2O and D_2O ($H_2O - D_2O$). (B) Trace a shows the spectrum of the $^{16}O_2$-bound truncated sensor domain in H_2O, and trace b is the $^{16}O_2 - {}^{18}O_2$ difference spectrum. (C) $^{16}O_2$-bound Y70F mutant HemAT-*Bs* in H_2O is shown by trace a, and the $^{16}O_2 - {}^{18}O_2$ difference spectrum is shown by trace b. (D) $^{16}O_2$-bound T95A mutant HemAT-*Bs* in H_2O is shown by trace a, and the $^{16}O_2 - {}^{18}O_2$ difference spectrum is shown by trace b. In all of traces a, the dashed and dotted lines were obtained by Gaussian band-fitting analyses. The dark red dotted lines denote contribution from ν_{Fe-O_2}. The gray dotted lines show heme modes, and two of them overlapped with ν_{Fe-O_2} bands were estimated from the spectra of the $^{18}O_2$-bound forms. The navy blue dashed lines are the sum of the component bands. All difference spectra are raw spectra without scaling. Results of band fitting analyses for $^{18}O_2$-bound mutants are shown in Figures S3−6 in Supporting Information.

of 21 cm^{-1}. In D_2O, the oxygen isotope-sensitive band(s) exhibited an upshift from 564 to 576 cm^{-1} as seen in the $H_2O - D_2O$ difference spectrum (Figure 1A (d)), indicating that an H-bond(s) is formed between the bound oxygen and a protein residue.[11] Thus, there would be multiconformations of weakly and strongly H-bonded forms, which are tentatively called as the open (566 and 572 cm^{-1}) and closed forms (554 cm^{-1}), respectively. The open form would provide the moderate oxygen affinity (K_d = 719 nM, here $K_d = k_{off}/k_{on}$) of HemAT-*Bs*,[7] because the open form would release bound oxygen easily. The heme−O_2 adducts were photo- and thermolabile, and careful experiments were required to investigate the multiple ν_{Fe-O_2} bands, as shown in Figures S1 and S2 in Supporting Information.

The low ν_{Fe-O_2} frequency of O_2-bound HemAT-*Bs* indicates that the sp^2 orbital of the proximal O atom of heme-bound O_2 is H-bonded from the distal side.[8] A water molecule nearby heme

10.1021/ja046896f CCC: $27.50

Scheme 1



found by the X-ray analyses[9] would be involved in such an H-bonding interaction in the closed form. Since the position of this water molecule can be slightly displaced to keep the same D-bond strength, the 554 cm^{-1} band does not show the H_2O/D_2O frequency shift despite strong H-bonding. In support of this idea, the T95A mutant, in which the water molecule may not be present in the hydrophobic environment, demonstrated a single oxygen isotope-sensitive band centered at 569 cm^{-1} as shown in Figure 1D (a). These results show that the O_2-bound T95A mutant has a single conformation of the distal heme pocket, which would correspond to the open form with ν_{Fe-O_2} = 572 cm^{-1} of WT where the interaction of Thr95 with the proximal O atom of heme-bound O_2 would be absent, and that Thr95 is an essential component for maintaining the closed form.

While the ν_{Fe-O_2} bands were affected by the mutation of Thr95, the mutation of Tyr70 showed little effect on the ν_{Fe-O_2} bands. The ν_{Fe-O_2} bands of the Y70F mutant were fitted with three bands at 555 and 566, and 573 cm^{-1} (Figure 1C (a)) which are almost the same positions as those of WT, indicating that Y70F mutant has the same closed and open forms as WT. These results suggest that H-bonding between Tyr70 and heme-bound O_2 does not exist even in the closed form and rather that the orientation of the phenyl ring at the position 70 (benzene ring for Y70F mutant) relative to the heme-bound O_2 would be responsible for distinguishing the closed form from the open forms. Although Tyr70 will be oriented toward the heme-bound O_2 in the closed form, the side chain of Tyr70 would be flipped out of the heme pocket in the open forms of O_2-bound HemAT-*Bs*, as observed in the unliganded structure of the senor domain.[9] Y70F mutant showed the same three forms as does WT, but the population ratios of the open to closed forms were altered between the WT and the Y70F mutant, as can be seen in Figure 1A (a) and Figure 1C (a). The ratio of the closed to open forms increased in Y70F mutant compared with WT, but the reason is not known at present. The position of Tyr70 in O_2-bound HemAT-*Bs* can be different from the one in CN-ligated HemAT-*Bs*, because Fe−O−O is naturally bent while Fe−C−N is linear, and both O atoms of heme-bound O_2 can be involved in an H-bonding network.

The schematic diagram of the multiconformations of O_2-bound HemAT-*Bs* is illustrated in Scheme 1. While CO-bound hemoproteins are known to form multiconformations (open (A_0) and closed (A_1 + A_3) forms),[12] this is the first example of an O_2-bound hemoprotein that demonstrates the multiple states in RR spectroscopy. Thr95 would be crucial for the specific sensing of heme-bound O_2, while Tyr70 would be essential for the signal transduction from the heme pocket to the signaling domain.[9] In both open forms, an H-bonding donor to the bound oxygen would be a protein residue, because the ν_{Fe-O_2} band around 566 cm^{-1} clearly demonstrated the H_2O/D_2O frequency shift.[13] Presumably, Thr95 is the H-bonding protein residue. Since the X-ray analyses of CN-ligated HemAT-*Bs* have shown that the carbonyl group of Leu92 is involved in an H-bonding network around the bound ligand through the interaction with the water molecule,[9] Leu92 may be important for the synchronous movement of Thr95 and Tyr70 for formation of the multiconformations of O_2-bound HemAT-*Bs*.

The RR spectrum of the truncated sensor domain is shown in Figure 1B (a), where the oxygen isotope sensitive bands of protein were fitted with two Gaussian bands centered at 563 and 571 cm^{-1}. Therefore, truncation of the linker and signaling domains affected only the closed form, which disappeared in this truncated mutant, although the open forms with ν_{Fe-O_2} at 566 and 572 cm^{-1} are little influenced. In other words, the closed form has a structural linkage with the signaling domain.

CO also binds to HemAT-*Bs*. The properties of the ν_{Fe-CO} (Fe−CO stretching) and δ_{Fe-C-O} (Fe−C−O bending) RR bands indicated that heme-bound CO does not interact with the distal side.[7] Indeed, the RR spectral features of the CO-bound form of the truncated sensor domain, T95A, and Y70F mutants are almost the same as those of WT, as shown in Figure S7 in Supporting Information. In the case of NO, the ratio of 6c and 5c heme−NO adducts is altered between WT and the truncated sensor domain, as shown in Figure S8 in Supporting Information. Thus, a proximal signaling pathway triggered by strain on Fe−His bond may be important for discrimination against NO.

In summary, we have shown here that bound oxygen was specifically sensed by the distal environment of HemAT-*Bs*, which has long-distance structural linkage with the signaling domain.

Acknowledgment. This study was supported by a JSPS Fellowship to T.O., by a Grant-in-Aid for Specifically Promoted Research to T.K. (14001004) and a Grant-in-Aid for Scientific Research to S.A. (12147203) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and by a Grant-in-Aid for Scientific Research to S.A. (14380303) from JSPS.

Supporting Information Available: Figures S1−8 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Aono, S.; Nakajima, H. *Coord. Chem. Rev.* **1999**, *190*−*192*, 267−282.
(2) Jain, R.; Chan, M. K. *J. Biol. Inorg. Chem.* **2003**, *8*, 1−11.
(3) Hou, S.; Larsen, R. W.; Boudko, D.; Riley, C. W.; Karatan, E.; Zimmer, M.; Ordal, G. W.; Alam, M. *Nature* **2000**, *403*, 540−544.
(4) Perutz, M. F. *Nature* **1970**, *228*, 726−734.
(5) Ballou, D. P.; Zhao, Y.; Brandish, P. E.; Marletta, M. A. *Proc. Natl. Acad. Sci.* **2002**, *99*, 12097−12101.
(6) Kasting, J. F. *Science* **1993**, *259*, 920−926.
(7) Aono, S.; Kato, T.; Matsuki, M.; Nakajima, H.; Ohta, T.; Uchida, T.; Kitagawa, T. *J. Biol. Chem.* **2002**, *277*, 13528−13538.
(8) Yeh, S.-R.; Couture, M.; Ouellet, Y.; Guertin, M.; Rousseau, D. L. *J. Biol. Chem.* **2000**, *275*, 1679−1684.
(9) Zhang, W.; Phillips, G. N., Jr. *Structure* **2003**, *11*, 1097−1110.
(10) Excitation light at 413.1 nm was obtained from a Kr^+ laser (Spectra Physics 2060). The laser power was 2 mW at the sample point, and the samples were cooled with a cold N_2 flow without freezing. The sample solutions for the Raman measurements were sealed in quartz cells, which were rotated at 1500 rpm. Typically, 55 μL aliquots of 30 μM protein solution in 50 mM Tris buffer, pH 8.0, were put into the cell. The scattered light was dispersed with a single polychromator (Ritsu (DG-1000) or Spex (750M)) equipped with a liquid nitrogen-cooled charge-coupled device (CCD) camera. The spectral slit width was 6 cm^{-1}. Raman shifts were calibrated using indene and CCl_4, providing an accuracy of ±1 cm^{-1} for intense isolated lines.
(11) Kitagawa, T.; Ondrias, M. R.; Rousseau, D. L.; Ikeda-Saito, M.; Yonetani, T. *Nature* **1982**, *298*, 869−871.
(12) Morikis, D.; Champion, P. M.; Springer, B. A.; Sligar, S. G. *Biochemistry* **1989**, *28*, 4791−4800.
(13) Upshift of the ν_{Fe-O_2} band would indicate that the D-bond to heme-bound O_2 is longer than the H-bond. This elongation of the D-bond can be explained as follows; due to difference in the zero-point energies in the asymmetric X−H(D) (X is an atom involved in the H(D)-donor amino acid residue) vibrational potential by anharmonicity, the X−D bond is shorter than the X−H bond, which would result in a longer D bond to O_2 than H bond.

JA046896F