Fluorescence Spectra of Trp53Phe and Trp110Ile Mutants of a Heme-regulated Phosphodiesterase from *Escherichia coli*

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Fluorescence bands of a Trp110Ile mutant of the isolated heme domain of a heme-regulated phosphodiesterase (*Ec* DOS) from *Escherichia coli* and its complex with 8-anilino-1-naphthalenesulfonic acid (ANS) were very weak, compared to the wild-type protein, suggesting that the fluorescence of the remaining Trp53 residue is quenched by interactions with heme, and that the Trp110 residue is exposed to the solvent.

The heme protein, heme-regulated phosphodiesterase, from *Escherichia coli* (*Ec* DOS) is composed of two functional domains, an N-terminal heme-bound domain and a C-terminal phosphodiesterase (PDE) domain.[1–5] PDE is active when the heme iron is in the Fe(II), but not in the Fe(III) complex form, suggesting that the heme is a sensor that regulates activity. A change in the heme redox state may alter the conformation of the heme-bound domain, resulting in transmission of signals to the catalytic domain and initiation of PDE activity.

The heme-bound N-terminal region of *Ec* DOS has a unique sequence, and the tertiary structure is characterized as a PAS (an acronym formed from the names of the following proteins: *Drosophila* period clock protein (PER), vertebrate aryl hydrocarbon receptor nuclear translocator (ARNT), and *Drosophila* single-minded protein (SIM)) domain. PAS domains are sensory modules in signal-transducing proteins, such as the bacterial oxygen sensor FixL,[6] photoactive yellow protein PYP,[7] and redox potential sensor NifL,[8] which control responses to various environmental stimuli. The unique PAS structure of *Ec* DOS is additionally important for transmitting signals from the heme domain to the PDE domain.

Fluorescence spectra[9,10] are often employed to examine the environments of aromatic amino acid residues, such as Trp. *Ec* DOS contains 13 Trp residues, whereas the isolated PAS domain (*Ec* DOS PAS) contains only 2 Trp residues (Trp53 and Trp110). These two residues are useful probes for detecting protein conformational changes in the PAS domain. Here we analyze the effects of Trp110 and Trp53 mutations on the protein structure of *Ec* DOS PAS.

Ec DOS PAS contains two Trp residues (Trp53 and Trp110). Intrinsic fluorescence bands of *Ec* DOS PAS (thick solid line) are observed at 333 nm (excitation either at 280 or 295 nm) (Figure 1). In general, the Trp residue is excited at 295 nm, while two other aromatic amino acid residues (Phe, Tyr) are excited at 280 nm. Thus, Trp residues appear to be the main contributors to the total fluorescence of both proteins.

Excited energy shift between aromatic compounds enhances fluorescence intensity.[9,10] Also, fluorescence of Trp in a polar environment is located at higher wavelengths than that in a non-polar environment.[9,10] The fluorescence maximum of *Ec* DOS PAS displayed a shift from 333 nm to 340 nm in the Trp53Phe mutant (thin solid line in Figure 1). This red shift in fluorescence suggests that the environment of the remaining Trp110 residue is more polar than that in wild-type *Ec* DOS PAS. Interestingly, the fluorescence spectrum derived from the Trp53 residue was very weak in the Trp110Ile mutant (broken line in Figure 1). This finding indicates that either the fluorescence intensity of Trp53 is very small or the residue is located near the heme cofactor, which quenches its fluorescence.[9,10] The redox states of the wild-type protein had little or no effect on fluorescence, implying that changes in the redox state do not affect the environment of Trp residues in *Ec* DOS PAS.

Fluorescence of 8-anilino-1-naphthalenesulfonic acid (ANS) in the presence of wild-type, Trp53Phe and Trp110Ile mutant *Ec* DOS PAS proteins was examined to analyze the hydrophobic cluster at the protein surface (Figure 2). ANS fluorescence at around 515 nm was dramatically enhanced by wild-type *Ec* DOS PAS and the Trp53Phe mutant, concomitant with a shift in the fluorescence maximum to around 470 nm. Notably, the increase in ANS fluorescence in the presence of Trp53Phe mutant *Ec* DOS PAS was more significant than that observed with wild-type *Ec* DOS PAS. However, no changes in the fluorescence spectrum were observed with the Trp110Ile mutant, indicating that the Trp110 residue interacts with ANS.

The observed intrinsic fluorescence intensity of Trp53 in the

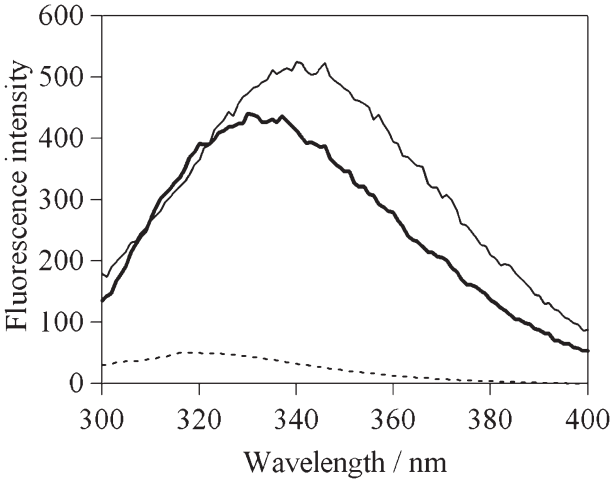

Figure 1. Intrinsic fluorescence spectra of wild-type (thick solid line), Trp53Phe (thin solid line), and Trp110Ile (broken line) mutants of *Ec* DOS PAS. Cloning, expression in *E. coli*, and purification of *Ec* DOS PAS (amino acids 1-147) were performed as described previously.[2–5] Excited at 280 nm. Fluorescence spectra excited at 295 nm were not different.

Trp110Ile mutant was very small, probably due to quenching by heme. According to the crystal structure (Figure 3),[12] Trp53 is located near the heme (the distance between the Trp53 side-chain and heme is 4.2 Å) and Trp110 is located at the surface of the molecule (Figure 3). Interestingly, the Trp53Phe mutant exhibited increased fluorescence intensity and a shift in the fluorescence maximum from 333 nm to 340 nm (Figure 1), signifying a change in the environment around Trp110 as a result of the substitution.

In the crystal structure the two Trp residues are relatively close to each other (the distance between the Cα atoms of the Trp53 side-chain and Trp110 is 11.4 Å), and the side-chain NE1 atom of Trp53 forms hydrogen bond with the main-chain O atom of Leu68. Loss of the hydrogen bond by mutation of Trp53 to Phe may induce conformational change around Trp110. Consequently, Trp110 in the Trp53Phe mutant protein is more exposed to the solvent. While the addition of the Trp53Phe mutant to ANS enhanced fluorescence intensity, the Trp110Ile mutant did not have a significant effect (Figure 2). These findings suggest that ANS interacts mainly with Trp110, which is located near the surface of the protein molecule. The finding led us to a proposal that Trp110 is located at the interface between the heme and catalytic domains, and contributes, in part, to stabilizing hydrophobic interactions between the two domains.[16]

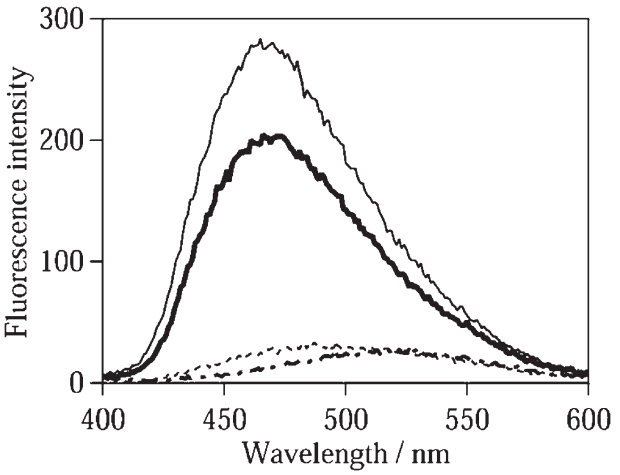

Figure 2. Fluorescence spectra of ANS in the absence of protein (broken line), and the presence of wild-type *Ec* DOS PAS (thick solid line), Trp53Phe (thin solid line) and Trp110Ile (dotted line) mutants of *Ec* DOS PAS. A stock solution of 4 mM ANS was prepared in 50 mM Tris-HCl buffer (pH 7.5). The dye concentration was determined by measuring absorption at 350 nm ($\varepsilon_{350} = 4.95 \times 10^3\ M^{-1}\ cm^{-1}$). The [ANS]/[protein] ratio in all experiments was maintained at a value of 5.

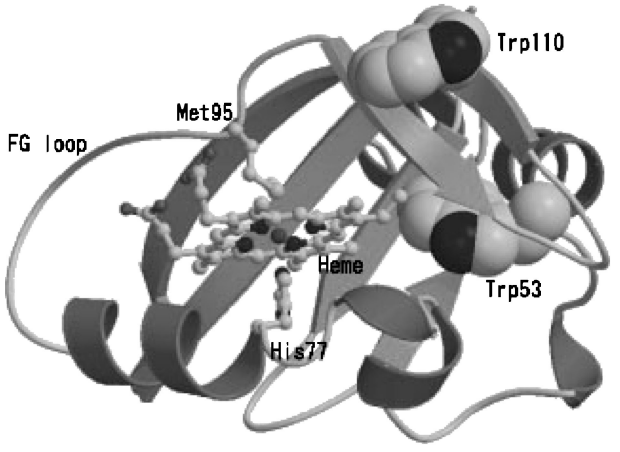

Figure 3. Structure of ferrous *Ec* DOS PAS (PDE code 1V9Z).[12] The two Trp residues are presented as a sphere model. Heme and two axial heme ligands, His77 and Met95, are presented as a ball-and-stick model. The figure was obtained using Molscript[13] and Raster3D.[14]

In summary, based on the fluorescence spectral findings on the Trp mutants, we suggest that: (1) Trp110 in *Ec* DOS PAS is exposed to the solvent, and (2) Trp53 is possibly located near the heme. Measurements of catalytic activities of the Trp53 and Trp110 mutants remain to be conducted to clarify role of these residues in catalysis.

References and Notes

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15 Angle between the heme plane and the side-chain of Trp53 is approximately 50°.
16 Note that the role Trp110 stated here is only a proposal. To verify the proposal, further experiments are needed.

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