# Heme Redox State Triggers Conformational Changes in the *Ec* DOS Protein: Ultraviolet Resonance Raman Spectroscopic Study

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The DOS protein from *Escherichia coli* (*Ec* DOS) is a heme-based signal transducer protein responsible for phosphodiesterase (PDE) activity. The *Ec* DOS is composed of two domains, an N-terminal sensor domain and a C-terminal PDE catalytic domain. PDE activity is dependent on the redox state of *Ec* DOS. The enzyme is active only when the heme is in the reduced state. The crystal structures of both the oxidized and reduced forms of the *Ec* DOS sensor domain (*Ec* DOS PAS) disclose that the heme axial ligand switching from His77/hydroxide anion to the His77/Met95 ligand pairs occurs upon heme reduction.

In the present study, we investigate the protein conformational changes of *Ec* DOS PAS accompanied by redox change with 229 nm excited ultraviolet resonance Raman spectroscopy (UVRR). The UVRR spectra of the wild-type *Ec* DOS PAS, W53F and W110I mutants of the oxidized and reduced states enable us to reveal the UVRR spectra of the Trp53 and W110 residues, separately. The difference spectra between the reduced and oxidized forms reflect the environmental changes of Trp residues. The W18, W17, W16, W7, and W3 bands of Trp53 near the heme vinyl side chain at position 2 exhibit significant intensity enhancement upon formation of the reduced form, while Trp110, which is located near to the surface of the protein displays small changes. Thus, Trp53 mainly undergoes environmental changes upon the formation of the reduced state. These results show protein conformational changes through the interaction between the heme vinyl group at position 2 and nearby residues such as Trp53 in the DOS PAS domain.