

## Heme Redox Cycling in Soybean Peroxidase: Hypothetical Model and Supportive Data

Akiha Takayama, Takashi Kadono and Tomonori Kawano\*

Laboratory of Chemical Biology and Bioengineering,  
Faculty and Graduate School of Environmental Engineering,  
The University of Kitakyushu, Kitakyushu, 808-0135, Japan

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In plants, the peroxidase family is involved in a great deal of oxidation reactions essential for living cells, using  $H_2O_2$  as an electron acceptor and a variety of substrates as electron donors. Nowadays, plant peroxidases are widely used as biological sensing materials in various areas such as medical diagnosis, biosensors, and nanotechnology. Among plant peroxidases, horseradish peroxidase (HRP) and soybean peroxidase (SBP) are widely used as model enzymes applicable to various purposes. For the purpose of providing a basis for future innovation by using SBP as a sensing material, we focus on the behavior of purified SBP in responses to known plant peroxidase substrates reportedly involved in superoxide-generating reactions. A chemiluminescence study showed that SBP can catalyze the generation of superoxide in the presence of salicylic acid and indole-3-acetic acid used as the tester substrates for examining the involvement of the conventional peroxidase cycle (involving native form, Compounds I and II) and oxygenase cycle (involving native form, ferrous form, and Compound III), respectively. Enzyme intermediates in the above two redox cycles were spectroscopically determined by recording the typical spectra reflecting the presence of Compound II (peroxidase cycle) and Compound III (oxygenase cycle). Interestingly, addition of nitric oxide in the absence of  $H_2O_2$  resulted in the formation of an intermediate resembling Compound II, the catalytically active form in the peroxidase cycle; thus, the novel gaseous regulation of SBP-mediated reaction was proposed.

\*Corresponding author: e-mail: kawanotom@gmail.com