E329 Formation of Flavin Semiquinone Radical in FMN-Containing Ascorbyl Free Radical Reductase from *Pleurotus ostreatus*

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Ascorbyl free radical (AFR) reductase containing FMN as a prosthetic group was purified from *Pleurotus ostreatus*. EPR study at liquid N_2 temperature showed that the addition of NADH to the enzyme under aerobic condition caused the formation of flavin semiquinone radical of a red anionic type with line width of 1.5 mT. This flavin radical was observed during the reduction and disappeared after the incubation with AFR. The signal intensity of flavin radical decreased as the temperature increased and showed power saturation behaviour. At 100 K, the signal of AFR was not observed, which precluded the possibility that the signal might originate in AFR. By comparison with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) standard, the spin concentration of flavin semiquinone was determined to be 9 ~ 11.5 % of that of FMN in *P. ostreatus* AFR reductase. These results imply that flavin radical may be generated by the reaction of the reduced enzyme with O_2. This reactivity toward molecular oxygen is an unusual feature among flavoprotein dehydrogenases.

E330 Chemical Modification of FMN-Containing Ascorbyl Free Radical Reductase from *Pleurotus ostreatus*

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Ascorbyl free radical reductase purified from *Pleurotus ostreatus* contained FMN as a prosthetic group and showed the characteristic absorption spectrum ascribed to the charge transfer interaction of thiolate anion with FMN. Chemical modification studies using thiol-specific reagents revealed that the enzyme had 1 mol thiol group/mol of subunit in the active site with 6.9 of pK_a. Treatment of the reductase with *p*-chloromercuribenzoic acid caused the loss of charge transfer absorbance and blocked the transfer of electron from NADH to FMN of the enzyme. Modification of histidine, lysine and arginine has led to the loss of the reductase activity. After 10 min of incubation of the enzyme with diethyl pyrocarbonate, there was an increase in absorbance at 244 nm, indicating the *N*-carbethoxylation of histidine residues. The arginine-modified enzyme was capable of quenching the fluorescence of NADH as the case with the active enzyme. However, the fluorescence quenching of NADH was not observed in the lysine-modified enzyme. This fact implies that lysine residues can participate in the interaction with NADH.