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Electron paramagnetic resonance spectra of amine oxidase from *Aspergillus niger*

Amine oxidase (monoamine:oxygen oxidoreductase (deaminating), EC 1.4.3.4) occurs remarkably in mycelium of fungi belonging to Aspergillus, Penicillium, Monascus and Fusarium when they are grown on media containing amines as sole nitrogen sources[1]. The enzyme has been crystallized from *Aspergillus niger*[2] and, similar to other amine oxidases, characterized as a copper-containing pyridoxalphosphate-dependent enzyme[3–6]. The role of copper in the oxidase reaction is not yet established. It is, however, possible that the copper may be directly involved in the electron transfer from the substrate, amines, to oxygen.

This communication describes the electron paramagnetic resonance (EPR) spectra of the amine oxidase from *A. niger* studied to obtain more information about the role of copper in the amine oxidase.

Crystalline amine oxidase was isolated from the mycelial extract of *A. niger* grown with *n*-butylamine as the sole source of nitrogen, according to the method of YAMADA *et al.*[2]. The reduced form of the enzyme was prepared by the addition of *n*-butylamine to the enzyme solution under anaerobic conditions by the procedure of YAMADA *et al.*[3]. Protein concentration was determined by measuring the absorbance at 280 nm. An $E^{1\%}_{1\,cm}$ value of 11.8 was used for the determination[3].

EPR spectra were measured at liquid nitrogen temperature (−180°) with a Varian Model V-4500X band spectrometer, operating near 9000 Mcycles/sec. The spectra were taken with a modulation field of 1 gauss.

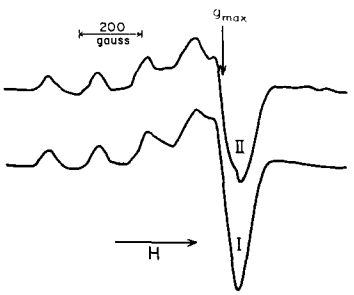

Fig. 1. EPR spectra of amine oxidase of *A. niger*; 73 nmoles in 0.3 ml of 0.03 M potassium phosphate buffer, pH 7.0; temp., −180°. Curve I, amine oxidase only; Curve II, amine oxidase *plus* 10 μmoles of *n*-butylamine under anaerobic conditions (the solution was frozen after 180 sec in room temperature).

Fig. 1 (Curve I) shows the EPR spectrum of the amine oxidase of *A. niger*. The g_{max} and $g_{||}$ values were determined by reference to 2 mM cupric sulfate in 25 mM disodium EDTA ($g_{max} = 2.09$; $g_{||} = 2.34$ (ref. 7)), to be 2.07 and 2.31, respectively, and the hyperfine separation was about 162 gauss. The spectrum is typical of cupric copper which is present as a complex of tetragonal symmetry, with oxygen, sulphur or more probably amino acid nitrogen bonded to the pyridoxal phosphate as ligands[6,8,9]. The concentration of cupric copper in the amine oxidase was determined by comparing the double integral of spectrum of the amine oxidase with that of cupric-EDTA of

known concentration as a standard. By this method*, about 1.9 atoms of cupric copper were found per mole of the enzyme, comparing with 3.0 atoms per mole estimated by the atomic absorption spectrometry. This discrepancy would indicate either that the amine oxidase of *A. niger* contains cuprous copper or that the cupric coppers in the enzyme are interacting with one another. Similar findings have been reported by YAMADA *et al.*[10] then by BUFFONI *et al.*[11], on similar oxidases from animal plasma.

On anaerobic addition of *n*-butylamine, the double integral of the EPR spectrum of the amine oxidase was not noticeably diminished; this means that the copper valence is not affected. However, small but reproducible changes were induced by the substrate, particularly in the main peak of the spectrum, as shown in Fig. 1, Curve II. This would possibly indicate that there are changes in the conformation of enzyme protein, by having the substrate bind, which are reflected in changes of the environment of the copper atoms. Similar changes have been reported by MONDOVI *et al.*[12], in the super-hyperfine lines of the EPR spectrum of pig kidney diamine oxidase, by adding each individual substrate.

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* The integration procedure was tested with a variety of samples. Individual integrations on different curves obtained with the same enzyme and standard under the same conditions agreed within 2.5% and 4.5%, respectively.