CHEMISTRY LETTERS, pp. 487-490, 1982.

COPPER BINDING SITE IN SERUM AMINE OXIDASE TREATED WITH SODIUM DIETHYLDITHIOCARBAMATE

Shinnichiro SUZUKI, Takeshi SAKURAI, Akitsugu NAKAHARA, Osamu ODA,+ Takashi MANABE,+ and Tsuneo OKUYAMA+

Institute of Chemistry, College of General Education, Osaka University
Toyonaka, Osaka 560
+Department of Chemistry, Faculty of Science, Tokyo Metropolitan University
Fukazawa, Setagaya-ku, Tokyo 158

The structure of copper binding site in bovine serum amine oxidase (BSAO) treated with diethyldithiocarbamate ion(DDC) was discussed by comparing its absorption and electron spin resonance spectra with those of a model copper complex. The structure of the model complex, [Cu(2,2'-bipyridine)(DDC)]$^+$, suggested that at least two nitrogen atoms are coordinated around the copper ion in the native BSAO. Some organic chromophore from which the pink color of BSAO stems was also observed as the difference of the absorption spectra in the region of 450 to 600 nm between BSAO-DDC and the model complex.

Bovine serum amine oxidase(BSAO) catalyzes oxidative deamination of various amines by accepting two electrons from amines and transferring them to molecular oxygen.[1] The molecule of this enzyme contains two paramagnetic copper(II) ions which are refered to type-2 or non-blue copper, exhibiting a pink color because of an intense absorption band at around 460 nm. This characteristic absorption band was supposed to be responsible for charge transfer transitions from ligating groups to Cu(II) and/or an electronic transition in some organic chromophore.[2] In a previous paper, we suggested as the results of the spectroscopic studies on cobalt(II)-substituted BSAO that the chromophore exhibits a distinct positive circular dichroism(CD) band at around 370 nm and a negative CD band at near 440 nm.[3]

This letter describes the structure of the copper site of BSAO treated with sodium diethyldithiocarbamate(BSAO-DDC) by comparing spectroscopic and ESR spectra of BSAO-DDC with those of a model copper complex. The DDC is usually a reagent for the separation of copper ion from copper proteins as $Cu(DDC)_2$ complex.[4] Yamada and Yasunobu reported that the copper in bovine plasma monoamine oxidase was mostly removed by dialysis against DDC with a concomitant loss of activity.[5] However, our BSAO-DDC sample was observed to keep ca. 80 % of copper at the active site with the same procedure.

BSAO was isolated from bovine serum and then twice crystallized.[1] The molecular weight of this pink enzyme was disclosed to be ca. 190,000, which contains

about 2 g atoms of copper. The BSAO-DDC was prepared by dialyzing BSAO(Cu : 8.3 x 10^{-5} mol dm^{-3}) against 0.2 mol dm^{-3} sodium phosphate buffer(pH 7.2) containing sodium diethyldithiocarbamate(0.05 mol dm^{-3}) for 26 h at 4 °C. The resulting brown BSAO-DDC sample was dialyzed against the DDC-free buffer(about 1.5 dm^3) in order to remove the excess DDC. The concentration of copper in BSAO or BSAO-DDC was determined by use of an atomic absorption spectrophotometer, and the protein concentration was determined by measuring the optical density at 280 nm. The visible electronic absorption and electron spin resonance(ESR) spectra of BSAO-DDC are shown in Fig. 1(a) and Fig. 2(a), respectively. The electronic absorption spectrum is characterized by an intense band at 380 nm and a discernible shoulder at around 480 nm. The ESR spectrum in the region of 270 - 340 mT displays the existence of a tetragonal copper(II) in the modified BSAO($g_{//}$ = 2.17, $g_{\perp}$ = 2.04, $A_{//}$ = 17.0 mT). The nitrogen superhyperfine structure observed at 315 - 330 mT indicates that Cu(II) is bound to some nitrogenous ligands. The very weak peak at around 265 mT is assigned to one of the four copper hyperfine lines of unreacted native BSAO,[2] although the amount of the native species is estimated to be extremely small through the peak area.

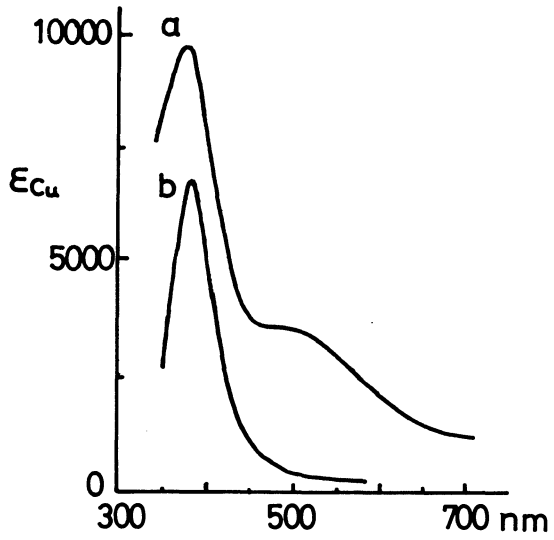

Fig. 1 Optical absorption spectra of BSAO-DDC(a) and $[Cu(bipy)(DDC)]^+$ complex(b) at room temperature.

a : copper concentration, 6.7 x 10^{-5} mol dm^{-3}; pH 7.2, 0.2 mol dm^{-3} phosphate buffer. b : solvent, aqueous ethanol(1 : 1 v/v).

In order to shed light on the structure of the copper site in BSAO-DDC, a model complex was prepared from $Cu(2,2'\text{-bipyridine})^{2+}$ complex* and sodium diethyldithiocarbamate in ethanol-water(1 : 1 v/v). The electronic absorption and the ESR

---

* 2,2'-bipyridine is abbreviated as bipy hereafter.

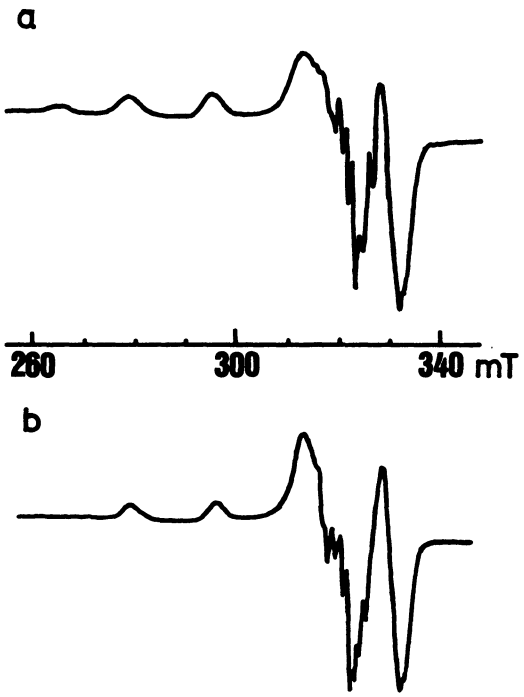

Fig. 2 ESR spectra of BSAO-DDC(a) and $[Cu(bipy)(DDC)]^+$ complex(b) at 77K.

a : pH 7.2, 0.2 mol dm^{-3} phosphate buffer.
b : aqueous ethanol(1 : 1 v/v).

spectra of the ternary copper complex, $[Cu(bipy)(DDC)]^+$,[6] are represented in Fig. 1(b) and Fig. 2(b), respectively. The intense band at 383 nm in Fig. 1(b) is supposed to be due to a S $\longrightarrow$ Cu charge transfer, being well contrasted to the intense band of BSAO-DDC at the same wavelength in Fig. 1(a). On the other hand, water-insoluble binary complex, $Cu(DDC)_2$, exhibited a charge transfer band at 428 nm in aqueous ethanol(1 : 1 v/v). Therefore the intense absorption band of BSAO-DDC at 380 nm suggests that there are two sulfur atoms of DDC and two nitrogen atoms of the endogenous groups(probably histidine imidazoles) as the ligating atoms of the copper site in BSAO-DDC. The shoulder band at around 480 nm is not supposed to be responsible for the copper center itself in the light of the fact that the model complex displays no absorption band in the 450 - 600 nm region. The shoulder band might be related to the chromophore under the influence of the copper(II). The ESR spectrum of $[Cu(bipy)(DDC)]^+$ complex, of which the spin Hamiltonian parameters are given as $g_{//} = 2.16$, $g_{\perp} = 2.04$, and $A_{//} = 17.1$ mT, indicates that the geometry of copper(II) is tetragonal,[8] and bears a strong resemblance to that of BSAO-DDC. A comparison of the ESR spectrum of BSAO-DDC with that of the model complex further afforded the evidence for the N_2S_2 donor set of the copper site in BSAO-DDC, as shown in Fig. 3.

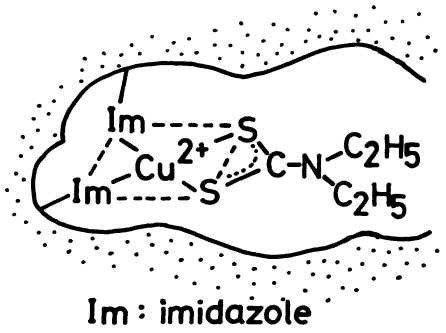

Fig. 3 Proposed structure of copper site of BSAO-DDC.

The present finding implies that the ligating groups around Cu(II) in native BSAO consist of at least two nitrogens, and that the shoulder at around 480 nm in Fig. 1(a) occurs not from the minor native species but definitely from some organic chromophore[3] under the influence of the copper in BSAO-DDC.

Acknowledgement: This work was supported by a Grant-in-Aid for Special Project Research(No. 56109009) from the Ministry of Education, Science and Culture of Japan.

References:

1) O. Oda, T. Manabe, and T. Okuyama, J. Biochem., 89, 1317 (1981).
2) S. Suzuki, T. Sakurai, A. Nakahara, O. Oda, T. Manabe, and T. Okuyama, FEBS Lett., 116, 17 (1980).
3) S. Suzuki, T. Sakurai, A. Nakahara, O. Oda, T. Manabe, and T. Okuyama, J. Biochem., 90, 905 (1981).
4) D. Cocco, L. Calabrese, A. Rigo, E. Argese, and G. Rotilio, J. Biol. Chem., 256, 8983 (1981).
5) H. Yamada and K. T. Yasunobu, ibid., 237, 3077 (1962).
6) [Cu(bipy)(DDC)]ClO_4: Found: C, 38.70; H, 4.14; N, 8.66; Cu, 14.3 %. Calcd for $C_{15}H_{18}N_3S_2CuClO_4$: C, 38.74; H, 3.88; N, 8.99; Cu, 13.9 %. The DDC molecule is coordinated to copper ion as a bidentate ligand.[7]
7) M. Bonamico, G. Dessy, A. Mugnoli, A. Vaciago, and L. Zambonelli, Acta Crystallogr., B 19, 886 (1965).
8) The ESR parameters, $g_{//}$ and $A_{//}$, for this complex fall in the region, where the ligands around copper(II) ions are two nitrogens and two sulfurs in the $g_{//}$-$A_{//}$ profile reported by Peisach et al..[9]
9) J. Peisach and W. E. Blumberg, Arch. Biochem. Biophys., 165, 691 (1974).

(Received January 20, 1982)