AN ESR STUDY OF PSEUDOMONAS COPPER-PROTEIN*

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Received November 28, 1962

Malmstrom and Vanngard have determined the electron spin resonance spectra of laccase and ceruloplasmin (1, 2) and found them to have exceptionally low hyperfine splitting constants, A = 0.009 cm^{-1} and 0.008 cm^{-1}, respectively. It was suggested that in these enzymes the copper is coordinated in some unique manner involving a high degree of delocalization of the unpaired electron (1) or perhaps interaction between Cu (II) and Cu (I), but not as one unpaired electron shared equally between two copper nuclei.

Beinert and his associates examined the electron spin resonance spectrum of cytochrome oxidase. They found that it was "similar to that of laccase and ceruloplasmin. The lack of nuclear hyperfine structure indicates that at least two copper ions are in close proximity in the enzyme and undergo exchange interaction" (3).

The hypothesis that pairs of copper atoms comprise the prosthetic centers of important copper-oxidases has been of considerable interest (cf. 4). In the present study we have investigated the possibility that electron spin resonance spectroscopy may be used to detect such structures by determining the electron spin resonance spectra of two copper-proteins which have only one copper atom per molecule.

Blue copper-proteins have been isolated from various pseudomonads (5, 6) and other microorganisms (7). They have unusually intense absorp-

* This study was supported by grants from the American Cancer Society and the United States Public Health Service, to whom we are greatly indebted.

tion in the region of 610 mu, molecular weights of about 16,000, and one atom of copper per molecule. Dr. P. Ambler generously provided two samples of the blue copper-protein from Pseudomonas aeruginosa, one of which contained 0.76 atom of copper per molecule, of which 0.67 atom was in the cupric form (8, 9) by chemical analysis, and 0.65 atom per molecule by quantitative ESR analysis using cupric-EDTA as a standard. His second sample contained 0.84 atom of copper per molecule; of this, 0.76 atom per molecule was in the cupric form (by chemical analysis), 0.70 atom by quantitative ESR spectroscopy. The second copper-protein was generously provided by Dr. H. Iwasaki; it was isolated from Pseudomonas denitrificans (10) and examined in the crystalline form.

The signals obtained from these two copper-proteins at -165°, using a Varian V-4500 spectrometer with 100 kc field modulation and field modulation amplitudes between 8 and 32 gauss, were almost identical and are illustrated by Fig. 1. They had g_{max} = 2.055, g-parallel = 2.157, and a hyperfine splitting constant A = 0.006 cm^{-1}, the lowest yet observed for any copper protein.

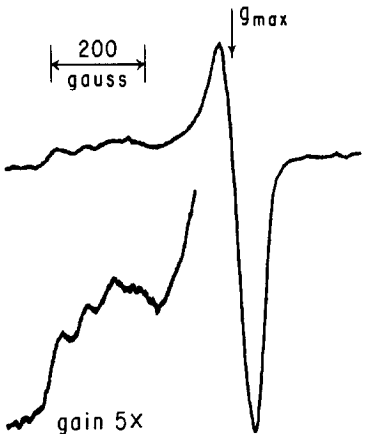

Figure 1. Electron spin resonance signal (derivative) of 1 mM copper-protein from Pseudomonas aeruginosa in 0.05 M ammonium acetate, pH 6.5, T = -165°.

These results mean that, for some copper-proteins at any rate, the explanation for small hyperfine splittings in the g-parallel region of the ESR spectrum, and for exhaltation of absorption in the 610 mu region, must be sought in a configuration which involves one copper atom only.

Thanks are due to Dr. N. Horie and Mr. Paul McMahill for assistance with analyses.

References

1. Malmstrom, B., and Vanngard, T., J. Mol. Biol., 2, 118 (1960).

2. Broman, L., Malmstrom, B., Aasa, R., and Vanngard, T., J. Mol. Biol., 5, 301 (1962).

3. Beinert, H., Griffiths, D. E., Wharton, D. C., and Sands, R. H.. J. Biol. Chem., 237, 2337 (1962).

4. Mason, H. S., Nature, 177, 79 (1956).

5. Horio, T., J. Biochem., Tokyo, 45, 195 (1958); 45, 267 (1958).

6. Coval, M. L., Horio, T., and Kamen, M. D., Biochem. Biophys. Acta. 51, 246 (1961).

7. Sutherland, I. W., and Wilkinson, H., Proc. Biochem. Soc., 84, 43P (1962).

8. Stark, G., and Dawson, C., Anal. Chem., 30, 191 (1958).

9. Griffiths, D. E., and Wharton, D. C., J. Biol. Chem., 236, 1857 (1961).

10. Suzuki, H., and Iwasaki, H., J. Biochem., Tokyo, 52, 193 (1962).