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Comment

The nature of the cupric site in nitrous oxide reductase and of Cu_A in cytochrome *c* oxidase

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We fully appreciate the arguments brought forth by Li et al. in favor of the now generally held explanation for the unusual properties of Cu_A in cytochrome *c* oxidase. However, it is probably fair to say that through the years there has been much controversy and uncertainty about the nature of Cu_A and some of this is still lingering on. The statement has been made that this Cu site is different from any other known to date [1]. Today, with spectroscopic and biochemical information on nitrous oxide reductase from *Pseudomonas stutzeri* accumulating [2–6], this view may no longer be valid. Note also that the primary structure of nitrous oxide reductase reveals a highly conserved consensus sequence with that of subunit II of cytochrome *c* oxidase [6,7].

Renewed uncertainty about the Cu components of cytochrome *c* oxidase arose through the finding in several laboratories of a third Cu per molecule, which seems to be in the Cu(I) state [8,9]. Also, the reported presence of only one cysteine in the ba_3 oxidase from *Thermus thermophilus* (which has a typical Cu_A) has cast doubt on the accepted picture of the Cu_A site [10]. Since in science progress in understanding complicated situations frequently comes from apparently unrelated work, we were prompted to draw attention to the similarities of EPR spectra of nitrous oxide reductase, which catalyzes a similar reaction, to those of cytochrome *c* oxidase. Perhaps this was premature, but we expect that our ongoing work on nitrous oxide reductase together with progress in determining the significance of the third Cu atom in cytochrome *c* oxidase will answer the question to what extent our suggestion was relevant.

To our knowledge, neither a four-line pattern for a mononuclear site nor a seven-line pattern for a binuclear site has been fully resolved in the g_{II} region (fig.1). Possibly the g_{II} region for cytochrome *c* oxidase is not resolved because: (i) the g_{mid} component of the heme of cytochrome *a* is superimposed onto the g_{II} region for S- and X-band data; (ii) the interaction of ferric sites in cytochrome *c* oxidase but not in nitrous oxide reductase affects the resolution of the lines; and (iii) the lines in the Q-band are broad and the hyperfine structure, irrespective of whether taken under passage conditions, is not resolved. Since the

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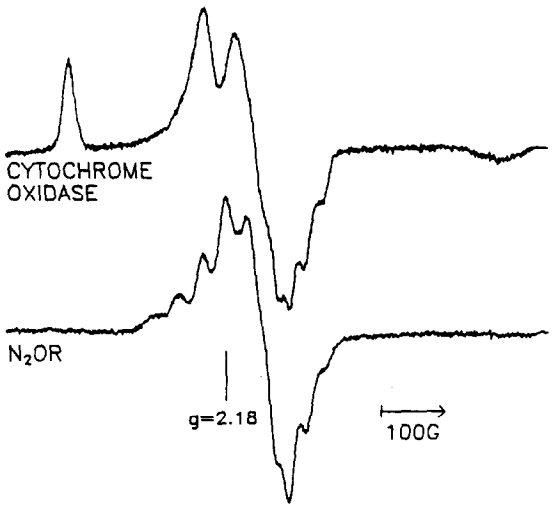

Fig.1. S-band first-derivative EPR spectra of cytochrome *c* oxidase and nitrous oxide reductase. Microwave frequency, 2.792 GHz; modulation frequency, 100 kHz; modulation amplitude, 0.3 mT; microwave power, 10 dB; temperature, 20 K. Cytochrome *c* oxidase [9] and nitrous oxide reductase [2] were prepared as described in the literature.

presence of a [Cu(II),Cu(I)] site implies the existence of a lower oxidation state, as correctly pointed out by Li et al., it appears more reasonable to suggest that the reduced hyperfine coupling reflects delocalization of the electron from the cupric site. Whether delocalization of the spin density is accomplished by the Cu–Cu interaction for nitrous oxide reductase or through the same or another interaction for cytochrome *c* oxidase needs further study.

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REFERENCES

[1] Mims, W.B., Peisach, J., Shaw, R.W. and Beinert, H. (1980) J. Biol. Chem. 255, 6843–6846.
[2] Coyle, C.L., Zumft, W.G., Kroneck, P.M.H., Koerner, H. and Jakob, W. (1985) Eur. J. Biochem. 153, 459–467.
[3] Dooley, D.M., Moog, R.S. and Zumft, W.G. (1987) J. Am. Chem. Soc. 109, 6730–6735.
[4] Riester, J., Zumft, W.G. and Kroneck, P.M.H. (1989) Eur. J. Biochem. 178, 751–762.
[5] Jin, H., Thomann, H., Coyle, C.L. and Zumft, W.G. (1989) J. Am. Chem. Soc., in press.
[6] Scott, R.A., Zumft, W.G., Coyle, C.L. and Dooley, D.M. (1989) Proc. Natl. Acad. Sci. USA, in press.
[7] Viebrock, A. and Zumft, W.G. (1988) J. Bacteriol. 170, 4658–4668.
[8] Bombelka, E., Richter, F.-W., Stroh, A. and Kadenbach, B. (1986) Biochem. Biophys. Res. Commun. 140, 1007–1014.
[9] Steffens, G.C., Biewald, K. and Buse, G. (1987) Eur. J. Biochem. 164, 295–300.
[10] Beinert, H. and Vaenngard, T. (1988) Chem. Scr. 28A, 127–131.