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Mutual Interactions between Macromolecular Reducing Substrates in *Pseudomonas aeruginosa* Nitrite Reductase

M. C. SILVESTRINI, M.G. TORDI

C.N.R. Center of Molecular Biology, c/o Inst. of Biochemistry, I University of Rome (La Sapienza), Rome, Italy

A. COLOSIMO and M. BRUNORI

Dept. of Biochemistry, Faculty of Medicine, II University of Rome (Tor Vergata), Rome, Italy

The existence "*in vitro*" of more than one macromolecular electron donor (azurin and cytochrome c_{551}) for the enzyme which reduces NO_2^- to NO in the respiratory chain of *Pseudomonas aeruginosa* (E.C. 1.9.3.2.), poses a number of problems relevant to a more complete understanding of the bacterial respiratory pathways [1]. From the biochemical point of view a crucial question is to define to what extent the molecular mechanisms of electron transfer from the two substrates to the enzyme are similar. The problem was attacked by kinetic methods under pre steady-state and steady-state conditions, using either only one or both substrates simultaneously. The results of these studies indicate that: i) cytochrome c_{551} is a more rapid electron donor, compared with azurin, by a factor of 4–5 [2]; ii) the two substrates display a partially competitive inhibition.

Given the presence of a very fast electron transfer between azurin and cytochrome c_{551} [3], however, the latter result by itself does not permit the definition of the nature of the reactive site(s) on the enzyme. A more direct answer to the problem may be obtained using Hg-substituted azurin [4], which is ineffective as a redox substrate, but still reacts with the specific binding sites on the enzyme. This may allow a straightforward interpretation of the observed kinetic patterns in mechanistic terms.

1 T. Yamanaka, *Nature, 204,* 253–257 (1964).
2 M. C. Silvestrini, M. G. Tordi, A. Colosimo, E. Antonini and M. Brunori, *Biochem. J., 203,* 445 (1982).
3 E. Antonini, A. Finazzi-Agrò, L. Avigliano, P. Guerrieri, G. Rotilio and B. Mondovì, *J. Biol. Chem., 245,* 4847 (1970).
4 A. Finazzi-Agrò, G. Rotilio, L. Avigliano, P. Guerrieri, V. Boffi and B. Mondovì, *Biochemistry, 9,* 2009 (1970).

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Endor of Metalloenzymes

BRIAN M. HOFFMAN

Department of Chemistry, Northwestern University, Evanston, Ill. 60201, U.S.A.

Much of the current understanding of metalloenzyme structure and function has been obtained by application of spectroscopic methods, in particular, electron paramagnetic resonance (epr) techniques. Nevertheless, epr studies often are of limited utility because hyperfine interactions from constituent or substrate nuclei are unobservable. Electron-nuclear double resonance (endor) is a high-resolution extension of epr that can often resolve these interactions, thus removing the limitation. In endor, nuclear resonance transitions are observed through their influence on the strength of the epr signal and, unlike ordinary nmr, they can be comparably observable for all magnetic nuclei. Thus, the technique offers some unique capabilities for the characterization of an enzyme catalytic center. We will describe the application of endor to metalloenzyme resting states and reaction intermediates by choosing examples from systems we have studied, which include cytochrome oxidase, peroxidases, and nitrogenase; resonances from ^{1}H, ^{14}N, ^{17}O, ^{57}Fe, $^{63,65}Cu$, and ^{95}Mo may be discussed.

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Bacterial Cadmium-binding Proteins

DENISE P. HIGHAM, PETER J. SADLER

Department of Chemistry, Birkbeck College, Malet Street, London, WC1E 7HX, U.K.

and MICHAEL D. SCAWEN

CAMR, Porton Down, Salisbury, Wilthire, U.K.

There is much current intererest in the metallothioneins. These are low molecular weight proteins (6–7,000 Daltons) which have a high cysteine (33%) and metal content (7 g atoms/mol). This, together with the absence of aromatic amino acids and histidine, makes this protein very unusual [1].

Metallothioneins have no defined functions, although many possible roles have been suggested including detoxification [2], and regulation of zinc [3] and copper metabolism [4].

Metallothioneins are widespread in mammals [1] and have also been isolated from fish [5], invertebrates [6], plants [7] and eukaryotic microorganismas such as yeast [8] and *Neurosporra crassa* [9].