PS1.2 — TU

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A NITRIC OXIDE ADDUCT OF THE BINUCLEAR IRON CENTER IN DEOXYHEMERYTHRIN FROM *PHASCOLOPSIS GOULDII*. ANALOGUE OF A PUTATIVE INTERMEDIATE IN THE OXYGENATION REACTION

The preparation and characterization of a nitric oxide adduct of the binuclear iron site in the non-heme oxygen-carrying protein, hemerythrin (Hr) [1] are reported. Addition of gaseous NO to an anaerobic solution of deoxyHr results in a nitric oxide adduct. The optical spectrum of this adduct is different from that of any other adduct of Hr in [Fe(II),Fe(II)] (deoxy), [Fe(III),Fe(III)] (oxy or met) or [Fe(II), Fe(III)] (semi-met) oxidation levels (Fig. 1). Samples of deoxyHr frozen within a few minutes after addition of gaseous NO yield an axial EPR spectrum with $g_{\parallel} = 2.76$, $g_{\perp} = 1.84$ (Fig. 2). Double integration of the spectrum yields 0.9 spins/2 Fe. Addition of excess N_3^- or CNO$^-$ but not several other anions results in immediate bleaching of the optical spectrum and disappearance of the EPR spectrum. The anion specificity is similar to that observed for bleaching of the oxyHr color and demonstrates the reversibility of the NO reaction. The ^{57}Fe Mössbauer spectrum of the NO adduct consists of two quadrupole doublets at 100 K but shows magnetic hyperfine splitting of both doublets at 4.2 K. The parameters of one of the doublets are characteristic of high spin Fe(II). The parameters of the remaining doublet are close to those of a non-heme ferrous nitrosyl complex having $S = 3/2$. The g values of the EPR spectrum can be fit by assuming antiferromagnetic coupling of the high spin Fe(II) ($S = 2$) with the $\{FeNO\}^7$ ($S = 3/2$) leading to a ground state, $S' = 1/2$. X-ray crystallography suggests that O_2 has direct access to only one of the iron atoms in Hr and binds with a bent Fe-O-O geometry [2]. These data suggest a [Fe(II),Fe(III)NO$^-$] formulation for the NO adduct of deoxyHr. This formalism is analogous to that of a presumed [Fe(II),Fe(III)O_2^-] intermediate in the oxygenation reaction:

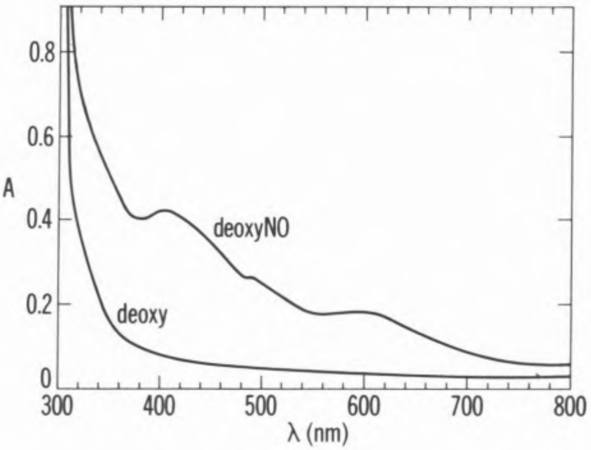

Fig. 1

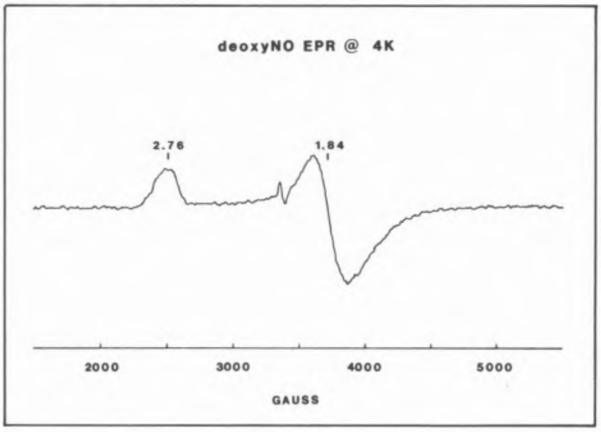

Fig. 2

$$[Fe(II),Fe(II)]\ (deoxy) + $$
$$+ O_2 \rightleftharpoons [Fe(III),Fe(III)O_2^{2-}]\ (oxy)$$

Our results suggest either that the iron atoms in deoxyHr are antiferromagnetically coupled or become coupled after addition of NO or O_2 to the exposed iron, but prior to formal oxidation of the second iron.

REFERENCES

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[2] R.E. Stenkamp, L.C. Sieker, L.H. Jensen, *J. Am. Chem. Soc.*, **106**, 618-622 (1984).

PS1.3 — TH

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CHARACTERIZATION OF A NOVEL COPPER ENZYME: BACTERIAL NITROUS OXIDE REDUCTASE

The respiratory capability of bacteria is highly diverse considering the terminal oxidants being used other than dioxygen. About 25 genera of taxonomically different groups of bacteria respire nitrate to N_2, NO or N_2O. Three or four terminal oxido-reductases accomplish this respiratory redox process. When dinitrogen is the final product of nitrate respiration, it is formed from N_2O, involving catalysis by a novel Cu protein. The enzyme from denitrifying *Pseudomonas perfectomarina* (ATCC 14405) has been isolated and purified to homogeneity [1]. Nitrous oxide reductase contains about eight copper atoms per molecular weight 120 000. The protein is a dimer of two presumably identical subunits. Several spectroscopically distinct forms of the enzyme have been identified. A "pink" form of the enzyme is obtained when the purification is done aerobically. The specific activity of this species is 15-35 nkat per mg protein as measured by the nitrous oxide-dependent oxidation of photochemically reduced benzyl viologen. The spectrum of the pink form has absorption maxima at 480, 530, 620 and 780 nm (Fig. 1a).

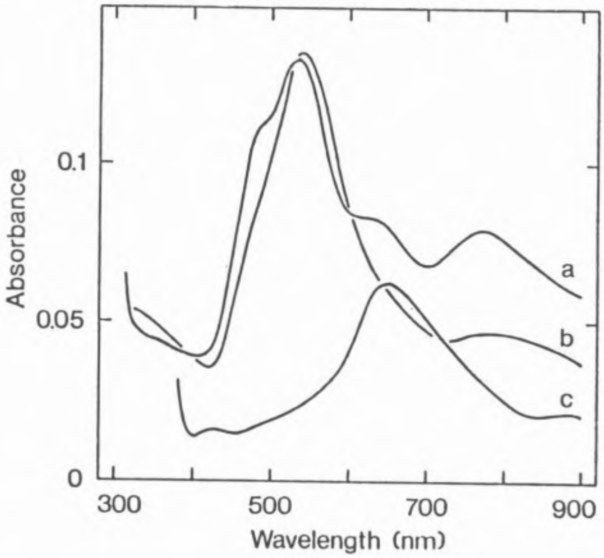

Fig. 1
Electronic spectra of (a) pink form of enzyme as isolated; (b) purple form as isolated; (c) dithionite-reduced form

Cells that were broken anaerobically and fractionated excluding oxygen from all chromatographic steps, yielded a "purple" form of the copper protein whose catalytic activity was consistently three to fivefold higher than that of the pink form. The spectrum of the purple form is shown in Fig. 1b. This form, as isolated, had absorption maxima at 540 and 780 nm. Maxima at 480 and 620 nm observed in the pink form were found only as slight shoulders.

EPR spectra of the pink and purple forms of the enzyme were similar, with the purple form providing better resolution. A representative spectrum of the oxidized purple protein is shown in Fig. 2a. The data suggest the presence of an unusual type 1 Cu. The type 1 Cu is unusual in the sense that although there is a relatively narrow hyperfine splitting ($A_{\parallel} = 35.33$ Gauss; $g_{\parallel} = 2.215$, $g_{\perp} = 2.033$) it does not appear to be associated