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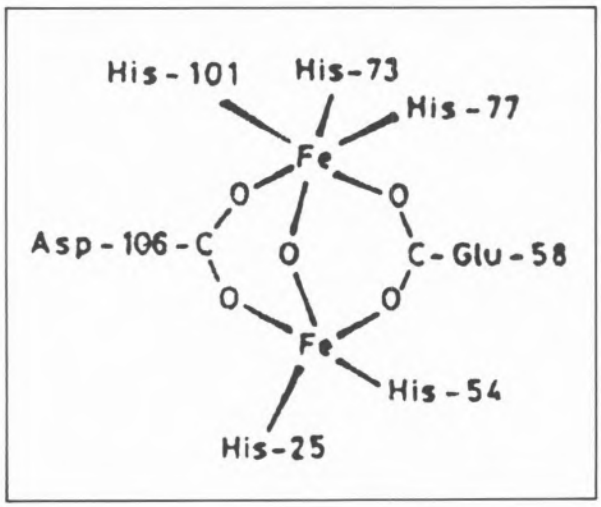
ACTIVE SITE CHEMISTRY OF OCTAMERIC AND MONOMERIC HEMERYTHRIN FROM *THEMISTE ZOSTERICOLA*

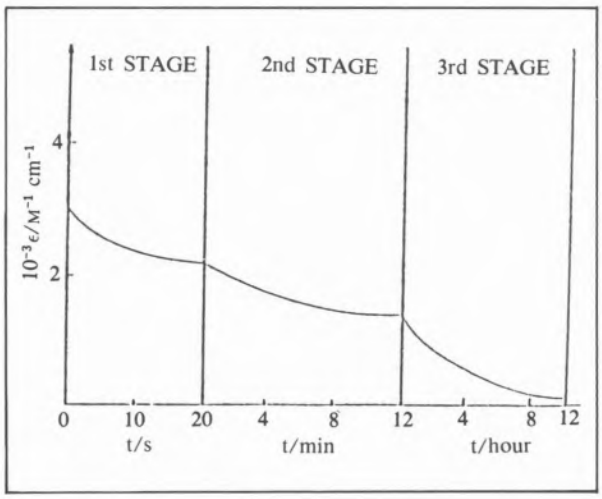
Hemerythrin [1,2] was obtained from the coelomic fluid of *Themiste Zostericola* as the octamer (M.Wt. 108,000), which has eight identical subunits, and from the retractor muscle as the monomer, metmyohemerythrin (M.Wt. 13,900). Each subunit is known to contain a binuclear Fe active site capable of binding O_2.

The structure of the active site of the met, Fe(III,III), form is as shown [3]. Coordination of hydroxide (corresponding to an acid dissociation of pK_a *ca.* 8.0) is known to occur at the five-coordination iron. Using EXAFS [4] it has been demonstrated that the deoxy, Fe(II,II), form has no μ-oxo bridge and there is an increase in the Fe-Fe distance.



Reduction of octameric methemerythrin has been studied by WILKINS *et al.* using dithionite and a semi-met, $Fe(II,III)_8$, intermediate has been successfully characterised. It has also been proposed that further reduction proceeds by long-distance intramolecular electron-transfer (*ca.* 30 Å yielding Fe(II,II) and Fe(III,III) subunits [5], the latter being rapidly re-reduced to Fe(II,III).

To further augment this study, and monitor precisely the latter stages of reduction, detailed investigations of the reduction of methemerythrin and metmyohemerythrin using as one-electron reductants the Sargeson cage complexes $[Co(sep)]^{2+}$ (E° -0.30 V) and $[Co(sarCl_2)]^{2+}$ (E° -0.13 V), the triazacyclononane complex $[Co(9\text{-}aneN_3)]^{2+}$ (E° -0.40 V) and $[Cr(bipy)_3]^{2+}$ (E° -0.26 V) have been carried out. Three stages are clearly seen in the reduction of methemerythrin (pH 6.3 - - 9.0). The figure shown is for a reaction monitored at 400 nm with $MetHr = 1.0\times10^{-4}$ M (expressed as monomer), $[Co(sep)]^{2+} = 1.4\times10^{-3}$ M, pH 6.3, $I = 0.15$ M (Na_2SO_4). The rate of the first stage shows a first-order dependence on reductant. Rate constants for stages 2 and 3 (3.7×10^{-3} and 1.2×10^{-4} s^{-1} respectively, pH 8.2) show no dependence on the concentration or nature of the reductant consistent with an intramolecular process. Hydrogen-ion dependencies are observed for the first and second stages. Rate constants for stage 3 are however independent of $[H^+]$.



Consumption of reductant in the different stages was determined using $[Cr(phen)_3]^{2+}$ which has an intense absorbance at 850 nm ($\epsilon = 6500$ M^{-1} cm^{-1}).

It was found that eight equivalents (per octamer) of reductant are consumed in the first stage followed by four equivalents in each of stages 2 and 3. These results were confirmed using the strong reductant $[Cr(edta)]^{2-}$ (E° −1.0 V) which enabled spectra to be recorded more precisely at the end of each stage.

Reduction of metmyohemerythrin also occurs in three stages at pH 8.2. The first stage gives a first-order dependence on reductant. The rate constants for stages 2 and 3 (4.0×10^{-3} and 9.0×10^{-4} s^{-1} respectively) show no dependence on the nature or concentration of the reductant. Further detail of these different stages will be presented.

The existence of a quarter-met intermediate in the reduction of methemerythrin (product of the second stage) indicates that the Fe(II,III) units generated at the end of the first and second stages are not identical. Furthermore, comparison of the rate constants for the second stage of the reduction of methemerythrin and metmyohemerythrin suggests a common rate-controlling conformational change in both cases.

REFERENCES

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PS2.25 — MO

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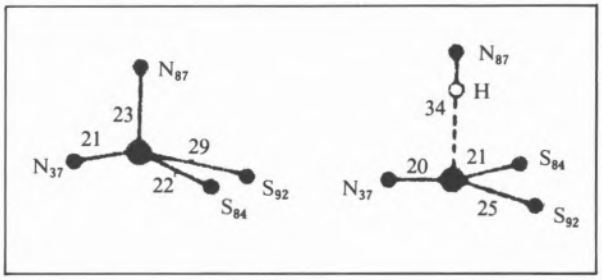
ACID DISSOCIATION CONSTANTS FOR THREE PLASTOCYANINS

The metalloprotein plastocyanin (M.Wt. 10,500) consists of 99 amino acids and a single (Type 1) Cu active site [1]. It is involved in electron transport ($E^\circ \sim 360$mV) in the chloroplast where it functions as an oxidant for cytochrome f and a reductant for P700. The Cu atom is coordinated by His37, Cys84, His87 and Met92 (NSNS donor atom set) in a distorted tetrahedral arrangement for both Cu oxidation states. Proton induced deactivation of the active site [2] has been shown to correspond to dissociation of His87 yielding a redox-inactive three-coordinate structure [3].



The acid dissociation constant, K_a, can be determined using NMR

$$HPCu(I) \overset{K_a}{\rightleftharpoons} H^+ + PCu(I) \qquad (1)$$

spectroscopy [4] which allows direct observation of the relevant protonation. The kinetic method employs oxidants such as $[Fe(CN)_6]^{3-}$ and $[Co(phen)_3]^{3+}$ to probe the reactivity of the protein in the pH range of interest, where PCu(I) but not HPCu(I) is redox active (2).