Poster Session: Models of Metalloproteins

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Electron Transfer in Catecholamines – Tetrapyridine-iron(III) Complex Ions Anchored to Polypeptides

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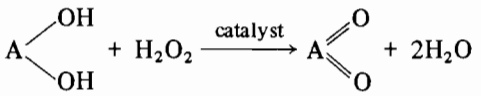
$[Fe(tetpy)(OH)_2]^+$ complex ions (tetpy = 2,2′,2″,2‴-tetrapyridyl) anchored to poly(L-glutamate) (FeL) or poly(D-glutamate) (FeD) through the γ-carboxylate groups of the polymers [1] exhibit stereoselective peroxidatic activity with a number of optically active substrates, according to the reaction (pH = 7.0, Tris buffer 0.05 *M*) [2, 3]:



Progressive binding of complex ions was found to determine a coil-to-α-helix transition in the charged polypeptide matrices, as well as aggregation phenomena with a freezing of iron molecules inbetween helical chains. Under these conditions, electron transfer from catecholamines (L-adrenalin and L-dopa) to the central iron(III) ion, which is rate-determining, proceeds stereoselectively because extensive and possibly specific interactions between substrate molecules and the peptidic residues in the close environment of the active sites ensure different steric constraints for the two diastereomeric precursor complexes. The rate constants of electron transfer processes can be expressed by nuclear and electronic factors which are highly sensitive to the separation of the redox centers [4]. Therefore, even small differences in steric hindrances between the diastereoisomers would affect differently the optimal mutual orientation of the reacting OH group of the substrates and the peripheral tetrapyridyl ligand of the active sites, whose π-system very likely acts as an electron-transfer agent [5]. Preliminary results on conformational analysis of substrate–catalyst adducts support such conclusions. This is reflected in the rate constants of the electron-transfer step, as illustrated by the Lineweaver–Burk plot of the reaction catalyzed by FeL or FeD systems at a complex to polymer-residue ratio [C]/[P] = 0.20, reported in Fig. 1. From the results, the turnover numbers for the oxidation of L-adrenaline are 0.56 ± 0.07 and 2.04 ± 0.23 min^{-1}, respectively, whereas those for the oxidation of L-dopa are 0.42 ± 0.05 and 1.14 ± 0.12

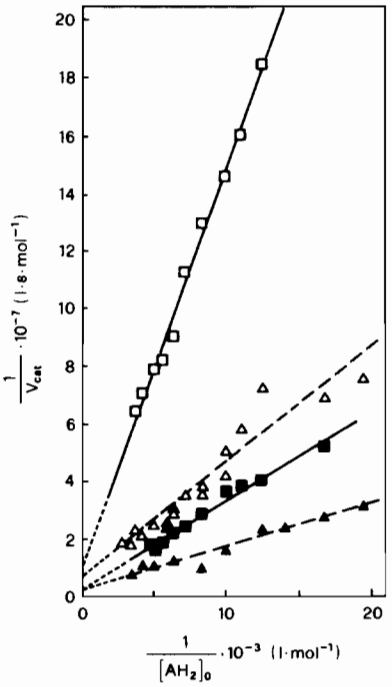


Fig. 1. Lineweaver–Burk plot for the electron transfer reaction between L-adrenaline (full lines) or L-dopa (broken lines) and FeL (empty symbols) or FeD (full symbols) systems at a complex to polymer ratio [C]/[P] = 0.20. T = 25.9 °C, pH = 7.0 (Tris buffer 0.05 *M*), $[C]_0 = 1 \times 10^{-5}$ (full lines) or 2×10^{-5} *M* (broken lines), $[H_2O_2]_0 = 1 \times 10^{-2}$ *M*. AH_2 denotes the substrate.

min^{-1} (25.9 °C). On the other hand, the Michaelis constants are $K_M = (1.30 \pm 0.18) \times 10^{-3}$ and $(1.05 \pm 0.15) \times 10^{-3}$ *M* in the former case, and $(8.01 \pm 1.12) \times 10^{-4}$ and $(5.50 \pm 0.48) \times 10^{-4}$ *M* in the latter.

According to Marcus theory [6], the ultimate rate of electron transfer depends on the rate of reorganization of the surrounding medium (precursor and successor complex formation). When the asymmetric polymers play an active role in the reaction, being involved in the formation of the precursor complex the polypeptide itself might experience local conformational changes. Since the time scale of these rearrangements is unknown, it is possible that the polymer reorganization retards the overall rate of the electron transfer process. This could explain the finding that stereoselectivity occurs at the expense of the efficiency of reaction, as shown in Fig. 2. Using catalysts at low [C]/[P] ratio, the active sites are exposed to the bulk solvent and a substrate–coordinated metal chelate forms without any

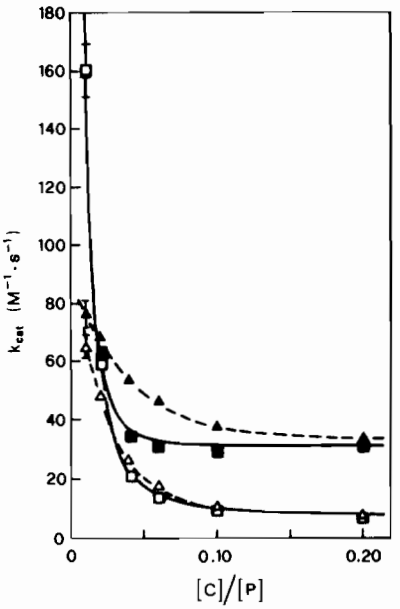


Fig. 2. Second-order rate constants for the electron transfer reaction between L-adrenaline (full lines) or L-dopa (broken lines) and FeL (empty symbols) or FeD (full symbols) as a function of [C]/[P] ratio of the catalytic systems. Experimental conditions as in Fig. 1; $[AH_2]_0 = 1 \times 10^{-4}$ (full lines) or 2×10^{-4} *M* (broken lines).

assistance from the coiled polypeptides [3]. This accounts for the lack of stereoselectivity in the oxidation reaction, which takes place at much higher rate however (Fig. 2).

1 M. Branca and B. Pispisa, *J. Chem. Soc. Faraday I, 73*, 213 (1977).
2 M. Barteri and B. Pispisa, *Biopolymers, 21*, 1093 (1982); *Makrom. Chem., Rapid Commun., 3*, 715 (1982).
3 B. Pispisa, 'Stereoselective Peroxidatic Activity of Iron(III) Complex Ions Supported on Polypeptides', in 'Coordination Chemistry of Metalloenzymes in Hydrolytic and Oxidative Processes', I. Bertini, R. S. Drago and C. Luchinat Eds., Nato Advanced Institutes Series, Reidel, Utrecht, in press.
4 N. Sutin, 'Electron Transfer Reactions of Metal Complexes in Solution', in 'Tunneling in Biological Systems', B. Chance *et al.* Eds., Academic Press, New York (1979), p. 201; *Acc. Chem. Res., 15*, 275 (1982).
5 C. E. Castro and H. F. Davis, *J. Am. Chem. Soc., 91*, 5405 (1969); J. K. Yandell, D. P. Fay and N. Sutin, *ibid., 95*, 1131 (1973); D. L. Toppen, *ibid., 98*, 4023 (1976).
6 R. A. Marcus, *Ann. Rev. Phys. Chem., 15*, 155 (1964).

N2

Model Complexes for the Active Sites of Reduced and Oxidized Sites of Hemocyanin and Tyrosinase. Structures of Binuclear Cu(I) and Cu(II) Complexes and Characterization of a Model Copper Monooxygenase Reaction

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It has been established that the dioxygen carrying copper containing protein hemocyanin and the copper monooxygenase tyrosinase both contain electronically coupled binuclear copper centers. In the deoxy protein, copper centers are thought to be two or three coordinate, while in their oxidized form, tetragonally coordinated Cu(II) ions are bridged by an endogenous protein group which is thought to be phenolate from tyrosine. Coordinated dioxygen (as peroxide) is also known to bridge the two coppers. The binding of other exogenous ligands (azide, chloride, *etc.*) to the binuclear copper centers has been used to probe the active site structures and some of these may also bridge the Cu(II) ions in methemocyanin or tyrosinase derivatives [1].

Extensive coordination model studies are being carried out [2–4] in order to 1) determine the nature of the ligand donors and coordination geometries of these active site copper centers; 2) mimick and understand the coordination chemistry of relevant Cu(I) systems; 3) mimick the Cu(II) binuclear centers in order to relate the spectroscopic and chemical properties of these models and their derivatives (with appropriate probes) to those of the proteins and 4) understand those factors necessary and the mechanism(s) for reversible dioxygen binding and 'activation' of dioxygen (*i.e.* specific hydroxylation of substrates by the copper monooxygenases).

As part of our own extensive investigations into the chemistry of binuclear Cu(I) and Cu(II) moieties, we have employed the binucleating ligand m-XYLpy, *I* (py = 2-pyridyl), where two tridentate donor groups are separated by a meta-xylyl bridge [5]. A novel binuclear cuprous complex, *II*, *(See next column)* containing well separated trigonally coordinated Cu(I) ions is isolated by the reaction of *I* with two equivalents of $Cu(CH_3CN)_4PF_6$ [6]. The coordination geometry and the type of ligands in *II* makes it a good model complex for the deoxy-sites in the proteins. In addition, *II* reacts with O_2 in a manner analogous to the copper monooxygenase tyrosinase. Specific hydroxylation of the xylyl ligand occurs producing the phenoxy- and hydroxy- (R = H) doubly bridged binuclear Cu(II) complex *III*.