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Stereoselective Effects in Electron-Transfer Reactions Catalyzed by Iron(III) Chelate Ions Anchored to Polypeptides

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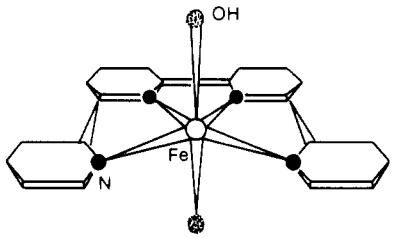
$[Fe(tetpy)(OH)_2]^+$ complex ions (tetpy = 2,2′:6′,2″:6″,2‴-quaterpyridyl) anchored to poly(L-glutamate) (FeL) or poly(D-glutamate) (FeD) catalyze the H_2O_2 oxidation of L-adrenaline (epinephrine) at pH 7. The catalytic sequence involves (1) formation of a substrate–catalyst precursor complex, (2) intramolecular electron transfer in this system, and (3) oxidation of both the lower valence metal chelate and adrenaline radical by H_2O_2 in subsequent fast steps. Stereoselective effects in the catalysis are coupled with the amount of α-helix in the polymeric supports (x_a), which in turn depends on the amount of bound iron(III) chelate ions. Thus, at a complex to polymer residue ratio [C]/[P] = 0.01 ($x_a < 0.05$) $k_{FeD} = k_{FeL} = 159.0 \pm 8.2$ $M^{-1}\cdot s^{-1}$ whereas at [C]/[P] = 0.20 ($x_a \approx 0.70$) $k_{FeD} = 31.2 \pm 2.0$ $M^{-1}\cdot s^{-1}$ and $k_{FeL} = 7.5 \pm 0.5$ $M^{-1}\cdot s^{-1}$ (26 °C). Evidence is presented that stereoselectivity is chiefly controlled by kinetic parameters. The rate constants of the electron transfer from the substrate to the central metal ion, which represents the rate-determining step, are $(2.9 \pm 0.3) \times 10^{-2}$ and $(1.2 \pm 0.1) \times 10^{-2}$ s^{-1} with FeD and FeL systems, respectively, at [C]/[P] = 0.10 and $(3.4 \pm 0.4) \times 10^{-2}$ and $(0.9 \pm 0.1) \times 10^{-2}$ s^{-1} when [C]/[P] = 0.20. Effects of the stereochemical features of the precursor complex on the observed phenomena are discussed. Evidence is also produced of a parallel complex ion uncatalyzed oxidation of L-adrenaline, whose rate is controlled by the chiral complex–polyelectrolyte systems.

Introduction

We have recently shown that hemin-like $[Fe(tetpy)(OH)_2]^+$ complex ions (I) anchored[1] to sodium poly(L-glutamate) (FeL)

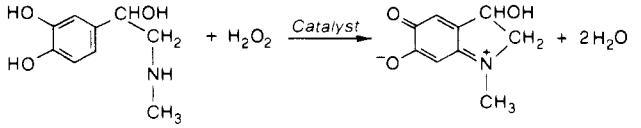


(I)

or poly(D-glutamate) (FeD) are efficient catalysts for the H_2O_2 oxidation of L-(+)-ascorbate anion to dehydroascorbic acid[2] (tetpy = 2,2′:6′,2″:6″,2‴-quaterpyridyl). It has been also reported that these enantiomeric systems act stereospecifically in the sense that they are able to distinguish the optically active substrate, the effect being the greater the larger is the amount of α-helical fraction in the polypeptide matrices.[3]

A number of transition-metal derivatives have been used as model compounds[4] for peroxidases,[5] though very little attention has been paid to systems formed by metal ions or metal chelates supported on polymers.[6-8] Furthermore, in all cases but one[7] achiral species were used so that there is a paucity of information pertaining to stereoselectivity in redox reactions.

We present here the results of the kinetic study on the oxidation of L-adrenaline (epinephrine, II) to adrenochrome (III), catalyzed by the above mentioned materials, according to the reaction[9,10]



(II) (III)

The aim of the work was twofold: first, to investigate the origin of stereoselectivity and, second, to elucidate the role played by the stereochemical characteristics of the precursor complex in the observed phenomena. Evidence is produced that stereoselectivity is almost entirely kinetically controlled. The effect very likely arises from the difference in the steric interference between the diastereomeric precursor complexes, which is reflected in a difference between the standard free energies of the transition states of the electron-transfer step.

A mechanistic interpretation of the experimental results will be discussed in the light of a few general considerations concerning the structural features of the catalytic systems.

Experimental Section

Materials. The pseudooctahedral Fe(III) chelate was prepared as already described.[14] It is an oxo-bridged dimeric compound in the solid state. In solution, at the pHs and concentrations with which we are primarily concerned, it is almost entirely in the low-spin mononuclear form.[14b] The polypeptides were purchased from Miles-Yeda (M_r = 30000) and converted to the sodium salt by 0.01 N NaOH. The stock solutions were then exhaustively dialyzed against water to eliminate excess sodium ions and the materials collected by freeze-drying. Concentration of the polymers [P], determined by UV absorption at 200 nm (ϵ = 5500 $M^{-1}\cdot cm^{-1}$), refers to the monomeric unit (monomol/L). Tris buffer (Sigma) was employed in the chloride form. Under the experimental conditions used (pH 7.01 ± 0.03) the degree of association of complex ions by polypeptides is higher than 93%, according to equilibrium dialysis experiments.[1] Analytical grade L-(−)-adrenaline (BDH) and stabilizer-free H_2O_2 (Erba) were used

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without further purification. Concentration of the substrate was determined spectrophotometrically (λ_{max} = 280 nm, ϵ_{max} = 2740). All measurements were performed on solutions freshly prepared by using doubly distilled water.

Methods. Kinetic experiments measuring the formation of adrenochrome at 320 nm (the cyclic oxidation product of adrenaline, λ_{max} = 308 and 480 nm[15]) were carried out under pseudo-first-order conditions with respect to H_2O_2. A typical run consisted of adding hydrogen peroxide by a microsyringe into the 1-cm optical cell containing 2 mL of catalyst and substrate, both systems being thermostated at 25.9 ± 0.1 °C. The experimental conditions normally used were as follows: pH 7.0 (0.05 M Tris buffer) $[AH_2] = 1 \times 10^{-4}$ M, $[H_2O_2] = 1 \times 10^{-2}$ M, $[C] = 0.5 \times 10^{-5}$–5×10^{-5} M, and [C]/[P] = 0.01–0.20 (AH_2, C, and P denoting adrenaline, complex ions, and polyelectrolytes, respectively). Measurements were also carried out with different initial concentrations of H_2O_2 and at another temperature (15.8 °C). Plots of log $(A_t - A_\infty)$ vs. t were linear over more than 2 half-lives, and the observed rate constants were obtained from the slopes. Under the experimental conditions used, adrenochrome undergoes a rearrangement reaction,[9,16] which has a rate constant 1 order of magnitude lower than that of its formation, the specific rate being around 0.3×10^{-3} s^{-1} (27 °C) as measured by the disappearance of the band at 480 nm. Four kinetic measurements were performed for each run to obtain consistency in results. Plots of k_{obsd} (s^{-1}) against complex concentration, at fixed [C]/[P] ratio, always gave straight lines, and the second-order rate constants k_{cat} ($M^{-1}\cdot s^{-1}$) were obtained from the slopes. Stereoselectivity is given by k_{FeD}/k_{FeL}, k_{FeD} and k_{FeL} ($M^{-1}\cdot s^{-1}$) being the observed specific rates of the catalysis. Initial reaction velocities, $V_0 = -d[AH_2]/dt$ ($M\cdot s^{-1}$), were evaluated from the slope of adrenochrome concentration against time curves at $t \rightarrow 0$, the extent of adrenaline oxidation being generally limited to about 20% of the original concentration. In agreement with the literature,[17] adrenaline in the aqueous buffered or complex-free poly(glutamate) solutions does not practically undergo oxidation, even in the presence of hydrogen peroxide.

Osmotic pressures of aqueous $[Fe(tetpy)(OH)_2]^+$–poly(L-glutamate) solutions (pH 7, 0.1 M Tris) at fixed [C]/[P] ratios, were measured as a function of polymer concentration (C_p = 0.15–0.045 g/100 mL) at 25 °C with a Hewlett-Packard 503 membrane osmometer. The data were treated according to the relationship[18] $\pi/C_P = RT(1/M_n + BC_P + ...)$, where π is the osmotic pressure (g/cm^2), C_P is the polymer concentration, R is the gas constant, T is the absolute temperature, M_n is the number-average molecular weight, and B is the second virial coefficient. Specific viscosities were obtained from the relative viscosities at 25 °C (C_P = 0.03 g/100 mL, pH 7, 0.02 M Tris).

Absorption measurements were carried out on a Beckman DBGT or a Cary 219 spectrophotometer. Circular dichroism spectra were recorded on a Cary 61 instrument with appropriate quartz cells. Other apparatuses were already described.[1,3]

Results

Structural Features of the Catalytic Systems. Association between $[Fe(tetpy)(OH)_2]^+$ ions and sodium poly(L-glutamate) was already reported to bring about a conformational transition in the polypeptide matrix even at pH 7 where the coil form normally predominates.[1] The effect was chiefly ascribed to a "site binding",[19] owing to the topological features of the complex molecules. They allow the metal ion to chelate with a γ-carboxylate group of a side chain of the polymer, which acts as unidentate ligand.[1,20] The extrinsic optical activity of the bound achiral complex ions originates, therefore, from both configurationally and conformationally induced

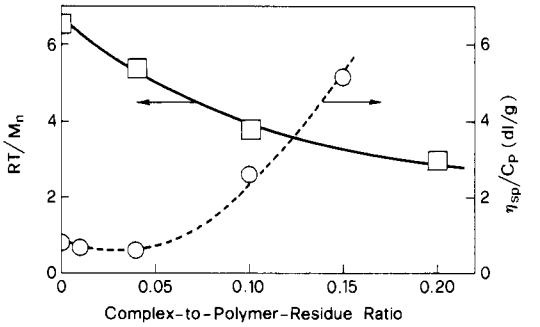

Figure 1. Molecular weight (from osmotic pressure measurements) and viscosity data of poly(L-glutamate) solutions, containing $[Fe(tetpy)(OH)_2]^+$ ions, as a function of [C]/[P] ratio (T = 25 °C; pH 7). Right-hand ordinate: η_{sp} is the reduced specific viscosity, C_p being the polymer concentration (g/100 mL). Left-hand ordinate: R is the gas constant, T the absolute temperature, and M_n the number-average molecular weight (see Experimental Section).

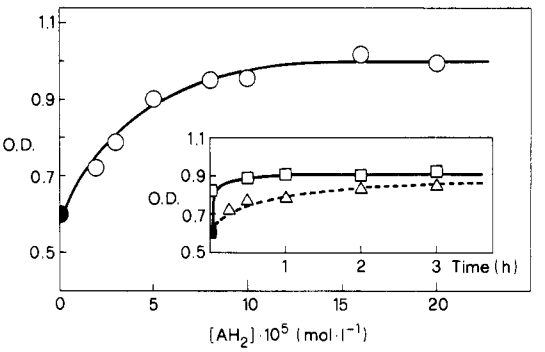

Figure 2. Absorption at 340 nm of FeL–adrenaline mixtures as a function of substrate concentration ([C]/[P] = 0.01, [C] = 5×10^{-5} M, optical path length 1 cm), reading being taken after about 60 min. Insert: variation of absorption at 340 nm of catalyst–adrenaline mixture as a function of time at two [C]/[P] ratios: 0.01 (□) and 0.20 (△) ($[AH_2]/[C]$ = 1; pH 7.0, 0.05 M Tris buffer).

Cotton effects.[1] In addition, aggregates of polymer chains form as the amount of bound complex ions increases. This is shown in Figure 1, where both molecular weight (based on osmotic pressure measurements; see Experimental Section) and viscosity of poly(glutamate) solutions containing $[Fe(tetpy)(OH)_2]^+$ ions are seen to increase as a function of [C]/[P]. Moreover, phase-separation measurements indicate that coacervation occurs well before a complete "neutralization" of the fixed charges on the polymer by the bound molecules is reached.[1b]

The change in the structural features of the catalytic systems with the complex to polymer ratio is reflected in the stereochemical characteristics of the substrate–catalyst adduct. For instance, addition of adrenaline into FeL or FeD solutions at low [C]/[P] ratio and in the deareated state gives rise to a new absorption band at around 340 nm, whose intensity increases with increasing substrate concentration (Figure 2). This finding suggests that a substrate-coordinated metal chelate forms under these conditions.[21] In contrast, when adrenaline is added to catalyst solutions at high [C]/[P] ratio the spectrum is much less perturbed. The 340-nm band reaches the maximum intensity only after a few hours (insert of Figure 2), which indicates that the formation of the mixed-ligand metal chelate is now controlled by diffusion. Indeed, the intensity of the band is seen to increase linearly with the square root of time, implying a dependence of the process on a Fickian diffusion mechanism. A substrate–catalyst complex must also form immediately under these conditions, as sug-

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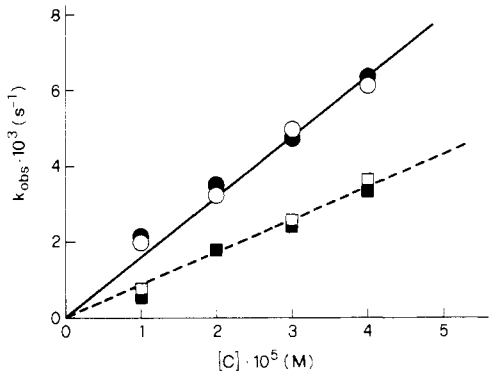

Figure 3. Catalytic effect for the oxidation of L-adrenaline in the presence of $[Fe(tetpy)(OH)_2]^+$ ions anchored to poly(L-glutamate) (empty symbols) or poly(D-glutamate) (full symbols), at [C]/[P] = 0.01 and 25.9 °C (solid line) or 15.8 °C (broken line) (pH 7.0, 0.05 M Tris buffer; $[AH_2]_0 = 1 \times 10^{-4}$ M, $[H_2O_2]_0 = 1 \times 10^{-2}$ M).

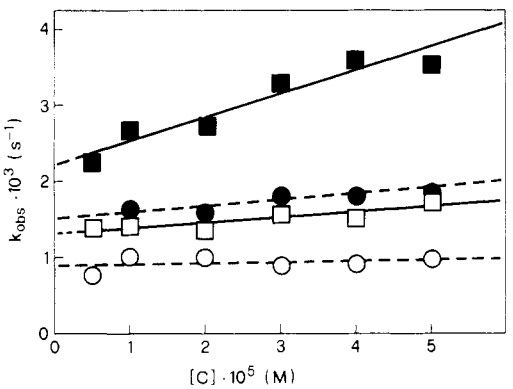

Figure 4. Catalytic effect for the oxidation of L-adrenaline in the presence of FeL (empty symbols) or FeD (full symbols) enantiomeric systems at [C]/[P] = 0.20 and at 25.9 °C (solid lines) or 15.8 °C (broken lines). Other experimental conditions are as in Figure 3.

gested by the Michaelis–Menten behavior of the initial reaction velocity against substrate concentration and by the remarkable stereospecific effects (see later). It appears, therefore, that in this case the substrate–catalyst adduct does not directly involve the metal chromophore.

Kinetic Data at Fixed Substrate Concentration. The pseudo-first-order rate constants k_{obsd} (s^{-1}) of the H_2O_2 oxidation of L-adrenaline (AH_2) at pH 7.0 (0.05 M Tris buffer) and $[H_2O_2]_0/[AH_2]_0 = 100$, as a function of complex concentration [C], are reported in Figures 3 and 4 ([C]/[P] ratio of 0.01 and 0.20, respectively). The linear variation of rate with the concentration of polymer-supported chelate ions at 25.9 and 15.8 °C indicates true catalytic behavior for the Fe(III) compound. Furthermore, at all [C]/[P] ratios investigated but 0.01 the straight lines have intercepts that differ significantly from zero; i.e., $k_{obsd} = k_0 + k_{cat}[C]$. These results are reminiscent of those obtained with ascorbic acid[3,11] and may be explained in terms of parallel pathways. One of these (k_{cat}, $M^{-1} \cdot s^{-1}$) refers to the electron transfer from adrenaline to the central metal ion, and the other (k_0, s^{-1}) corresponds to a complex ion uncatalyzed route to products, which becomes negligible as ([C]/[P]) → 0 (Table I). Furthermore, according to the data reported in Table I it appears that at [C]/[P] around 0.01 the catalysis exhibits no stereoselectivity whereas it becomes stereoselective as the [C]/[P] ratio increases. At the same time, the rate of the parallel complex ion uncatalyzed reaction increases and shows some stereoselectivity, though much smaller than that observed in the catalytic pathway.

The coupling between the binding-induced conformational phenomena on the charged polypeptide matrices by the complex iron(III) ions and stereospecific effects in the catalytic oxidation of L-adrenaline is clearly illustrated in Figure 5, where the stereoselectivity ratio k_{FeD}/k_{FeL} is plotted against [C]/[P] together with the α-helical fraction of the polypeptide (x_a) on binding of $[Fe(tetpy)(OH)_2]^+$ ions. The relationship between the parameter x_a and the amount of bound complex molecules, under experimental conditions of binding equilibrium similar to those of this investigation, has been already reported by us.[1b] It was essentially based on a statistical treatment by a two-state model for the polypeptide.[22] In the same figure the activation energy of the catalytic reaction is also reported, showing an S-shaped variation as a function of the [C]/[P] ratio.

Finally, the oxidation of L-adrenaline was also studied at low concentration of hydrogen peroxide, in the range $[H_2O_2]_0/[AH_2]_0 = 0.5$–2. A typical plot of k_{obsd} as a function of [C], at [C]/[P] = 0.10 and varying H_2O_2 concentration, is illustrated in Figure 6. The observation that the slope of the straight lines remains constant, within experimental errors, while the intercepts increase with increasing $[H_2O_2]_0$ implies that, under the conditions used, the rate of catalytic reaction does not depend on hydrogen peroxide concentration,[3,4a,b] at variance with that of the "uncatalyzed" process.

From the results, the following empirical rate expression may be formulated:

$$-\frac{d[AH_2]}{dt} = k_{0_{app}}[AH_2][H_2O_2] + k_{cat}[AH_2][C] \quad (1)$$

where $k_{0_{app}}$ is the second-order rate constant of the complex

Table I. Rate Constants for the Catalytic and the Complex Ion Uncatalyzed Oxidation of L-Adrenaline at Different Complex to Polymer Residue Ratios [C]/[P] (T = 25.9 °C; pH 7.0, 0.05 M Tris Buffer; $[H_2O_2]_0 = 1 \times 10^{-2}$ M, $[AH_2]_0 = 1 \times 10^{-4}$ M)

[C]/[P]	k_{FeD}, $M^{-1} \cdot s^{-1}$	k_{FeL}, $M^{-1} \cdot s^{-1}$	k_{FeD}/k_{FeL}[a]	$10^3 k_{0D}$,[b] s^{-1}	$10^3 k_{0L}$,[b] s^{-1}
0.01	158.0 ± 7.6	159.9 ± 8.8	1.0	<0.1	<0.1
0.02	63.1 ± 2.9	58.9 ± 2.6	1.1 ± 0.1	0.5	0.5
0.04	33.7 ± 2.3	21.0 ± 1.5	1.6 ± 0.2	1.1	0.9
0.06	30.1 ± 1.9	13.9 ± 0.9	2.2 ± 0.2	1.3	1.0
0.10	29.7 ± 1.8	9.4 ± 0.6	3.1 ± 0.3	1.7	1.2
0.20	31.2 ± 2.1	7.5 ± 0.5	4.2 ± 0.4	2.2	1.3

[a] Stereoselectivity of the catalytic reaction. [b] Standard deviations within 10%.

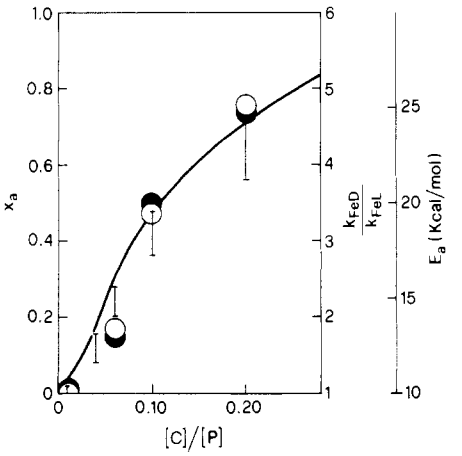

Figure 5. Variations of the binding-induced α-helical fraction in the polypeptide support (x_a, solid line), of stereoselectivity ratio (k_{FeD}/k_{FeL}, vertical bars), and of activation energy (circles) of the catalytic oxidation of L-adrenaline as functions of complex to polymer residue ratio. The different symbols refer to the enantiomeric catalysts used (pH 7.0, 0.05 M Tris buffer); see text.

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Table II. Kinetic Parameters of the Lineweaver-Burk Plot for the Electron Transfer from L-Adrenaline to Iron(III) Ions in FeL or FeD Systems (25.9 °C; $[C]_0 = 1 \times 10^{-5}$ M; pH 7.0)

catalyst	[C]/[P]	$10^{-3}T$,[a] s^{-1}	$10^{-6}I$,[a] $s \cdot M^{-1}$	$10^{2}k_3$, s^{-1}	$10^{3}K_M$, M
FeL	0.10	9.48 ± 0.86	8.33 ± 0.74	1.20 ± 0.13	1.14 ± 0.16
FeL	0.20	13.81 ± 1.05	10.60 ± 0.87	0.94 ± 0.11	1.30 ± 0.18
FeD	0.10	3.20 ± 0.31	3.40 ± 0.35	2.94 ± 0.29	0.94 ± 0.13
FeD	0.20	3.08 ± 0.26	2.94 ± 0.26	3.40 ± 0.38	1.05 ± 0.15

[a] Obtained by the least-squares method.

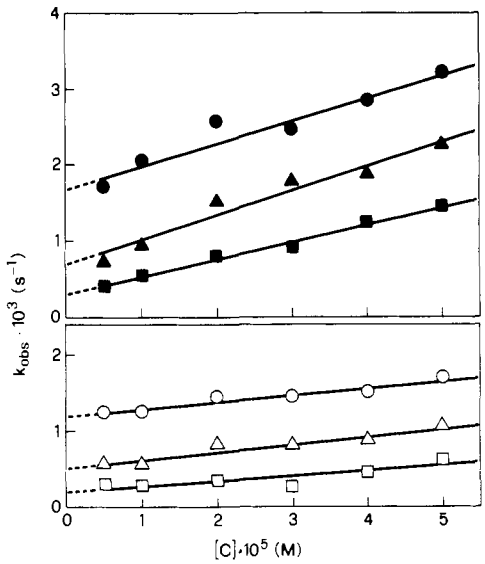

Figure 6. Catalytic effect for the oxidation of L-adrenaline in the presence of FeL (empty symbols) or FeD (full symbols) enantiomeric systems at [C]/[P] = 0.10 and varying initial concentrations of hydrogen peroxide. $[H_2O_2]_0 = 1.0 \times 10^{-2}$ M (circles), 2.0×10^{-4} M (triangles), 1.0×10^{-4} M (squares) (T = 25.9 °C). Other experimental conditions are as in Figure 3.

ion uncatalyzed reaction, which at [C]/[P] = 0.10 has the values $k_{0D_{app}} = 3.4 \pm 0.3$ an $k_{0L_{app}} = 2.5 \pm 0.2$ $M^{-1} \cdot s^{-1}$. From these values and $k_{FeD} = 28.3 \pm 2.0$ and $k_{FeL} = 9.0 \pm 0.7$ $M^{-1} \cdot s^{-1}$ (which are the average values of the slopes of the straight lines of Figure 6), the reaction velocities were calculated according to eq 1. Comparison with the experimental rates is rather satisfactory. For instance, when $[H_2O_2]_0 = 1.0 \times 10^{-4}$, $[AH_2]_0 = 1.0 \times 10^{-4}$, and $[C] = 5.0 \times 10^{-5}$ M, the experimental initial rates are $V_{FeD} = 1.60 \times 10^{-7}$ and $V_{FeL} = 0.63 \times 10^{-7}$ $M \cdot s^{-1}$, whereas the calculated ones are $V_{FeD} = 1.76 \times 10^{-7}$ and $V_{FeL} = 0.70 \times 10^{-7}$ $M \cdot s^{-1}$ (25.9 °C). In this connection, it is worth mentioning that the efficiency in the decomposition of hydrogen peroxide catalyzed by the same materials is definitely lower than that observed in the oxidation of adrenaline.[23a]

Kinetic Data as a Function of Substrate Concentration. The dependence of the overall initial rate of the H_2O_2 oxidation of L-adrenaline on the initial concentration of substrate, at fixed complex concentration $[C]_0 = 1 \times 10^{-5}$ M, is illustrated in Figure 7 (pH 7.0, $[H_2O_2]_0/[AH_2]_0 \approx 100$, $[C]_0/[AH_2]_0 \ll 1$). At low $[AH_2]_0$ the reaction velocity follows first-order saturation kinetics,[23b] i.e., the straight lines have unitary slope, whereas at high concentration of adrenaline all curves show the tendency toward saturation. Under the experimental conditions used, the initial rate of reaction can be written as

$$V_0 = k_0[AH_2] + V_{cat} \qquad (2)$$

from which V_{cat} may be evaluated. Typical curves of V_{cat} as a function of the initial concentration of adrenaline are presented in Figure 8 ([C]/[P] = 0.10, $[C]_0 = 1 \times 10^{-5}$ M).

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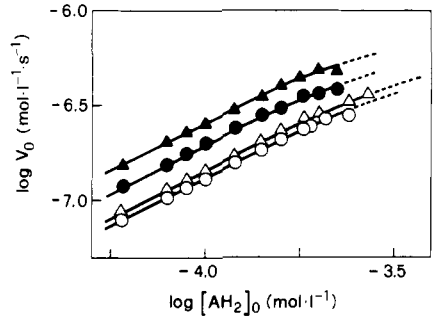

Figure 7. Overall initial rate of the H_2O_2 oxidation of L-adrenaline as a function of the initial concentration of substrate, at fixed complex ion concentration of 1×10^{-5} M. Catalyst: FeL (empty symbols) and FeD (full symbols) at [C]/[P] = 0.10 (circles) and 0.20 (triangles) (T = 25.9 °C; $[H_2O_2]_0/[AH_2]_0 \approx 100$; pH 7.0, 0.05 M Tris buffer).

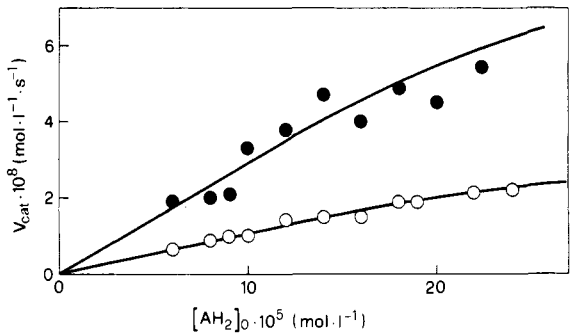

Figure 8. Dependence of the rate of the catalytic oxidation of L-adrenaline on the initial concentration of substrate at fixed $[C]_0 = 1 \times 10^{-5}$ M. Catalyst: FeL (empty symbols) and FeD (full symbols) at [C]/[P] = 0.10. Other experimental conditions are as in Figure 7.

The rate of catalysis also follows first-order saturation kinetics, i.e.

$$V_{cat} = V_m[AH_2]/([AH_2] + K_M) \qquad (3)$$

where V_m is the "saturation" velocity and K_M the Michaelis constant. The Lineweaver–Burk plot of these data ($1/V_{cat}$ against $1/[AH_2]_0$) gives, therefore, straight lines, whose intercepts I and tangents T (obtained by the least-squares method using V_{cat}^2 weight factors) for two [C]/[P] ratios are reported in Table II.

On the basis of these results, the following mechanism may be taken into account:

$$Fe^{III}L^+ + AH_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Fe^{III}LAH + H^+$$

$$Fe^{III}LAH \xrightarrow[slow]{k_3} Fe^{II}L + A^{-}\cdot + H^+$$

$$Fe^{II}L + {}^1/_2H_2O_2 \xrightarrow[fast]{} Fe^{III}L^+ + OH^-$$

$$A^{-}\cdot + H^+ + {}^1/_2H_2O_2 \xrightarrow[fast]{} A + H_2O$$

$$2A^{-}\cdot + 2H^+ \xrightarrow[fast]{} A + AH_2$$

where $Fe^{III}L^+$ (throughout the paper called C) denotes [Fe-

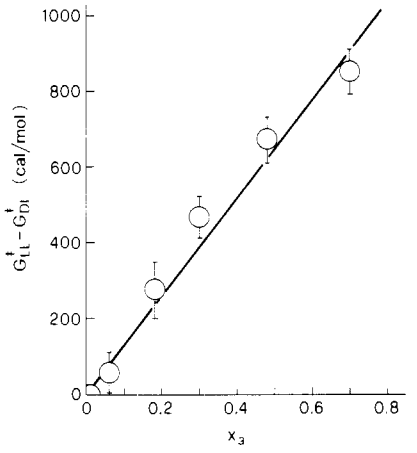

Figure 9. Dependence of the difference in the standard free energies of the diastereomeric transition states on the α-helical fraction of polypeptide matrices (T = 25.9 °C; pH 7).

$(tetpy)(OH)_2]^+$ ions anchored to poly(glutamates). This scheme predicts that a precursor complex forms in a preequilibrium step and that electron transfer from the substrate to the central metal ion, which is rate determining, takes place intramolecularly in this intermediate. Both the reduced iron ion and adrenaline radical are then oxidized by H_2O_2 in subsequent fast steps. $A^{-}\cdot$ may also disproportionate, as does the ascorbate radical.[24]

Under conditions where the intermediate complex FeLAH fulfills the steady-state approximation, the rate of catalysis will be given by eq 3 in which $K_M = (k_{-1}[H^+] + k_3)/k_1$ and $V_m = k_3[C]_0$. The kinetic parameters of the Lineweaver–Burk plot are then $I = 1/k_3[C]_0$ and $T = K_M/k_3[C]_0$. When $[AH]_2 << K_M$, V_{cat} reduces to $(k_3/K_M)[C]_0[AH_2] = k_{cat}[C][AH_2]$ (see eq 1). From the results, one obtains the values of k_3 and K_M reported in Table II. Comparison of k_3/K_M with k_{cat} ($[AH_2] = 1 \times 10^{-4}$ M) is rather good. For example, $k_{3L}/K_{ML} = 10.5 \pm 1.9$ and $k_{3D}/K_{MD} = 31.3 \pm 5.3$ $M^{-1}\cdot s^{-1}$ as compared to $k_{FeL} = 9.4 \pm 0.6$ and $k_{FeD} = 29.7 \pm 1.8$ $M^{-1}\cdot s^{-1}$, respectively ([C]/[P] = 0.10), whereas $k_{3L}/K_{ML} = 7.2 \pm 1.3$ and $k_{3D}/K_{MD} = 32.4 \pm 5.9$ $M^{-1}\cdot s^{-1}$ as compared to $k_{FeL} = 7.5 \pm 0.5$ and $k_{FeD} = 31.2 \pm 2.5$ $M^{-1}\cdot s^{-1}$ ([C]/[P] = 0.20). In addition, stereoselectivity is almost entirely kinetically controlled, the turnover numbers (moles of substrate transformed per mole of catalyst per minute) of FeL and FeD at, e.g., [C]/[P] = 0.20 being 0.56 and 2.04 min^{-1}. These values are of the same order of magnitude of those found in the copper poly(histidine) catalyzed oxidation of homogentisic acid and *p*-hydroquinone.[6]

According to the theory of absolute reaction rate, the second-order rate constants of the reaction between the FeL and FeD isomers of the asymmetric catalyst and the L isomer of the substrate are related by

$$k_{FeD}/k_{FeL} = \exp[-(\Delta G^{\ddagger}_{DL} - \Delta G^{\ddagger}_{LL})/RT] \qquad (4)$$

where $\Delta G^{\ddagger}_{DL}$ and $\Delta G^{\ddagger}_{LL}$ are the standard free energies of activation of the diastereomeric reactions. In agreement with the foregoing results, one may neglect as a first approximation the difference in the standard free energy of interaction between FeL–L-adrenaline and FeD–L-adrenaline systems. Equation 4 then becomes

$$k_{FeD}/k_{FeL} = \exp[(G^{\ddagger}_{LL} - G^{\ddagger}_{DL})/RT] \qquad (5)$$

where $G^{\ddagger}_{LL}$ and $G^{\ddagger}_{DL}$ are the standard free energies of the diastereomeric transition states of the electron-transfer step. Equation 5 was originally used by Prelog,[13] who assumed that the difference $(G^{\ddagger}_{LL} - G^{\ddagger}_{DL})$ reflects the difference in the steric hindrance between the two diastereomeric transition states. In our case, as already found in the oxidation of L-ascorbic acid,[3] the difference happens to be linearly dependent on the α-helical fraction of the polypeptide matrices (x_a), as shown in Figure 9. This finding suggests that with an increase in the amount of ordered polypeptide the stereochemistry of the Michaelis complex would be such that the DL diastereoisomer experiences smaller steric hindrances than does the LL diastereoisomer, in agreement with the idea that the process resulting in less steric interference would occur at a higher rate.[12,13]

Complex Ion Uncatalyzed Reaction. Adrenaline in aqueous buffered or complex-free polypeptide solutions (pH 7) does not undergo any appreciable oxidation, even in the presence of hydrogen peroxide.[17] On the other hand, evidence is produced that a complex ion uncatalyzed electron-transfer process between the substrate and H_2O_2 occurs in the presence of complex–polypeptide systems (eq 1), the specific rate being dependent on both the complex to polymer ratio and the chirality of the polymeric material (Table I). Similar findings were already observed in the H_2O_2 oxidation of L-ascorbic acid[3,11] and were interpreted as due to the fact that the charged complex–polypeptide systems behave as a second phase, in which the concentration of reactants is different from that in the bulk of solution because of electrostatic factors.[25,26] We are inclined to think that a similar explanation also holds true in the present case, though further experiments are required to elucidate better the phenomenon, which probably reflects a variation in the redox potential of the reacting species, too.

Discussion

Spectroscopic data suggest that a substrate-coordinated metal chelate forms when L-adrenaline is added to catalyst solutions at low complex to polymer residue ratio. Under these conditions, the bound complex ions exhibit an "open" (axial) site[1,20] to which an OH group of the catechol end of adrenaline may coordinate. Iron(III) ions have been reported, in fact, to chelate preferentially with the catechol end of catecholamines.[21] Transfer of one electron from the substrate to the central metal ion can then take place directly. The hypothesis of a direct-attack mechanism of the substrate on the iron(III) ion is consistent with the relatively low activation energy of the reaction[4a,27] and may account for the very small stereoselectivity (Figure 5). The mixed-ligand metal chelate should be conformationally mobile, owing to the degrees of freedom of internal rotation of the bulky substituent in the substrate. One can predict, therefore, a negligible difference in the steric interference between the two diasteromeric processes. This must be reflected in a very small difference between the second-order rate constants,[13] as experimentally observed (Table I).

With an increase in the complex to polymer residue ratio the polypeptides undergo a coil-to-α helix transition.[1] At the same time, extensive aggregation of polymer chains occurs, most of $[Fe(tetpy)(OH)_2]^+$ ions being very likely engaged as bridging groups between helical segments of the polymers (Figure 1). Under these conditions, the formation of the substrate-coordinated metal chelate is largely hindered (insert of Figure 2), and the former reaction is inhibited. The chiral residues of the helical polypeptide represent now the primary site of binding for the substrate molecules. For instance, hydrogen bonding between the γ-carboxylate groups of poly(glutamate) and the secondary amino group of adrenaline

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may take place, probably rigidifying the whole set of bonds in the adduct.[28] That the different steric constraints in the diastereomeric precursor complexes could have an ultimate influence on the specific rates of the electron-transfer step of the two reactions is reasonable in view of the fact that both nuclear and electronic factors of the rate constants of electron-transfer processes are highly sensitive to the separation and orientation of the redox centers.[29,30] This is indeed the case, as shown in Table II. Unfortunately, the complexity of the systems investigated makes it difficult at present to attempt any calculation of rate constants. A further complication arises from the fact that electron transfer between the substrate bound to the helical polypeptide in the surrounding of the catalytic centers and the iron(III) ion can proceed only by a remote-attack mechanism, probably making use of the π system of the peripheral quaterpyridine ligand of the active sites. This pathway is feasible on stereochemical grounds[20] and is consistent with the change in the reactivity patterns of the catalysis[31a] in going from low to high [C]/[P] ratios (Table I and Figure 5). It is also reminiscent of the mechanism proposed for a number of redox reactions between ferriporphyrins or metalloproteins and different reductants.[4c,31,32]

We may therefore conclude with some confidence that the stereoselective route to adrenochrome does not involve substitution on the iron center but that the reaction proceeds through an electron-transfer site far from the central metal ion, possibly the edge of quaterpyridyl group.

To summarize, depending upon the structural features of the catalysts, the oxidation of L-adrenaline can proceed by direct or remote attack, each pathway giving rise to quite different stereospecific effects because of the different stereochemical characteristics of the precursor complex. The kinetics of the catalysis are reminiscent of some enzymic peroxidatic reactions. The similarity is even greater if one considers that hemin bound to glutamyl residues is supposed to be the active site of catalases.[33] This similarity is, however, rather formal as the precursor complexes are different in the two cases, that of the oxidation reactions on peroxidases being probably a ternary adduct. Nevertheless, the specific effects of polypeptide matrices on the stereoselective pattern of the catalytic process may perhaps contribute to certain enzymic reactions.

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Notes

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Stereochemistry of $[Cd_4(SC_6H_5)_{10}]^{2-}$, a Cage Complex Related to the Cadmium–Cysteinate Aggregates in Metallothioneins

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As observed earlier,[1] the adamantane-like cage $M_4(\mu\text{-}S)_6$ is the most frequently encountered structural unit of polynuclear metal thiolates. It has been established by X-ray analysis in $[M_4(SPh)_{10}]^{2-}$ (M = Mn(II),[2] Fe(II),[1,3] Co(II),[4] Zn(II)[5]), $[Fe_4(SPh)_6Cl_4]^{2-}$,[6] $[Zn_4(SPh)_8Cl_2]^{2-}$,[7] and $[Zn_4(SPh)_8(MeOH)]_n$.[8] In our ongoing development of metal–thiolate chemistry we have demonstrated the utility of the Fe(II)[9,10] and Co(II)[11] complexes as synthetic precursors of M–S–SR clusters. The adamantane-like structure of $[Fe_4(SEt)_{10}]^{2-}$[12] demonstrates that the cage can be stabilized by a ligand other than benzenethiolate bridging two M(II) sites. The scope of cage formation with transition-element ions has also been examined. The preparation of a compound corresponding to the formulation $(Et_4N)_2[Cd_4(SPh)_{10}]$ and associated NMR studies[1] have provided evidence, although not definitive, for the existence of the cage structure containing a metal ion with a tetrahedral radius (0.92 Å[13]) some 0.12–0.20 Å larger than those of the preceding first-transition-series ions. The species $[Cd_4(SPh)_{10}]^{2-}$ has been mentioned on earlier occasions by Dance,[7,8] and the preparation of the Me_4N^+ salt has been described briefly by Choy et al.,[14] who depicted the anion as an adamantane-like cage. Structural data were not reported. A possible relationship between the structures of $[Cd_4(SPh)_{10}]^{2-}$ and the cadmium(II)–cysteinate aggregates in metallothionein proteins as deduced from ^{113}Cd NMR studies[15-19] has been raised by ourselves[1] and others.[8,20] To provide

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