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Coupling between Binding-induced Conformational Phenomena and Stereospecific Effects in Asymmetric Reactions

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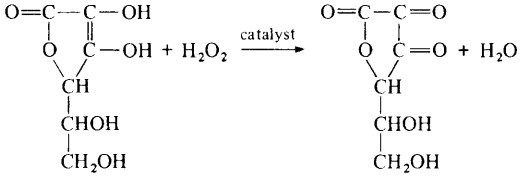
The oxidation by H_2O_2 of L-(+)-ascorbate anion in the presence of 2,2′,2″,2‴-tetrapyridineiron(III) complex ions anchored to poly(L-glutamate) (FeL) or poly(D-glutamate) (FeD) has been studied at pH 7.0 and varying complex-to-polymer-residue ratio [C]/[P]. The reaction follows two parallel routes; one corresponds to an electron-transfer process within a substrate–catalyst adduct and the other refers to an uncatalysed pathway to the dehydroascorbic acid. Unusual phenomena are observed in the catalysis in the sense that only the conformational dissymmetry of the active sites, arising from the binding-induced coil-to-α-helix transition of polypeptide matrices by Fe^{III} complex counter-ions, is able to impart stereospecific effects in the reaction.

Evidence is produced to show that stereoselectivity is driven by activation entropy. The effect probably arises from the formation of a rather rigid precursor complex, with the optically active substrate molecules bound to the chiral residues of the ordered polymer surrounding the active sites. The stereochemistry of such an intermediate allows the reaction to proceed only by a remote electron-transfer pathway, through the quaterpyridine ligand of the metal chelate. Evidence suggests that the asymmetric $[Fe(tetpy)(OH)_2]^+$–polyelectrolyte systems also behave as environmental controllers of the uncatalysed oxidation of the L-(+)-ascorbate anion. This effect is briefly discussed in terms of the role played by macroions in ionic reactions in solution.

Active molecules anchored to polymeric matrices are useful systems for the investigation of catalytic phenomena. For example, transition metal ions or complex ions bound to macromolecules have been used as enzymatic models[1-3] or active catalysts for a number of reactions in solution.[4,5]

We have recently reported that haemin-like 2,2′,2″,2‴-tetrapyridyliron(III) complex ions anchored to sodium poly(L-glutamate) (PLG) or poly(D-glutamate) (PDG) are efficient catalysts for the oxidation of L-(+)-ascorbic acid,[6] according to the reaction



Moreover, when the polypeptide matrices are in an α-helical conformation oxidation of the optically active substrate by the enantiomeric catalysts $[Fe(tetpy)(OH)_2]^+$–PLG (FeL) and $[Fe(tetpy)(OH)_2]^+$–PDG (FeD) becomes stereoselective.[6] Since an ordered structure in PLG or PDG can be achieved by either lowering the pH or increasing the amount of bound counter-ions,[7] the effect of both these parameters on the kinetics of the reaction has been extensively investigated. We report here the results obtained

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in a study of the peroxidase-like activity[8] of the aforementioned catalysts with L-(+)-ascorbic acid at fixed pH (7.0) and varying complex-to-polymer-residue ratio. The aims of the work were as follows: first to elucidate the mechanism of electron transfer catalysed by systems which also decompose hydrogen peroxide efficiently,[9] and secondly, to investigate the relationship between binding-induced conformational dissymmetry of the active sites and stereoselectivity in the catalysis.

EXPERIMENTAL

MATERIALS

Poly(L-glutamic) acid and poly(D-glutamic) acid were purchased from Miles–Yeda (m.w. = 30000). They were converted into the sodium salts using 0.1 mol dm^{-3} NaOH. Stock solutions were exhaustively dialysed against water to eliminate excess sodium ions and the materials recovered by freeze-drying. Concentrations of the polymers were determined by u.v. absorption at 200 nm ($\varepsilon = 5500$). The pseudo-octahedral *trans*-iron(III) derivative is an oxo-bridged dimeric compound in the solid state.[10a] In solution, at the pH values and concentrations with which we are concerned it is almost entirely in the mononuclear form $[Fe(tetpy)(OH)_2]^+$.[10] Concentrations of the iron(III) chelate were determined at 352 or 300 nm ($\varepsilon = 12190$ and 13430, respectively; pH 7.0). Tris(hydroxymethyl)aminomethane (Sigma Chemicals) was used as buffer in the chloride form (tris buffer) at a concentration of 0.05 mol dm^{-3} (pH 7.00 ± 0.03). Interaction between polymer or complex ions and buffer was ruled out on the basis of preliminary optical measurements. Under the experimental conditions used the degree of association of $[Fe(tetpy)(OH)_2]^+$ counter-ions by polypeptides is $> 93\%$, according to equilibrium dialysis experiments.[7] Both L-(+)-ascorbic acid (Merck) and stabilizer-free H_2O_2 (C. Erba) were analytical-grade reagents. All measurements were performed on freshly prepared solutions, using doubly distilled water with conductivity $< 2 \times 10^{-6}$ Ω^{-1} cm^{-1} (20 °C).

METHODS AND APPARATUS

Kinetic experiments were carried out spectrophotometrically under pseudo-first-order conditions, measuring the disappearance of ascorbic acid at 265 nm.[11] A typical run consisted of adding hydrogen peroxide *via* a microsyringe into the 1 cm optical cell containing 2 cm^3 of catalyst and substrate mixture, both systems being thermostatted at 25.9 ± 0.1 °C. The experimental conditions normally used were $[AH^-] = 1 \times 10^{-4}$ mol dm^{-3}, $[H_2O_2] = 1 \times 10^{-2}$ mol dm^{-3}, $[C] = (0.5\text{-}7) \times 10^{-5}$ mol dm^{-3} and $[C]/[P] = 0.01\text{-}0.20$, AH^-, C and P denoting L-(+)-ascorbate anion, complex and polymer, respectively. [The pK_{a1} value of ascorbic acid is reported to be 4.04 ($\mu = 0.1$ mol dm^{-3} KNO_3, 25 °C)[12a] or 4.03 ($\mu = 1.0$ mol dm^{-3} $NaClO_4$, 20 °C)[12b]. Polymer concentration [P] is referred to the monomer, *i.e.* monomol dm^{-3}. Measurements were also carried out with different initial concentrations of H_2O_2 and at another temperature (16.0 °C). Plots of log $(A_t - A_\infty)$ as a function of time were linear over several half-lives. The observed pseudo-first-order rate constants k (s^{-1}) were obtained from the slopes of the straight lines. At least four kinetic measurements were performed from each run to obtain consistent results. A slight oxidation of the substrate by hydrogen peroxide was detected in the presence of the sole polypeptide matrices. Its rate amounted to at most *ca.* 20% of the total rate. All first-order rate constants were properly corrected for this effect. Plots of k against complex concentration [C], at fixed complex-to-polymer-residue ratio, [C]/[P], always gave straight lines and the observed second-order rate constants k_{FeD} and k_{FeL} (dm^3 mol^{-1} s^{-1}) were obtained from the slopes. The stereoselectivity factor of the catalysis is given by k_{FeD}/k_{FeL}.

Absorption spectra were determined on a Berkman DBGT or Cary 219 spectrophotometer. The pH measurements were made with a Radiometer 26 pH-meter using standard semimicroelectrodes.

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RESULTS

The pseudo-first-order rate constants k of the H_2O_2 oxidation of L-(+)-ascorbate anion (pH 7.0, tris buffer 5×10^{-2} mol dm^{-3}) as a function of complex concentration [C], at fixed [C]/[P] values of 0.01 and 0.10 and at two temperatures, are reported in fig. 1 and 2. Within the range of complex concentration explored, the specific rates for the electron transfer increase with increasing [C]. The linear variation of rate with the concentration of (polymer-supported) complex ions at 25.9 and 16.0 °C indicates true catalytic behaviour for the iron(III) chelate. However, at [C]/[P] > *ca.* 0.01 extrapolation of the straight lines for [C] → 0 gives intercept values on the specific

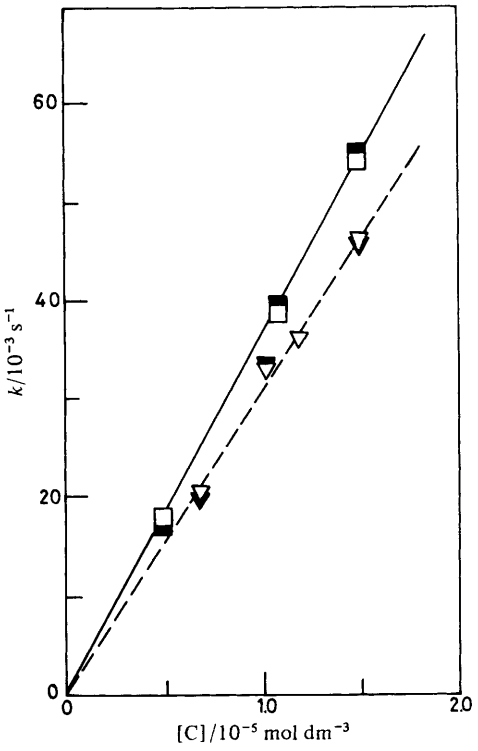

FIG. 1.—Catalytic effect for the oxidation of L-(+)-ascorbate anion in the presence of iron(III) complex ions bound to poly(L-glutamate) (empty symbols) or poly(D-glutamate) (full symbols), at [C]/[P] = 0.01 and two temperatures (25.9 °C, full line; 16.0 °C, broken line); pH 7.0 (tris buffer 5×10^{-2} mol dm^{-3}). k = difference between first-order rate constants in the presence and absence of bound complex counter-ions.

rate ordinate which are not zero (fig. 2). Thus k is a composite rate constant reflecting contributions from parallel pathways ($k = k_0 + k_{cat}$[C]), of which one (k_{cat}/dm^3 mol^{-1} s^{-1}) corresponds to the catalytic process and the other (k_0/s^{-1}) refers to an uncatalysed route for electron transfer, becoming negligible at very low complex-to-polymer-residue ratios. The slopes and intercepts of the plots of k against [C], corresponding to k_{cat} and k_0, respectively, are reported in table 1. (Subscripts FeD and FeL denote the enantiomeric catalytic systems whereas D and L denote the asymmetric polymeric matrices, see below.) Table 1 shows that the complex-to-polymer-residue ratio markedly affects both parallel pathways for the oxidation of

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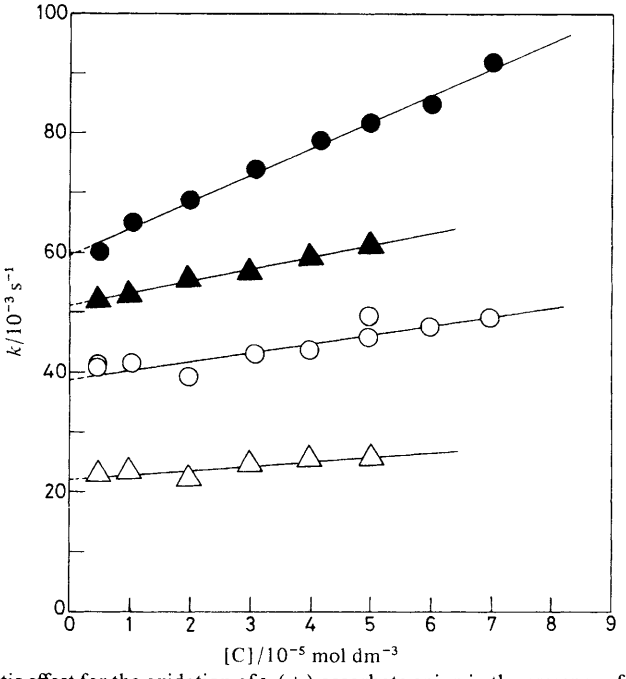

FIG. 2.—Catalytic effect for the oxidation of L-(+)-ascorbate anion in the presence of FeL (empty symbols) and FeD (full symbols) enantiomeric systems, at [C]/[P] = 0.10 and two temperatures: 25.9 (○, ●) and 16.0 °C (△, ▲); pH 7.0 (tris buffer 5×10^{-2} mol dm^{-3}). k = difference between first-order rate constants in the presence and absence of bound counter-ions.

TABLE 1.—RATE CONSTANTS FOR THE CATALYTIC (k_{FeD}, k_{FeL}/dm^3 mol^{-1} s^{-1}) AND NON-CATALYTIC ($k_{0,D}$, $k_{0,L}$/s^{-1}) OXIDATION OF L-(+)-ASCORBATE ANION AT DIFFERENT [C]/[P] VALUES
T = 25.9 °C, pH 7.0, tris buffer 5×10^{-2} mol dm^{-3}, $[H_2O_2]_0 = 1 \times 10^{-2}$ mol dm^{-3}, $[AH^-]_0 = 1 \times 10^{-4}$ mol dm^{-3}.

[C]/[P]	k_{FeD}	k_{FeL}	k_{FeD}/k_{FeL}[a]	$k_{0,D} \times 10^3$	$k_{0,L} \times 10^3$	$k_{0,D}/k_{0,L}$
0.01	3705 ± 116	3608 ± 119	1.0	*ca.* 0.6	*ca.* 0.6	1.0
0.02	1144 ± 62	1160 ± 53	1.0 ± 0.1	22.1	20.0	1.1
0.04	485.5 ± 40.3	317.7 ± 28.0	1.5 ± 0.2	41.0	32.7	1.2
0.06	409.3 ± 39.8	208.7 ± 17.9	2.0 ± 0.3	46.8	34.9	1.3
0.10	442.3 ± 42.9	153.6 ± 12.6	2.9 ± 0.5	59.6	38.7	1.5
0.20	414.0 ± 33.4	106.4 ± 9.2	4.0 ± 0.5	70.9	39.9	1.8

[a] Stereoselectivity factors of the catalytic reaction.

ascorbate anion. At [C]/[P] ≈ 0.01, not only is the rate of the non-catalytic process negligibly small compared with that of the catalytic one, but also the catalysis exhibits no stereoselectivity ($k_{FeD} = k_{FeL} = 3.66 \times 10^3$ dm^3 mol^{-1} s^{-1}). However, the observed second-order rate constant compares very favourably with those obtained using other Fe^{III} complexes as catalysts for the oxidation of the same substrate, although at lower pH.[13, 14a] When [C]/[P] is increased the reaction becomes stereoselective, k_{FeD} being definitely larger than k_{FeL}. In addition, the higher is the complex-to-polymer ratio the greater is the stereoselectivity factor k_{FeD}/k_{FeL}. At the same time, the rate of the parallel uncatalysed reaction increases and also shows some stereospecificity.

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On the basis of these results, the following empirical rate expression may be formulated:

$$\text{rate} = k_0[AH^-] + k_{cat}[AH^-][C] \quad (1)$$

where AH^- denotes the L-(+)-ascorbate anion[12] and k_0 is an apparent first-order rate constant, which vanishes as $[C]/[P] \rightarrow 0$.[15]

The oxidation of ascorbic acid was also studied as a function of the initial concentration of hydrogen peroxide in the range $[H_2O_2]_0/[AH^-]_0 = 0.5\text{-}100$. The second-order rate constants for the catalytic process were found to be independent of $[H_2O_2]$ for all values of $[C]/[P]$ (fig. 3 and table 2). This result agrees with that

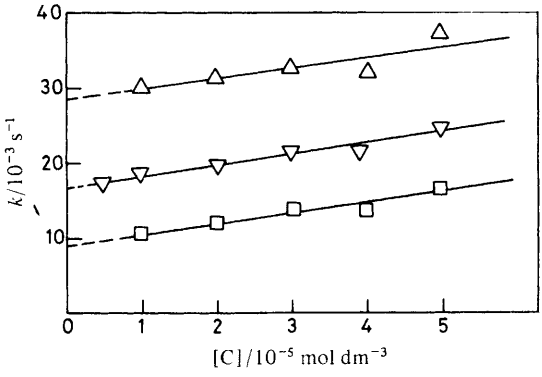

FIG. 3.—Catalytic effect for the oxidation of L-(+)-ascorbate anion in the presence of FeL system at different initial concentrations of hydrogen peroxide (see table 2): △, 1.1×10^{-3}; ▽, 1.1×10^{-4}; □, 5.4×10^{-5} mol dm^{-3} $[C]/[P] = 0.10$, $T = 25.9$ °C, pH 7.0 (tris buffer 5×10^{-2} mol dm^{-3}). k = difference between first-order rate constants in the presence and absence of bound complex counter-ions.

TABLE 2.—RATE CONSTANTS (dm^3 mol^{-1} s^{-1}) FOR THE CATALYTIC OXIDATION OF L-(+)-ASCORBATE ANION AS A FUNCTION OF THE INITIAL CONCENTRATION OF HYDROGEN PEROXIDE (mol dm^{-3}) AT TWO [C]/[P] VALUES

$T = 25.9$ °C, pH 7.0, tris buffer 5×10^{-2} mol dm^{-3}, $[AH^-]_0 = 1 \times 10^{-4}$ mol dm^{-3}.

	[C]/[P] = 0.01			[C]/[P] = 0.10	
$[H_2O_2]_0$	k_{FeD}	k_{FeL}	$[H_2O_2]_0$	k_{FeL}	k_{FeD}
1.0×10^{-2}	3705	3608	1.0×10^{-2}	442.3	153.6
1.2×10^{-3}	3922	3794	1.1×10^{-3}	408.8	129.2
1.2×10^{-4}	3372	3471	1.1×10^{-4}	405.1	156.1
4.9×10^{-5}	3661	3716	5.4×10^{-5}	471.0	140.7
av.	3665 ± 196	3647 ± 121	av.	431.8 ± 30.6	144.9 ± 10.8

obtained by Grinstead on the H_2O_2 oxidation of the same substrate on EDTA–Fe^{III} complex[13] and is similar to those obtained by Martell *et al.*,[14a] who used O_2 as an oxidant and diverse ferric chelates as catalysts. In contrast, the parallel uncatalysed reaction depends on $[H_2O_2]$ (fig. 3 and 4), so that eqn (1) can be written as follows:

$$-\frac{d[AH^-]}{dt} = k_{0,\,app}[AH^-][H_2O_2] + k_{cat}[AH^-][C] \quad (2)$$

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where $k_{0,\,app}/dm^3\ mol^{-1}\ s^{-1}$ is still a complicated function of the complex to polymer ratio, vanishing as $[C]/[P] \rightarrow 0$. From the slopes of the straight lines of fig. 4 one obtains $k_{0,\,D,\,app} = 230.6$ and $k_{0,\,L,\,app} = 157.0\ dm^3\ mol^{-1}\ s^{-1}$. Using these parameters and $k_{FeD} = 431.8\ dm^3\ mol^{-1}\ s^{-1}$ and $k_{FeL} = 144.9\ dm^3\ mol^{-1}\ s^{-1}$ (table 2), the reaction velocities were calculated at $[C]/[P] = 0.10$ and $[H_2O_2]_0/[AH^-]_0 \approx 1$. Comparison with the experimental rates shows a rather good agreement (table 3).

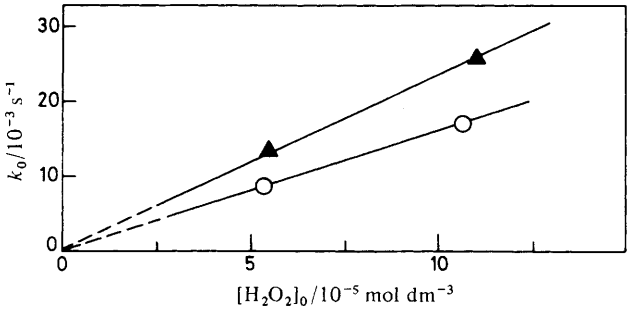

FIG. 4.—Dependence of pseudo-first-order rate constants of the uncatalysed oxidation of L-(+)-ascorbate anion on the initial concentration of H_2O_2 {empty symbols, FeL; full symbols, FeD system ([C]/[P] = 0.10)}. $T = 25.9$ °C, pH 7.0 (tris buffer 5×10^{-2} mol dm^{-3}); see text.,

TABLE 3.—COMPARISON BETWEEN EXPERIMENTAL AND CALCULATED [EQN (2)] REACTION VELOCITIES (10^{-6} mol dm^{-3} s^{-1}) OF THE OXIDATION OF L-(+)-ASCORBATE ANION IN THE PRESENCE OF THE ENANTIOMERIC SYSTEMS FeD AND FeL, AT [C]/[P] = 0.10

$T = 25.9$ °C, pH 7.0, tris buffer 5×10^{-2} mol dm^{-3}, $[C] = 5 \times 10^{-5}$ mol dm^{-3}; see text.

$[AH^-]_0$ /10^{-4} mol dm^{-3}	$[H_2O_2]_0/[AH^-]_0$	expt.		calc.	
		V_{FeD}	V_{FeL}	V_{FeD}	V_{FeL}
0.6	0.9	2.43	1.15	2.04	0.94
0.6	1.8	3.35	1.21	2.82	1.47
1.1	0.5	3.26	2.02	3.74	1.73
1.1	1.0	6.04	3.32	5.16	2.70

From the empirical rate law [eqn (2)] it appears that electron transfer in the non-catalytic reaction takes place between ascorbic acid and H_2O_2, whilst in the catalytic process it occurs between substrate and the transition metal complex, possibly within a Michaelis adduct. In the latter case, oxidation of both the lower valence metal chelate and $A^{\cdot -}$ (or $AH^{\cdot}$) species by H_2O_2 takes place in subsequent fast steps. $A^{\cdot -}$ may also disproportionate very rapidly.[16]

Two further findings deserve a few comments. First, when the stereoselectivity factors k_{FeD}/k_{FeL} are plotted as a function of [C]/[P] ratio a trend similar to that followed by the α-helical fraction of the polymeric support (x_a) on binding of $[Fe(tetpy)(OH)_2]^+$ ions is observed (fig. 5). The relationship between the parameter x_a and the amount of bound complex counter-ions, under experimental conditions of binding equilibrium similar to those of this investigation, has been already reported by us.[7b] It was essentially based on a statistical treatment by a two-state model for the polypeptide[17] and a preferential association of complex ions to the helical conformation of the polyelectrolyte, in agreement with the cooperative behaviour of

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the binding isotherm.[7] The results illustrated in fig. 5 clearly indicate that the conformational dissymmetry of the active sites is chiefly responsible for stereospecificity in the catalysis. Secondly, when the activation energies of the catalytic reaction are reported as a function of [C]/[P] an S-shaped curve is obtained, similar to that shown by the stereoselectivity factors (see above). Furthermore, at each value of [C]/[P] the activation energies for both enantiomeric catalysts are equal within experimental error (fig. 6). Finally, according to the theory of absolute reaction rates, the second-order

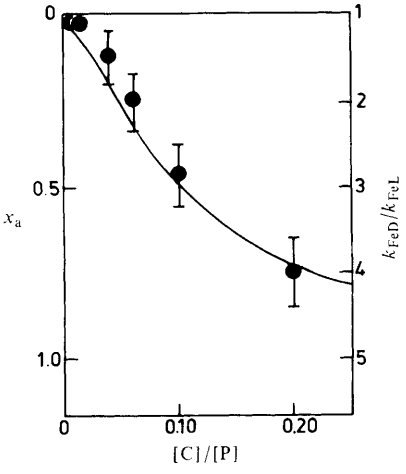

FIG. 5.

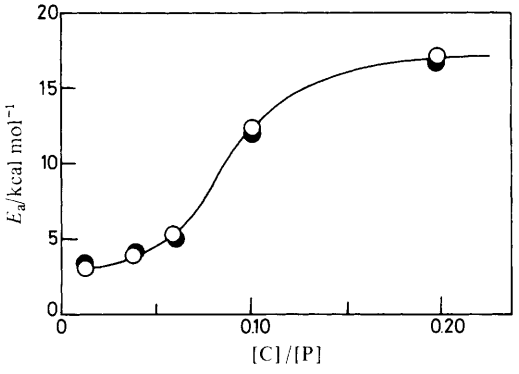

FIG. 6.

FIG. 5.—Variation of the stereoselectivity factor, k_{FeD}/k_{FeL} (●), of the catalytic oxidation of L-(+)-ascorbate anion at 25.9 °C and of the α-helical fraction of polypeptide matrix, x_a (solid line), induced by the binding of *trans*-Fe^{III} molecules, as a function of [C]/[P] (degree of association > 93%); see text and ref. (7*b*).

FIG. 6.—Variation of the activation energies of the catalytic oxidation of L-(+)-ascorbate anion, measured between 16 and 26 °C, as a function of [C]/[P]. The different symbols refer to the enantiomeric catalysts used; pH 7.0 (tris buffer 5×10^{-2} mol dm^{-3}).

rate constants of the reaction between the FeD or FeL isomers of the asymmetric catalyst and the L isomer of the substrate are related by

$$\frac{k_{DL}}{k_{LL}} = \exp[-(\Delta G^{\neq}_{DL} - \Delta G^{\neq}_{LL})/\boldsymbol{RT}] \qquad (3)$$

where $\Delta G^{\neq}_{DL}$ and $\Delta G^{\neq}_{LL}$ are the standard free energies of activation, and the notation DL and LL are used instead of FeD and FeL, respectively, to emphasize the diastereomeric character of the reacting systems under consideration. Assuming ideal behaviour, owing to the very low concentration of reagents, eqn (3) becomes

$$\frac{k_{DL}}{k_{LL}} = \exp[(G^{\neq}_{LL} - G^{\neq}_{DL})/\boldsymbol{RT}] \qquad (4)$$

where $G^{\neq}_{LL}$ and $G^{\neq}_{DL}$ are the standard free energies of the diastereomeric transition states. Eqn (4) was originally used by Prelog,[18] who assumed that the difference $(G^{\neq}_{LL} - G^{\neq}_{DL})$ reflects the difference in steric hindrance between the two diastereomeric transition states, although this interpretation has recently been questioned.[19] In our case, the difference is linearly dependent on the α-helical fraction of the polymeric support (x_a), as shown in fig. 7. This finding suggests that the Michaelis complex also

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involves the chiral polymer residues surrounding the active sites. On increasing the amount of ordered polypeptide, the local stereochemistry would be then such that the LL diastereoisomer experiences larger steric hindrances than does the DL diastereoisomer, which thus produces a less efficient electron-transfer process.

The overall results lead to the conclusion that: (i) stereoselectivity is coupled with the amount of α-helix in the polymer support, which in turn depends on [C]/[P] (fig. 5 and 7); (ii) the effect is remarkable although it occurs at the expense of the catalytic efficiency, because the rate constants decrease by *ca.* one order of magnitude with respect to that of the non-stereospecific reaction (fig. 8); (iii) the diverse efficiency in the electron-transfer process between the FeD–L-(+)-ascorbate (DL) and FeL–L-(+)-ascorbate (LL) diastereomeric systems is ascribable to a difference in entropies of activation, since at each [C]/[P] $\Delta H^{\neq}_{DL} = \Delta H^{\neq}_{LL}$, within experimental error (fig. 6); (iv) besides their catalytic role, the asymmetric [Fe(tetpy)$(OH)_2$]$^+$–polyelectrolyte materials play the unusual role of environmental controller of the uncatalysed oxidation of L-(+)-ascorbate anion in terms of specific rate as well as stereospecific effects (table 1 and fig. 4), the latter being much smaller than those observed in the catalytic process, however.

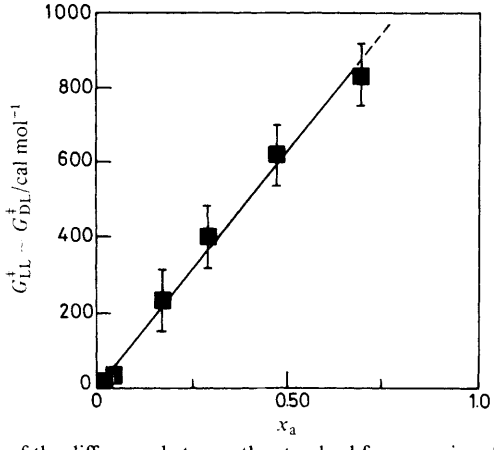

FIG. 7.—Dependence of the difference between the standard free energies of the diastereomeric transition states ($G^{\neq}_{LL} - G^{\neq}_{DL}$) on the α-helical fraction of polypeptide matrices; see text.

DISCUSSION

Evidence is produced that the H_2O_2 oxidation of L-(+)-ascorbate anion in the presence of [Fe(tetpy)$(OH)_2$]$^+$–polyglutamate systems follows parallel routes, one of which corresponds to an electron transfer process between substrate and central metal ion and the other refers to an uncatalysed pathway to products [eqn (2)]. Furthermore, the observation that even the uncatalysed process exhibits stereoselective effects, although smaller than those of the catalytic reaction, indicates that complexes between counter-ions and polyelectrolytes play a role additional to that as catalyst.

For sake of clarity, we discuss the data of the two parallel reactions separately.

CATALYTIC OXIDATION

The relevance of the results described here is that the enantiomeric systems [Fe(tetpy)$(OH)_2$]$^+$–PLG (FeL) and [Fe(tetpy)$(OH)_2$]$^+$–PDG (FeD) act as stereospecific catalysts in the oxidation of L-(+)-ascorbate anion only when the polypeptide

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supports are in α-helical conformation, a situation that at fixed pH (7.0) can be achieved by increasing the amount of bound counter-ions.[7] As [C]/[P] was increased both the stereoselectivity factor k_{FeD}/k_{FeL} and the activation energy for catalysis were found to increase following a sigmoid trend, clearly reflecting the helix–coil transition of the polymeric support (fig. 5 and 6). These observations indicate that at least two different factors are involved in determining the catalytic reaction rate; one is favoured by a decrease and the other by an increase in [C]/[P]. It is thus reasonable that these factors are the amounts of random coil and α-helix in the polypeptide matrices.

At very low [C]/[P] the (negatively charged) random-coil form of the support predominates. Under these conditions the anionic substrate probably interacts with the catalyst only through the bound, positively charged, iron(III) molecules, possibly forming a mixed-ligand chelate complex.[14, 20] By analogy with simpler redox systems, electron transfer may be regarded as taking place between the two species directly. The *direct* attack mechanism of the substrate molecule on the central metal ion can account for the observed low activation energy of the reaction[14, 21] (fig. 6), and is consistent with the idea that the precursor complex is conformationally mobile, owing to the size of the substituent in the ascorbate anion. According to this hypothesis, some of the conformers may favour the LL and others the DL reaction so that, on average, low stereoselectivity should be observed. This is indeed the case, as shown in table 1 and fig. 5.

At high [C]/[P], the fixed charges in the polymeric matrix are effectively shielded and the support acquires rigidity, now being predominantly in the α-helical conformation.[7] Both these effects allow extensive interactions between substrate molecules and the catalyst, probably involving the chiral polymer residues surrounding the active sites. In this case a rigid intermediate would form because of hydrogen bonding between hydroxy groups of the ascorbate anion substituent and γ-carboxy groups of the side-chains of the polypeptide. Conformational rigidity of the reacting species should enhance stereospecific effects in asymmetric processes,[19] so that different rate constants for the oxidation of the optically active substrate on enantiomeric catalysts may be expected. This hypothesis is consistent with the experimental results illustrated in fig. 5 and 7. Furthermore, according to molecular models the stereochemistry of such an adduct allows electron transfer to take place only through the peripheral quaterpyridine ligand of the active sites, a mechanism for this type of reaction already found in metalloproteins and ferriporphyrin systems.[22-25] This is probably a route with an activation energy higher than that of the direct attack mechanism (see above), as experimentally observed (fig. 6). We may therefore conclude that the stereospecific electron transfer catalysis does not involve substitution on the iron centre but that the reaction proceeds by remote attack, making use of an electron-transfer site far from the central metal ion, possibly the edge of tetrapyridyl group.

Further support for the above conclusions is provided by the following observation. The dependence of the second-order rate constants of the overall catalytic process (k_{cat}) on [C]/[P] (hereafter denoted by X) was found to be satisfactorily described by the empirical expression

$$k_{cat} = k_1 \frac{1}{1+bX+b'X^2} + k_2 \frac{bX+b'X^2}{1+bX+b'X^2} \qquad (5)$$

where k_1 is the specific rate constant at some low value of X (around 0.01), k_2 is the specific rate constant at $X \approx 0.20$ (where k_{cat} exhibits an almost asymptotic value and stereoselectivity approaches the maximum value), and b and b' are adjustable constants. The data of fig. 8 were fitted with an average deviation of 6%, which

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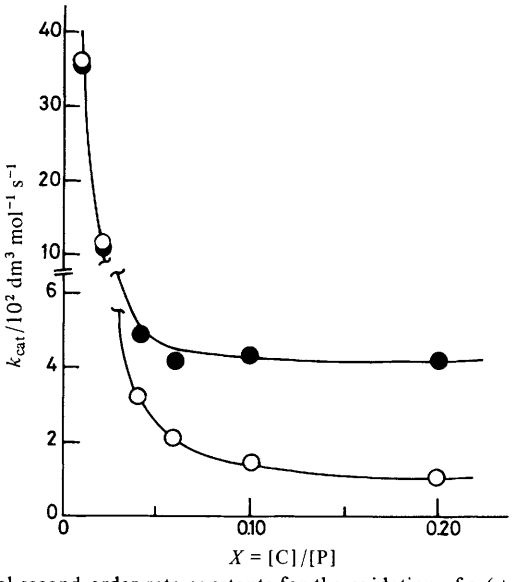

FIG. 8.—Experimental second-order rate constants for the oxidation of L-(+)-ascorbate anion catalysed by FeL (empty symbols) and FeD (full symbols) systems and calculated curves according to eqn (5) (solid lines). T = 25.9 °C, pH 7.0 (tris buffer 5×10^{-2} mol dm^{-3}); see text.

is of the same order of magnitude of the standard deviation of k_{cat}, using $k_1 = 4.02 \times 10^3$ dm^3 mol^{-1} s^{-1} for both catalysts and $k_{2L} = 100$ and $k_{2D} = 420$ dm^3 mol^{-1} s^{-1} (solid lines in the fig. 8). [The constants b and b' were -108 and 1.20×10^4, respectively. In fitting the data obtained on the FeD system, $(bX + b'X^2)$ was replaced by $(cX + c'X^2 + c''X^3)$ in order to get the same agreement, with $c = -59.4$, $c' = 3.57 \times 10^3$ and $c'' = 4.85 \times 10^5$.] According to eqn (5), the reactant molecules can be divided into two groups which follow parallel pathways. One group would contain those particles which are oxidised with rate constant k_1, irrespective of the catalyst used. This fraction, designed by $(1 + bX + b'X^2)^{-1}$, is obviously the larger the lower is X, so that it predominates when the support is highly disordered. The other group would contain those substrate molecules which are oxidised with rate constant k_{2D} or k_{2L}, depending on the catalyst employed. This fraction, designed by $(bX + b'X^2)$-$(1 + bX + b'X^2)^{-1}$, predominates at high values of X, *i.e.* when the amount of α-helix in the polypeptide matrices is large. In agreement with the above-mentioned data, the stereoselectivity increases at the expense of catalytic efficiency because k_{2D} and k_{2L} are much smaller than k_1. Furthermore, the maximum stereoselectivity is found to be $k_{2D}/k_{2L} = 4.2 \pm 0.5$.

In conclusion, $[Fe(tetpy)(OH)_2]^+$–polypeptide systems may be considered as a simple model of the allosteric effects attributed to enzymes. The progressive binding of complex counter-ions determines the coil-to-α-helix transition in the charged polymeric matrices. This in turn brings about a change in the mechanism of oxidation of the L-(+)-ascorbate anion, leading to marked stereospecific effects in the catalysis. At very low values of [C]/[P] the reaction is scarcely stereospecific and proceeds predominantly by a route involving direct attack of the ascorbate anion on the iron(III). On the other hand, at high [C]/[P] the electron-transfer process is slower but exhibits stereoselectivity, which is greater the larger the amount of α-helix in the polypeptide matrices. In this case the precursor complex probably sees the substrate molecules bound to the chiral polymer residues surrounding the active sites and the

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reaction proceeds predominantly by a remote pathway, through the quaterpyridine ligand. In the sense that the complex–polymer system contains both binding sites and catalytic sites it may be considered to be an enzymic model.

NON-CATALYTIC OXIDATION

The data reported in table 1 and fig. 4 clearly indicate that the uncatalysed H_2O_2 oxidation of ascorbate anion

$$AH^- + H_2O_2 \rightarrow A + OH^- + H_2O$$

takes place in the domains of the complex–polyelectrolyte systems. The rate constant of the reaction varies with both [C]/[P] and the chirality of the polypeptide matrix used.

Enhancement of the specific rates of ionic reactions carried out in the presence of macroions bearing opposite charges to those of the reacting particles has been widely reported. The phenomenon, known as 'polyelectrolyte catalysis',[26–28] is ascribable to electrostatic interactions between the species in solution. According to this view our results could be rationalised if the $[Fe(tetpy)(OH_2)]^+$–polyglutamate system were to behave as a cationic polyelectrolyte. This is in fact the case, since the γ-carboxylate anions of the side-chains of the polymer, acting as unidentate ligands, are coordinated by the *trans*-iron(III) derivative through an apical site.[7] As a result, the negative charges on the polypeptide chains are effectively shielded by the bulky complex counter-ions, but a *polycationic* material simultaneously forms *in situ*, since the bound molecules preserve on coordination their formal univalent positive charge. This hypothesis can account for the dependence of k_0 on [C]/[P], owing to the fact that the charge density of the 'new' polyelectrolyte increases with increasing number of bound counter-ions,[29] as well as the finding that stereoselectivity of the uncatalysed reaction is lower than that of the true catalytic process (table 1). In the latter case the formation of a substrate–catalyst complex allows closer contact between the asymmetric partners,[30] which is reflected in a much larger difference between the standard free energies of the diastereomeric transition states.

The true rate constants of complicated reactions, such as those involving enzymes, can be evaluated only when the overall kinetic data are corrected from the contribution of the primary salt effect due to the presence of charged macromolecules.[28b] As far as we know, the results reported here represent the first example in which the contribution of the so-called 'catalytic' effects of the polyelectrolyte is clearly discriminated from that of the real active sites. Further experiments are, however, needed to clarify this phenomenon.

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