



PS5.18 — MO

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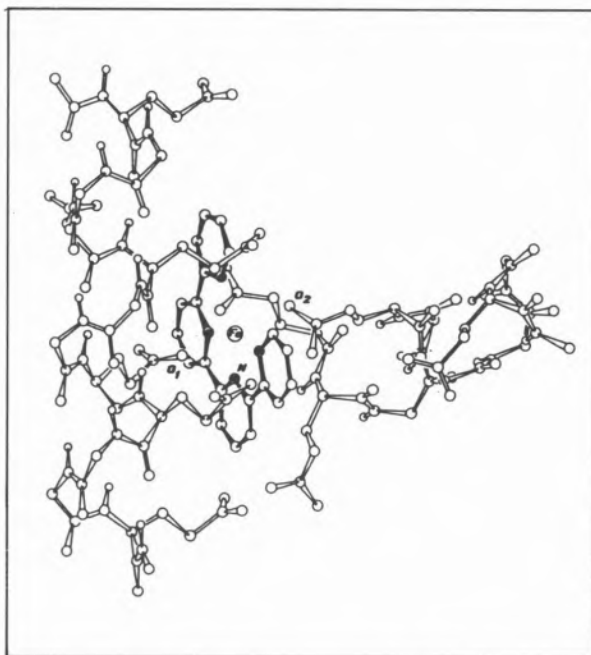
CHIRAL DISCRIMINATION IN ELECTRON TRANSFER REACTIONS BETWEEN ASYMMETRIC SPECIES

A simple, though realistic, synthetic model of enzymic material for stereoselective oxidation reactions has been prepared by us anchoring hemin-like $[\text{Fe}(\text{tetpy})(\text{OH})_2]^+$ ions to poly(L-glutamate) (FeTL) or poly(D-glutamate) (FeTD) ($\text{tetpy} = 2,2',2'',2'''$ -tetrapyrrolyl) [1,2]. The structural features of FeTL system, under conditions where it exhibits stereoselective activity, are illustrated in Figure. The molecular model was obtained by conformational energy calculations, partially based on available X-ray data, and is fully consistent with a number of experimental findings [3].

Oxidation of chiral catecholamines, such as L-dopa and L-adrenaline, by FeTL and FeTD enantiomeric systems was found to proceed stereoselectively [2], as shown in Table, where thermodynamic data for the formation of the diastereomeric precursor complexes are also reported. From the results, it appears that: i) stereoselectivity is largely controlled by kinetic effects, $\Delta(\Delta G^\ddagger)$ being definitely higher than $\Delta(\Delta G^0)$, and ii) the energetics of chiral discrimination, $\Delta(\Delta H)$, is of the order of magnitude that one would have been expected on the basis of $\Delta(\Delta G^0)$ values, on

the reasonable assumption that the entropies of association of the diastereomeric pairs are nearly identical.

We have also investigated the geometric and steric constraints which control the formation of the diastereomeric adducts by conformational energy calculations, based on nonbonding, electrostatic and hydrogen bonding energy terms [3]. The most relevant molecular parameters of the hypothetical models of the diastereomeric noncovalent electron-transfer complexes, corresponding to the deepest minimum in the total interaction energy, are reported in the same Table. Inspection of the Table indicates that: i) the redox centers in the diastereoisomers experience a different separation distance (and mutual orientation), as one would predict for kinetically-controlled stereoselectivity, ii) the difference in the total energy between LL



and DL pairs agrees surprisingly well (both in magnitude and sign) with the experimentally determined $\Delta(\Delta H)$ values, and iii) stereoselectivity calculated [3] by the molecular parameters of the models is in satisfactory agreement with that observed.

These findings, and the indirect tests carried out in searching vainly an agreement between calculated and experimental stereoselectivity using molecular parameters corresponding to other relative

Table
Kinetic, Calorimetric and Molecular Parameters of the Diastereomeric Electron-Transfer Complexes

Reductant	Diaster.	$G_{LL}^{\ddagger}-G_{DL}^{\ddagger}$ ^{a,b)}	$G_{LL}^o-G_{DL}^o$ ^{c,b)}	$\Delta H_{LL}-\Delta H_{DL}$ ^{d)}	$\bar{R}^{e,f)}$	$R'^{g,f)}$	$U_{LL}-U_{DL}$ ^{h,f)}	Stereoselectivity _{cld^{i,l)} cld^{i,m)} expⁿ⁾}		
L-dopa	DL	568 ± 110	253 ± 120	130 ± 50	7.0 ± 0.1	7.1 ± 0.1	60 ± 90	4.2 ± 0.8	2.6 ± 0.5	3.9 ± 0.8
	LL				5.8 ± 0.1	7.5 ± 0.1				
L-adrenaline	DL	711 ± 100	161 ± 110	250 ± 100	7.0 ± 0.1	7.5 ± 0.1	245 ± 185	4.3 ± 0.8	4.6 ± 0.7	4.5 ± 1.1
	LL				7.5 ± 0.1	7.8 ± 0.1				

- a) Difference (cal/mol) in the standard free energies of the diastereomeric transition states of the electron-transfer step;
b) from kinetic data in the steady-state approximation (25.9°C);
c) difference (cal/mol) between the standard free energies of the diastereomeric precursor complexes;
d) difference (cal/mol) between the enthalpies of formation of the diastereomeric pairs, from differential calorimetric measurements at 25°C, under conditions where the association of substrates is virtually complete;
e) closest catecholic-O⁻...Fe separation distance (Å);
f) from conformational energy calculations in the deepest minimum of total interaction energy, given as a sum of all pairwise non-bonded, electrostatic and hydrogen bonding interactions (average values as obtained using two types of nonbonded potential functions and four different sets of values for the interatomic interaction parameters);
g) closest catecholic-O⁻...Fe separation distance (Å) *via* tetrapyrrolyl ring, estimated from the molecular geometry of the models;
h) difference (cal/mol) in the total potential energy between LL and DL diastereoisomers in the deepest minimum of total energy;
i) overall stereoselectivity calculated by $(R_{DL}/R_{LL})^2 \exp [(U_{LL}-U_{DL})/RT](k_{etDL}/k_{etLL})$, where $R_{DL(LL)}$ refers to \bar{R} or R' and k_{et} is the specific rate for the intramolecular electron-transfer process, as obtained by kinetic data in the steady-state conditions;
l) using \bar{R} values;
m) using R' values;
n) 25.9°C, pH 7, 0.05 M Tris buffer, [C]/[P]=0.20, α -helical fraction in the polypeptide matrices ≈ 0.70 , as determined by chiroptical measurements.

minima of the total energy as well, lead us to consider the present hypothetical models as a good representation of the actual diastereomeric electron-transfer complexes. They confirm the idea that chiral discrimination in the reactions investigated is coupled with a remote attack mechanism on the central metal ion *via* the peripheral tetrapyrrolyl ligand of the active site [2,3]. In the sense that FeTL (or FeTD) system contains both binding and catalytic sites, it may be considered as an enzymic model.

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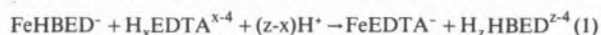


PS5.19 — TU

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**AN INVESTIGATION OF THE KINETICS
AND MECHANISM OF IRON(III)
RELEASE FROM *N,N'*-DI(2-HYDROXY-
BENZYL)ETHYLENEDIAMINE-*N,N'*-
DIACETIC ACID AS A MODEL FOR IRON
EXCHANGE FROM TRANSFERRIN**

The kinetics and mechanism of the release of iron(III) from *N,N'*-di(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED) [1] has been investigated. Because the donor groups of HBED (phenol, carboxylate, amine) provide a hexadentate binding site which has a high affinity for iron(III) and which has certain similarities to transferrin [2], this reaction may serve to mimic iron dissociation reactions from transferrin. Our initial study of this complex was made by investigating the following iron(III) exchange reaction,



The rate law for this exchange reaction over the pH range from 2.5 to 6.0 expressed in terms of EDTA^{4-} is as follows.

$$\text{Rate} = (k_2[\text{H}^+]^2 + k_3[\text{H}^+]^3 + k_4[\text{H}^+]^4 + k_5[\text{H}^+]^5)[\text{FeHBED}^-][\text{EDTA}^{4-}] \quad (2)$$

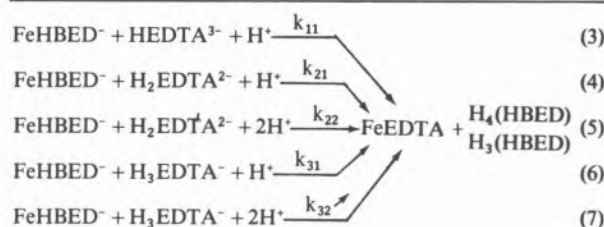
These observations are consistent with five parallel paths to products as shown in the Reaction Scheme.

The values for the microscopic rate constants and pH range where each path contributes significantly to product formation are as follows: (3) pH 6.0-4.5, $k_{11} = 1.4 \text{ M}^{-2}\text{s}^{-1}$; (4) pH 6.0-2.5, $k_{21} = 1.0 \text{ M}^{-2}\text{s}^{-1}$; (5) pH 4.5-2.5, $k_{22} \leq 7.5 \times 10^2 \text{ M}^{-3}\text{s}^{-1}$;

(6) pH 3.8-2.5, $k_{31} = 1.6 \text{ M}^{-2}\text{s}^{-1}$; (7) pH 3.8-2.5, $k_{32} \leq 2.5 \times 10^3 \text{ M}^{-3}\text{s}^{-1}$. Apparently the microscopic rate constants are not influenced by the degree of protonation of EDTA. This is consistent with a carboxylate group, unprotonated over the entire pH range, being the site of primary attack by the EDTA leading to ternary complex formation. Protonation of one or both of the phenolate groups of HBED further assists the iron exchange.

The influence of acetohydroxamic acid as a catalyst for reaction (1) was investigated. A significant rate enhancement was observed at physiological pH. Possible mechanisms for this catalytic effect will be discussed.

Reaction Scheme



In order to determine the usefulness of HBED as a kinetic model for transferrin, certain experiments previously reported for transferrin [3,4] were duplicated using FeHBED^- . Initial rate studies for iron(III) exchange between FeHBED^- and EDTA, thioglycolate, and pyrophosphate were carried out at physiological pH. These results are compared with results from the corresponding transferrin reactions in the Table. Also included in the Table are redox reactions using dithionite and thioglycolate reductants and bathophenanthroline-disulfonic acid (BPS) as a trap for the dissociated iron(II). The transferrin system reacts 10^2 to 10^5 times faster than FeHBED^- toward competing chelating agents. In the reduction reactions, however, the thioglycolate reaction proceeds at comparable rates and the dithionite reaction is 10^2 faster for FeHBED^- than transferrin. Furthermore, the FeHBED^- system exhibits a wider reactivity range than does transferrin (variation in k by 10^6 for FeHBED^- and 40 for transferrin). Apparently HBED is not a good kinetic model for transferrin, despite the binding site similarities.