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Chiral Recognition and Stereoselective Reduction of Horse Ferricytochrome c by Optically Active Iron(II) Complexes [1]

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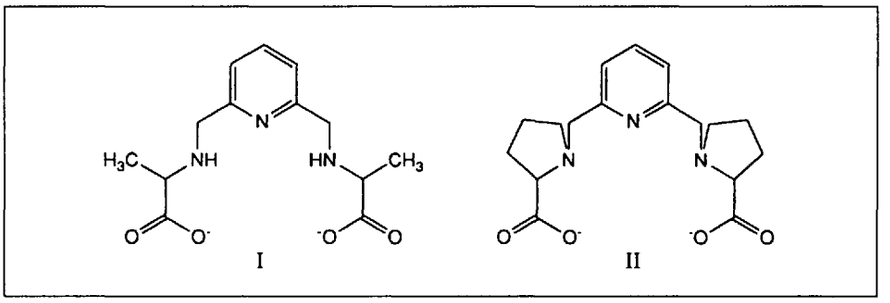
Abstract. The reduction of ferricytochrome c with the chiral complexes [Fe(alamp)] (alamp = *N,N'*-[(pyridine-2,6-diyl)bis(methylene)]bis[(*R*)- or (*S*)-alanine]) and [Fe(promp)] (promp = *N,N'*-[(pyridine-2,6-diyl)bis(methylene)]bis[(*R*)- or (*S*)-proline] is moderately stereoselective. The temperature dependence of the stereoselectivity shows $\Delta\Delta H^{\#}_{\Delta\text{-}\Lambda}/\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$ compensation behaviour giving crossing *Eyring* plots at 28° and 36° for alamp and promp, respectively. The activation parameters of the two ligands show inverted signs indicating that electron transfer with the corresponding Fe^{II} complexes probably does not occur in an identical geometrical environment.

Introduction

Class I cytochromes are among the best known electron-transfer proteins, and their reactions with small inorganic transition metal complexes as redox agents have been thoroughly studied [3]. The non-physiological reagents used belong to well defined groups, and most of them are either positively or negatively charged. Since cytochrome c itself carries a high positive charge, electrostatic interactions will affect the rate of electron-transfer by their influence on the stability of the precursor complex [4]. One of the main questions in the electron-transfer studies involving small inorganic complexes is the exact location of the site on the protein surface at which electron transfer occurs, as well as the distance at which the reagent is found in the transition state. According to NMR measurements, metal complexes preferentially bind to three regions of cytochrome c [5], and kinetic studies with chemically modified derivatives of the protein showed that two of these regions are relevant for electron transfer which takes place near the solvent exposed heme edge [6][7].

Since our first communication on the stereoselectivity of electron transfer between plastocyanin and optically active Fe^{II} complexes [8], some more reports have been published on stereoselective electron transfer involving the reduced forms of plant ferredoxin [9] and cytochrome c [10–12]. In these cases, inert, optically active Co^{III} complexes were used as oxidation agents. These complexes were chiral due to the arrangement of the achiral bidentate ligands. In the present paper, we report some results of kinetic stereoselectivity (used as defined in [13]) in the electron-transfer reaction between ferricytochrome c and optically active Fe^{II} complexes formed with the linear pentadentate ligands alamp(I) and promp(II). When the Co^{III} complexes of these ligands were synthesized, only one isomer could be isolated [14] and it is, therefore, assumed that the reactions with labile metal ions like Fe^{II} and Co^{II} are stereospecific, leading to only one isomer of given geometry and chirality.



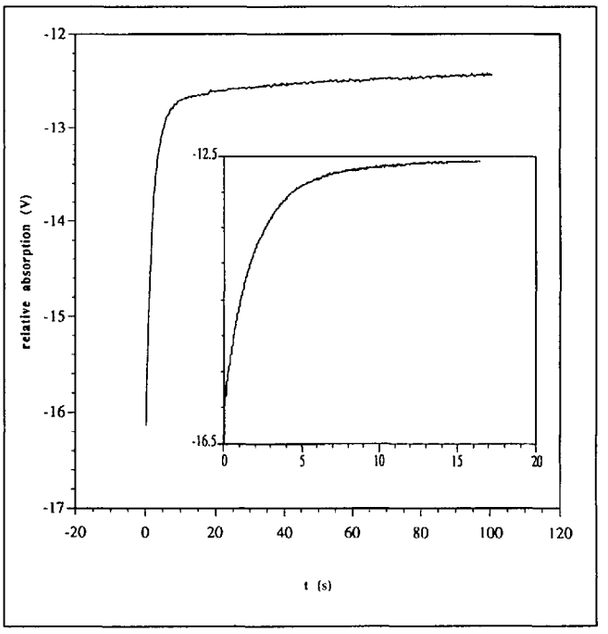


Fig. 1. *Relative absorption change at* $\lambda = 550$ *nm for the reduction of cytochrome c by Λ-[Fe((S,S)-promp)].* $C_{cyt.c} = 1.2 \cdot 10^{-5}$ M; $C_{Fe} = 1.5 \cdot 10^{-3}$ M; $C_{promp} = 2.2 \cdot 10^{-3}$ M; pH = 7.5 (Tris/HCl 0.1 M); $T = 25°$.

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Results

The trace of the optical change at λ = 550 nm during the reaction of ferricytochrome c with Λ-[Fe((*S,S*)-promp)] shown in *Fig. 1* reveals a biphasic reaction. Both reactions observed are first-order in cytochrome c and in the Fe^{II} complex (*Fig. 2*). Secondary rate constants with Λ-[Fe((*S,S*)-promp)] are $419\,M^{-1}\cdot s^{-1}$ for the first and $45\,M^{-1}\cdot s^{-1}$ for the subsequent reaction. The second reaction becomes more important at higher temperature as well as in H_2O/EtOH mixtures. In the present communication, we report results concerning the first, rapid reaction. As shown in *Fig. 3*, small but significant stereoselectivity is observed for promp at 25°. Measurements of the reaction rate at various temperatures gave a linear relationship $\ln(k/T) = f(1/T)$ from 5 to 45°, from which the activation parameters were determined. For both ligands, alamp and promp, a $\Delta\Delta H^{\#}_{\Delta\text{-}\Lambda}/\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$ compensation behaviour is observed leading to crossed *Eyring* plots. The temperature of inversion $T_i = \Delta\Delta H^{\#}_{\Delta\text{-}\Lambda}/\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$ is 36° and 28° for promp and alamp, respectively. Above and below these temperatures the stereoselectivity of the reaction increases. This behaviour is illustrated in *Fig. 4* which represents the measured variation of the stereoselectivity as a function of temperature for the two ligands. Rate constants, activation parameters and stereoselectivity data are given in the *Table*.

Discussion

$\Delta\Delta H^{\#}_{\Delta\text{-}\Lambda}/\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$ compensation in electron-transfer reactions with metalloproteins and enantiomeric reagents has been observed for plastocyanin and plant ferredoxin [13]. Such behavior has tentatively been discussed in terms of a closer approach of the reagent and stronger desolvation of the protein surface during the formation of the transition state with one of the enantiomers compared to the other [9]. This may be a general behavior in electron-transfer reactions involving metalloproteins. On the other hand, it should be mentioned that, contrary to previous observations, the sign of both $\Delta\Delta H^{\#}_{\Delta\text{-}\Lambda}$ and $\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$ is inverted for the two complexes [Fe(alamp)] and [Fe(promp)] (*Fig. 4*). A similar observation has been made for the reaction of plastocyanin with complexes of Fe^{II} and Co^{II} with identical ligands [15]. This inversion of the relative contribution of the activation parameters to stereoselective effects with reagents of identical chirality and very similar chemical structures could be an indication that electron transfer between metalloproteins

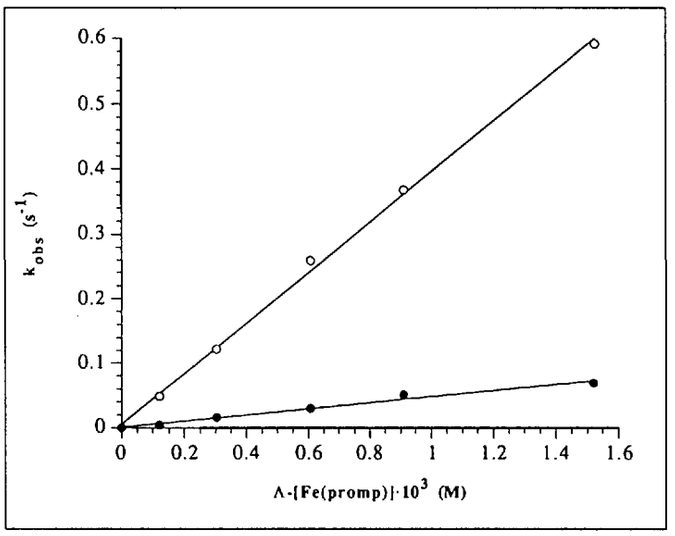

Fig. 2. *Dependence of* k_{obs} *for the reduction of cytochrome c on Λ-[Fe((S,S)-promp)] concentration:* (○) *fast reaction,* (●) *slow reaction.* $C_{cyt.\,c}$ = $1.2\cdot10^{-5}$ M; pH = 7.5 (Tris/HCl 0.1 M); T = 25°.

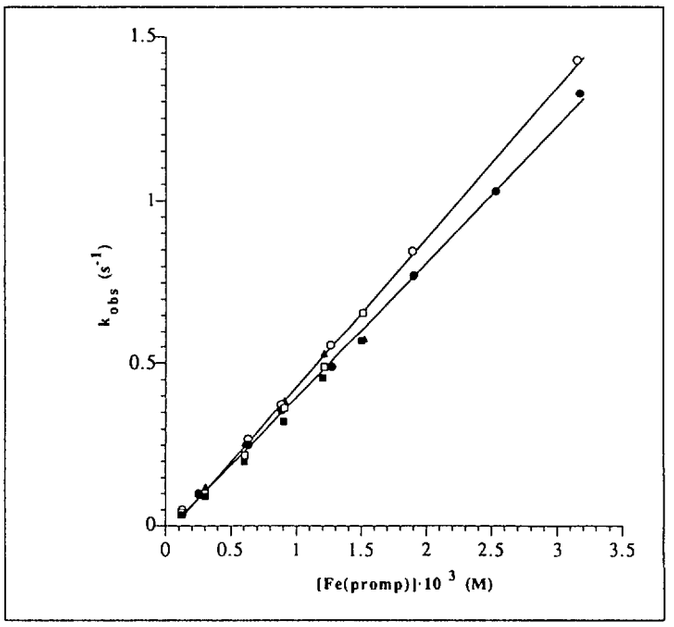

Fig. 3. *Dependence of* k_{obs} *on Λ-[Fe(promp)]* (full symbols) *and Δ-[Fe(promp)]* (open symbols) *concentration for the reduction of cytochrome c by the two enantiomers* (the different symbols indicate different series of measurements). $C_{cyt.\,c} = 1.2\cdot10^{-5}$ M; pH = 7.5 (Tris/HCl 0.1 M); T = 25°.

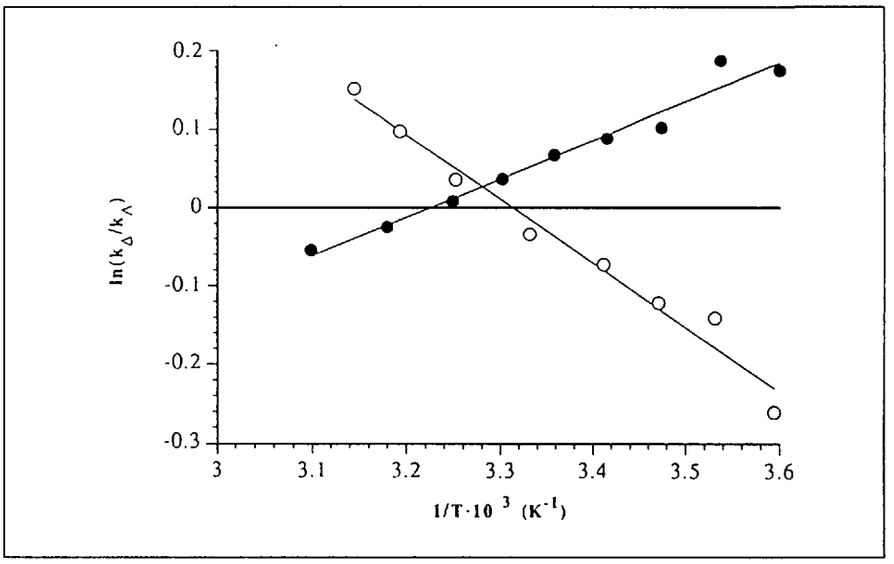

Fig. 4. *Inverse temperature dependence of the stereoselectivity for the reduction of cytochrome c by the enantiomers of [Fe(alamp)]* (○) *and [Fe(promp)]* (●). $C_{cyt.\,c} = 1.2\cdot10^{-5}$ M; $C_{Fe} = 1.5\cdot10^{-3}$ M; $C_{promp} = C_{alamp} = 2.2\cdot10^{-3}$ M; pH = 7.5 (Tris/HCl 0.1 M).

Table. *Rate Constants, Stereoselectivity, and Activation Parameters for the Reduction of Cytochrome c by Optically Active Fe^{II} Complexes*

Complex	k[a])	$\Delta H^{\#}$	$\Delta S^{\#}$	$\Delta\Delta H^{\#}_{\Delta\text{-}\Lambda}$	$\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$	$T\Delta\Delta S^{\#}_{\Delta\text{-}\Lambda}$[a])
	$[10^2\cdot M^{-1}\cdot s^{-1}]$	$[kJ\cdot mol^{-1}]$	$[J\cdot K^{-1}\cdot mol^{-1}]$	$[kJ\cdot mol^{-1}]$	$[J\cdot K^{-1}\cdot mol^{-1}]$	$[kJ\cdot mol^{-1}]$
Δ-[Fe(alamp)]	9.0±0.2	36.3±1.0	−67±3			
				+7.5	+25	+7.45
Λ-[Fe(alamp)]	9.1±0.2	28.8±1.0	−92±3			
Δ-[Fe(promp)]	4.58±0.07	31.4±0.7	−89±2			
				−4.7	−15	−4.47
Λ-[Fe(promp)]	4.15±0.07	36.1±0.5	−74±2			

[a]) At 25°C.

and metal complexes does not take place at a defined position. As recently suggested [16], the reagent may have some mobility on the protein surface and the point allowing fastest electron transfer may not be necessarily the most favorable from a stereochemical point of view.

As mentioned above, the *Eyring* plots cross at 36° and 28° for promp and alamp, respectively, illustrating in a striking manner the meaning of stereoselectivity data collected at different temperatures. The important chiral recognition between cytochrome c and [Fe(alamp)], demonstrated by a difference in the activation enthalpy of *ca.* 20% between the two enantiomers, would be completely overlooked, if the kinetic stereoselectivity measurements were made at 25° only.

The final remarks concern the biphasic character of the reaction, which has been observed for various reducing agents like $[Fe(edta)]^{2-}$ [17], ascorbic acid [18], and $[Fe(CN)_6]^{4-}$ [19], and which was attributed to an equilibrium between two forms of the protein, cyt_N and cyt_A [20] which are reduced at different rates and the interconversion of which is slower than the reduction. The behavior of [Fe(alamp)] and [Fe(promp)] is in agreement with the mechanism proposed for $[Fe(edta)]^{2-}$ but the first reaction is *ca.* 60 times slower for the neutral complexes compared to the negatively charged $[Fe(edta)]^{2-}$, whereas the second, slower reaction takes place at almost the same rate. This suggests that electrostatic interactions are much more important for the first reaction than for the second.

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Experimental

1. *General.* All solns. were prepared with bidistilled water in buffer pH 7.5 (Tris/HCl; μ = 0.1) and conserved under Ar.

Kinetic measurements were made on a *High Tech SF-3L* stopped flow spectrophotometer at λ = 550 nm under pseudo-first order conditions. The reacting solns. were $1.2\cdot10^{-5}$ M in cytochrome c and $1.2\cdot10^{-4}$ M to $3\cdot10^{-3}$ M in Fe^{II} complex. Rate constants are mean values of six runs calculated for 4 half-lives.

2. *Products.* Horse heart cytochrome c was obtained from *Fluka* 80%$_{(GE)}$, M_r = 13000 and used without further purification. All other commercially available products were of anal. grade.

N,N'-[(Pyridine-2,6-diyl)bis(methylene)]-bis[(*R*)- or (*S*)-alanine] ((*R,R*)-or (*S,S*)-alamp) and *N,N'*-[(pyridine-2,6-diyl)bis(methylene)]-bis[(*R*)- or (*S*)-proline] ((*R,R*)- or (*S,S*)-promp) were obtained as described in [14][21].

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