38. Stereoselectivity and Chiral Recognition in the Electron-Transfer Reaction between Spinach Ferredoxin and Optically Active Cobalt(III) Complexes[1])

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The kinetics of the electron transfer between reduced spinach [2Fe-2S]-ferredoxin and the optically active complexes [Co((*R*,*R*)- or (*S*,*S*)-alamp)py]$^{+}$ (**I**), [Co((*R*,*R*)- or (*S*,*S*)-promp)H_2O]$^{+}$ (**IIa**), and [Co((*R*,*R*)- or (*S*,*S*)-promp)py]$^{+}$ (**IIb**) have been investigated. The reactions are stereoselective, and for **I** and **IIa**, the stereoselectivity strongly depends on temperature due to large differences in the activation enthalpy between enantiomeric reagents. Isokinetic behaviour is observed between enantiomers, the $\Delta\Delta H^{\#}_{\Delta-\Lambda}$ values being largely compensated by the $\Delta\Delta S^{\#}_{\Delta-\Lambda}$ values. The compensation behaviour is explained by the combination of stereochemical interactions and desolvation processes on formation of the precursor complex or the transition state.

Introduction. – Plant ferredoxins, [2Fe-2S]-proteins involved in the photosynthetic electron transport in oxygenic photosynthetic organisms, are small metalloproteins (mol.wt. *ca.* 10000), characterized by a strongly negative charge (−17 in the oxidized state). The structure of the protein isolated from the blue-green algae *Spirulina platensis* shows that the [2Fe-2S] cluster is located near the surface in a hydrophobic pocket [2]. From similarities in the behaviour of this protein and the ferredoxins isolated from spinach or parsley leaves, it has been concluded [3] [4] that equivalent structural features should exist in the latter compounds. Investigations of the redox behaviour of ferredoxins with small synthetic reagents [4–9] have shown that the reaction with 2+ or more positively charged reagents leads to the formation of a precursor complex followed by intramolecular electron transfer. For 1+, neutral, or negatively charged reagents, no precursor formation could be observed, and the reaction is believed to occur on close contact of the reactants [10] [11].

In preceding communications [1] [12], we showed that the electron transfer between plastocyanin and certain optically active Fe(II) complexes is stereoselective and that this stereoselectivity is the consequence of an incomplete compensation between the activation enthalpy and the activation entropy of the reaction. These results prompted us to extend stereoselectivity studies to other metalloproteins in order to find out if the isokinetic behaviour of plastocyanin with enantiomeric reagents is a general feature of reactions involving metalloproteins.

In the present work, we report some results of the reaction between spinach ferrodoxin and the enantiomers of three Co(III) complexes.

[1]) Part XIII of the series 'Stereoselectivity in Reactions of Metal Complexes'. For Part XII, see [1].

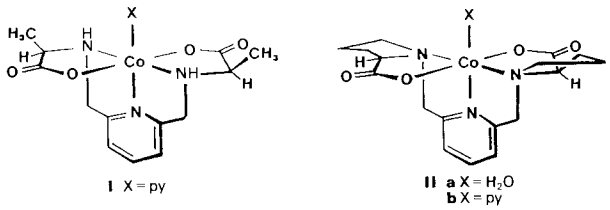

Fig. 1. *Absolute configuration of Δ-[Co(alamp)py]*$^+$ (**I**) *and Δ-[Co(promp)X]*$^+$ (**II**; X = H_2O, pyridine)

Results. – *Reagents.* The reagents used in this work are the *Λ*- or *Δ*-(pyridine){*N,N'*-[(pyridine-2,6-diyl)bis(methylene)]bis[(*S*)- or (*R*)-alaninato]}cobalt(III) ([Co(alamp)-py]$^+$), the *Λ*- or *Δ*-aqua- and the *Λ*- or *Δ*-(pyridine){*NN'*-[(pyridine-2,6-diyl)bis (methylene)]bis[(*S*)- or (*R*)-proline]}cobalt(III) ([Co(promp)X]$^+$; X = H_2O, pyridine)[2]). The *Δ* compounds **I** and **II** with the two optically active ligands are shown in *Fig. 1*. The structure of **I** and **II** whose absolute configuration has been described previously [13] [14] shows C_2 symmetry with the side chain at C(α) of the amino-acid moiety, in the *'exo'*-position, pointing in the direction of the monodentate ligand located at the sixth coordination site.

Kinetics. Reaction rates of the oxidation of reduced ferredoxin have been measured as a function of the Co(III) complex concentration and of the temperature as described in detail in the *Exper. Part.* The reaction with [Co(promp)H_2O]$^+$ is shown as an example in *Fig. 2*. A linear relationship is obtained between observed pseudo-first-order rate con-

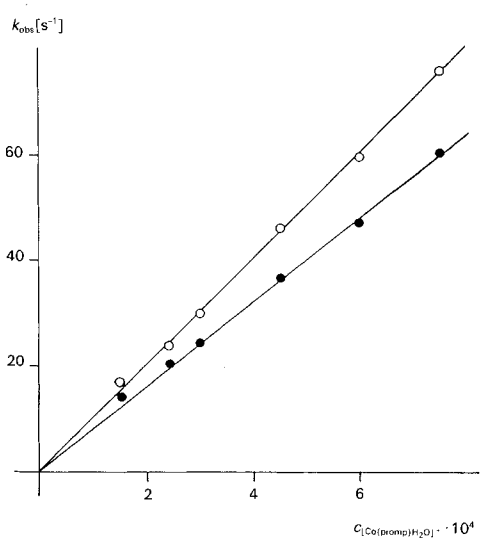

Fig. 2. *Observed pseudo-first-order rate constants for the oxidation of reduced spinach ferredoxin by Δ-[Co((*R,R*)-promp)H_2O]*$^+$ (○) *and Λ-[Co((*S,S*)-promp)H_2O]*$^+$ (●). pH 8.0 (*Tris*-HCl); μ = 0.1 (NaCl); T = 25°.

[2]) The anionic ligands were formerly named as carboxy-substituted azaalkyl derivatives [13] [14].

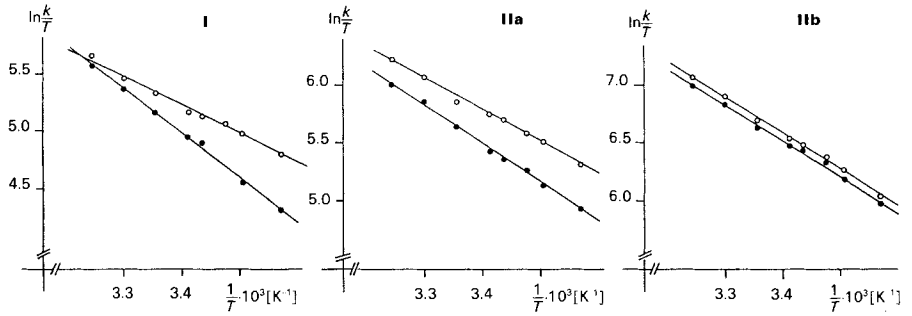

Fig. 3. Eyring *plots for the reduction of spinach ferredoxin by [Co(alamp)py]*$^+$ (**I**), *[Co(promp)H*$_2$*O]*$^+$ (**IIa**), *and [Co(promp)py]*$^+$ (**IIb**). ○: *Δ*-(*R,R*)-isomer; ●: *Λ*-(*S,S*)-isomer. $c_{Co(III)} = 2.41 \cdot 10^{-4}$ M, pH 8.0 (*Tris*-HCl), $\mu = 0.1$ (NaCl).

stants and oxidant concentration in a concentration range from $1.5 \cdot 10^{-4}$ to $7.5 \cdot 10^{-4}$ M. As expected for monopositively charged reagents, association between the protein and the reagent should be weak. From these results, mean values of the second-order rate constants are calculated. The variation of the reaction rate with temperature is determined for a single concentration of the oxidant under pseudo-first-order rate conditions ($c_{Co(III)} \approx 14 \cdot c_{ferredoxin}$) and for a temperature range from 7 to 35°. Unfortunately, irreversible alteration of the protein occurs, when the temperature is rised above 35°. The *Eyring* plots for the enantiomers of the three complexes used are represented in *Fig. 3*. The calculated activation parameters together with second-order rate constants obtained at 25° by variation of the oxidant are summarized in the *Table*.

Discussion. – The most striking observation which can be made from the results given in the *Table* is the surprisingly large difference of the activation enthalpy between the enantiomers for the reaction with [Co(alamp)py]$^+$ and – to a somewhat lesser extent – with [Co(promp)H$_2$O]$^+$. On the other hand, $\Delta H^{\neq}$ values are identical for the enantiomers of [Co(promp)py]$^+$.

As it was observed in the case of the stereoselective electron transfer with plastocyanin [1], the $\Delta\Delta H^{\neq}_{\Delta-\Lambda}$ value for a given couple of enantiomeric reagents is largely compensated by the corresponding $\Delta\Delta S^{\neq}_{\Delta-\Lambda}$ value, and the observed stereoselectivity is, therefore, relatively small compared to the difference in the heat of activation of the process.

From this compensation behaviour, two important consequences emerge: *i*) the high $\Delta\Delta H^{\neq}$ values cause a large temperature dependence of the observed stereoselectivity, and *ii*) because $\Delta\Delta H^{\neq}$ and $\Delta\Delta S^{\neq}$ give opposite contributions to the difference of the free activation energy, a temperature must exist at which the sense of the stereoselectivity is reversed. In the case of [Co(alamp)py]$^+$ this happens at 37°; below this temperature, stereoselectivity is in favour of the *Δ*-isomer, whereas above 37° the *Λ*-isomer should react faster.

$\Delta H^{\neq}/\Delta S^{\neq}$ compensation is well known in reactions involving proteins [15] and has also been observed for electron-transfer reactions between metalloproteins and various reagents [7] [16]. It is, therefore, interesting to notice that this compensation behaviour is not only found for reagents of different chemical nature, but also for enantiomers. *Fig. 4* shows that the results obtained in this work fit quite well into a $\Delta H^{\neq}/\Delta S^{\neq}$-compensation

Table. *Second-Order Rate Constants, Stereoselectivity, and Activation Parameters for the Reduction of Spinach Ferredoxin by Optically Active Cobalt(III) Complexes.* $C_{ferredoxin} = 3.5 \cdot 10^{-5}$ M, pH 8 (*Tris*/HCl), $\mu = 0.1$ (NaCl).

Complex		$k_{et}/10^4$ [a])	$\Delta H^{\neq}$ [kJ/mol]	$\Delta S^{\neq}$ [J/mol/K]	k_Δ/k_Λ	$\Delta\Delta H^{\neq}_{\Delta-\Lambda}$ [kJ/mol]	$T\cdot\Delta\Delta S^{\neq}_{\Delta-\Lambda}$ [kJ/mol]	E [d]) [V] *vs.* NHE
$[Co(alamp)py]^+$	Δ	6.1 ± 0.2	19.8 ± 0.9	-87 ± 3	1.27[a])	11.8	11.3[a])	0.02
	Λ	4.8 ± 0.1	31.6 ± 0.8	-49 ± 3	1.90[b])		10.4[b])	
$[Co(promp)H_2O]^+$	Δ	9.9 ± 0.2	22.4 ± 0.6	-73 ± 3	1.32[a])	5.6	5.1[a])	0.04
	Λ	7.5 ± 0.2	28.0 ± 0.6	-56 ± 3	1.56[b])		4.6[b])	
$[Co(promp)py]^+$	Δ	20.0 ± 0.6[c])	25.8 ± 0.6	-56 ± 2	1.13[c])	0	0.3	0.13
	Λ	17.7 ± 0.3[c])	25.8 ± 0.7	-55 ± 2				

[a]) 25°.
[b]) Calculated for 0°.
[c]) 15°.
[d]) Measured by cyclic voltametry.

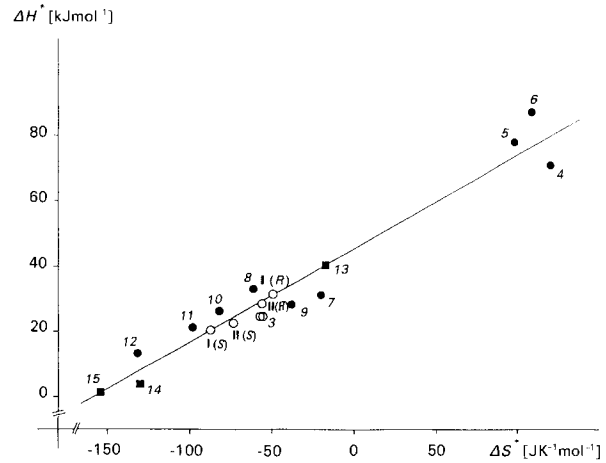

Fig. 4. *$\Delta H^{\#}/\Delta S^{\#}$-compensation plot for the oxidation of [2Fe-2S]-ferredoxins by various Co(III) and Fe(III) complexes.* **I** (*R*): *Λ*-[Co((*R*,*R*)-alamp)py]$^+$; **I** (*S*): *Λ*-[Co((*S*,*S*)-alamp)py]$^+$; **II** (*R*): *Λ*-[Co((*R*,*R*)-promp)H_2O]$^+$; **II** (*S*): *Λ*-[Co((*S*,*S*)-promp)H_2O]$^+$; *3*: [Co(promp)py]$^+$; *4*: $[(H_3N)_5CoNH_2Co(NH_3)_5]^{5+}$; *5*: $[Co(NH_3)_6]^{3+}$; *6*: $[Co(en)_3]^{3+}$; *7*: $[Co(NH_3)_5Cl]^{2+}$; *8*: $[Co(NH_3)_5C_2O_4]^+$; *9*: $[Co(Hdmg)_2(C_6H_5NH_2)_2]^+$; *10*: $[Co(acac)_3]$; *11*: [Co(edta)]$^-$; *12*: $[Co(C_2O_4)_3]^{3-}$; *13*: ferricytochrome c; *14*: [Fe(edta)]$^-$; *15*: [Fe(Hedta)]. ○: this work; ●: from [7] (parsley); ■: from [5] (spinach).

plot, together with the other oxidizing agents used so far for the reaction with ferredoxin, giving a slope (compensation temperature) of 288 K.

Electron-transfer reactions between metalloproteins and small reagents are generally considered to be of the outer-sphere type, and the activation enthalpy thus includes the thermal energy required to bring the reactants close enough together to allow electron transfer as well as the energy needed to break up the hydration shell of the reacting species [16]. The entropy term of the activation process, on the other hand, contains the contribution due to rearrangement of the solvent molecules. $\Delta H^{\#}/\Delta S^{\#}$ compensation may be a consequence of this behaviour: the nearer the reagents are brought together in the transition state, the more solvent molecules are displaced during the activation process. This is in agreement with the high $\Delta H^{\#}$ and the positive $\Delta S^{\#}$ for the three highly positively charged reagents (see *Fig. 4*), for which the association constants with the protein have been determined and which show that desolvation should be an important factor in the reaction.

When this model is applied to reactions with enantiomeric reagents, which lead to diastereoisomeric transition states, then the higher activation enthalpy measures the energy barrier of the enantiomer coming closer to its reaction partner, a phenomenon generally called 'chiral recognition'. This situation is schematically represented in *Fig. 5*. Since there is no difference in the chemical properties of enantiomeric reagents, the $\Delta H^{\#}/\Delta S^{\#}$-compensation behaviour can only be the result of energetic differentiation by chiral interactions in the formation of the diastereoisomeric transition state. Stereoselectivity on the other hand, as the ratio of the rate constants, is not only the consequence of chiral recognition between the reactive species but the macroscopic expression of all the

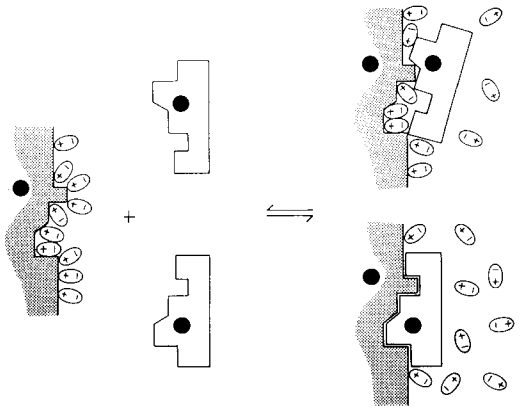

Fig. 5. *Schematic representation of the formation of a precursor complex or a transition state between a metalloprotein and the enantiomers of an optically active complex*

energetic factors which characterize the system, and it may, therefore, happen that under given conditions, the pair of reactants showing better chiral recognition reacts more slowly. This is an important result which shows that in the electron transfer involving metalloproteins chiral recognition and stereoselectivity cannot be compared directly. The question then arises, whether the results reported here, concerning electron transfer in aqueous solution between metalloproteins and small reagents, represent a particular case, or if on the contrary, similar arguments should be applied whenever given chemical reactivity is the consequence of particular structural properties of the reacting compounds. Stereoselectivity measurements provide certainly an excellent way to obtain more information about this question. Unfortunately, very few examples are known [17] where activation parameters have been determined for stereoselective reactions.

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Experimental Part

1. *Products.* 1.1. *Reagents.* Both enantiomers of the optically active Co(III) complexes used were prepared as described previously [13] [14]. All other reagents were of anal. grade.

1.2. *Ferredoxin.* Ferredoxin was isolated from fresh or frozen market spinach leaves *(Spinacea oleracea)*. Proteins were extracted and fractionated by addition of acid to pH 4.8 and $(NH_4)_2SO_4$ to 80% saturation according to [18]. The supernatant of this precipitation which contained most of the ferredoxin was collected on an anion-exchange column *(Whatman DE-23)*, equilibrated in 50 mM *Tris*-HCl pH 7.9 containing $(NH_4)_2SO_4$ at 80% saturation according to [19]. The column was washed with 4 volumes of 50 mM *Tris*-HCl pH 7.9 containing $(NH_4)_2SO_4$ at 60% saturation and eluted with 5 volumes of the same buffer containing $(NH_4)_2SO_4$ at 30% saturation. The coloured fractions containing ferredoxin were combined and their volume reduced by ultrafiltration on a *YM-5* membrane *(Amicon)*. The concentrated protein soln. was chromatographed on a *Sephadex G-50* fine column equilibrated with 50 mM *Tris*-HCl pH 7.3 containing 50 mM NaCl. The ferredoxin-containing fractions were combined and applied to a DEAE-trisacryl column equilibrated with 50 mM *Tris*-HCl pH 7.3 containing 50 mM NaCl. Proteins were eluted with a linear gradient in 50 mM *Tris*-HCl pH 7.3. Ferredoxin was detected spectrophotometrically, and fractions with a 420 nm/276 nm absorbance ratio of > 0.45 were combined, concen-

trated, and diafiltrated with 25 mM MOPS-KOH (3-morpholinopropanesulfonic acid) pH 7.6 on a *YM-5* membrane and stored frozen at –20°.

2. *Solutions.* Solutions were prepared using bidistilled H_2O and were freed from dissolved O_2 by gently bubbling N_2 through the soln. for at least 45 min. N_2 was purified by passing through a column filled with BTS-CuO catalyst heated to 150°.

2.1. *Buffer.* It was prepared from *Tris* (0.0181M), HCl (0.01M), and NaCl (0.09M) giving a soln. of pH 8.0 at 25° and ionic force $\mu = 0.1$.

2.2. *Co(III) Complexes.* Identical amounts of each enantiomer of the different complexes were dissolved in the buffer. In each case, the UV/VIS spectra of enantiomeric solns. were superimposable, and the CD spectra were perfect mirror images.

2.3. *Ferredoxin.* Just before use, 20 ml of a $3.5 \cdot 10^{-5}$ M soln. was prepared by diluting the concentrated stock soln. in the buffer.

3. *Measurements.* All manipulations were performed under N_2 purified as described in *2*. The $3.5 \cdot 10^{-5}$ M ferredoxin (10 ml) was placed into the reservoir syringe of the *High-Tech SF-SL* stopped-flow spectrophotometer and reduced by 0.5 ml of $1.4 \cdot 10^{-2}$ M $Na_2S_2O_4$ (the 20-fold excess of reducing agent does not perturb the measurements because the reduction of ferredoxin by dithionite under identical conditions is much slower than the oxidation by the Co(III) complexes used [20]). After 5 min, the yellow soln. was transferred to one of the injection syringes, the second being filled alternatively by one of the two enantiomers of the oxidant, in order to measure both enantiomers under strictly identical conditions. The reactions were followed by monitoring the absorption change at 420 nm; each trace consisted of *ca.* 2500 digitized voltages and times. Calculated rate constants are given as mean values of at least six runs. Each series was performed at least twice. Variable-temp. measurements were always started at the lowest temp. which was then increased progressively. Rate constants were evaluated using a *SORD* laboratory computer as described in [21].

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