Stereoselectivity in the Reaction of Spinach Plastocyanin with Optically Active Reducing Agents

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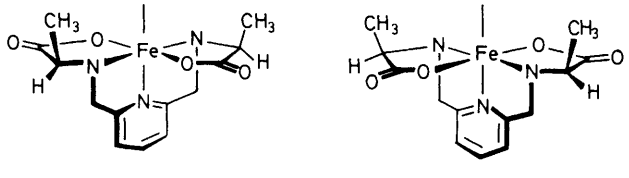
The kinetics of the reduction of spinach plastocyanin by optically active iron(II) complexes of 2,6-bis[3-(*S*)- or 3-(*R*)-carboxy-2-azabutyl]pyridine [(*S,S*)- or (*R,R*)-ALAMP] have been studied and the complex with (*R,R*)-ALAMP (Δ-configuration) reacts 1.6 to 2.0 times faster at different values of pH and temperature than the (*S,S*)-enantiomer; the activation parameters show that this observed stereoselectivity is a consequence of the differences in the activation entropies ($\Delta\Delta S^{\ddagger}_{(\Delta-\Lambda)} = +15$ J mol^{-1} K^{-1}), which over-compensates the effect of the activation enthalpy, the latter being in favour of the complex with the Λ-configuration ($\Delta\Delta H^{\ddagger}_{(\Delta-\Lambda)} = +3.0$ kJ mol^{-1}).

The electron transfer reaction between plastocyanin, a Type 1 copper protein, and several natural or synthetic oxidizing or reducing agents has been intensively studied recently,[1,2] and at least two reactive sites have been identified on the surface of the protein: one at His 87, which is co-ordinated to the copper atom in its oxidized state, and the other near Tyr 83, a region characterized by a high negative charge density about 18 Å away from the metal centre. Electron transfer can take place on one of these sites,[3—5] or simultaneously on both.[1,2,6]

One of the striking features frequently observed in the electron-transfer reactions involving blue copper proteins and non-physiological reagents is their highly negative activation entropy. It seemed of interest to study the kinetics of the reaction between metalloproteins and synthetic reagents having identical chemical properties, such as thermodynamic stability, electrochemical potential *etc.*, but showing different stereochemical interactions with the protein in the transition state.



Λ - [Fe(*S,S*)-ALAMP] Δ - [Fe(*R,R*)-ALAMP]

Figure 1

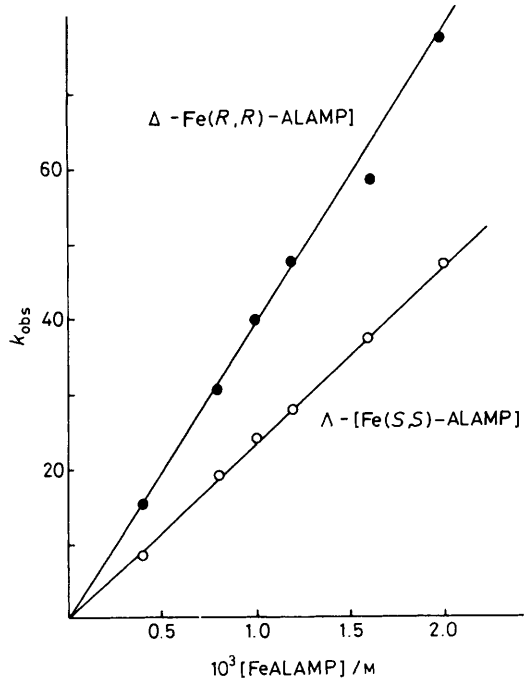


Figure 2. Dependence of k_{obs} on [FeALAMP]-concentration for the reduction of Cu^{II}-plastocyanin. [plastocyanin] = 2.3×10^{-5} M, pH = 7.0 (phosphate buffer); $\mu = 0.1$; $T = 25$ °C.

We have shown[7] that such a chiral recognition can give rise to an important differentiation in the rate of electron transfer between optically active metal complexes. Here we report first results of the stereoselectivity of reduction of spinach plastocyanin with optically active Fe^{II} complexes.

As the optically active ligand for these complexes we used 2,6-bis[3-(*S*)- or 3-(*R*)-carboxy-2-azabutyl]pyridine which is known to react stereospecifically when it forms an inert Co^{III} complex, *i.e.*, each enantiomer of the ligand forms only one of the four theoretically possible diastereoisomers.[8] By analogy with this result we deduced that an identical stereospecificity exists when the ligand reacts with metal ions forming labile complexes, *e.g.* Fe^{2+}, Figure 1.

Reaction rates were measured for the reduction of spinach plastocyanin with both enantiomers of the Fe^{II} complex, the latter being in a 30—100 fold excess with respect to plastocyanin concentrations. A linear relationship is obtained for observed first order rate constants, Figure 2. Activation parameters were obtained by measuring the reaction rates over the range 10—35 °C.

It appears from these results that the system shows an important stereoselectivity. In the pH range from 5.0 to 7.0 the reaction with the Δ-isomer is 1.6—2.0 times faster than the one with its enantiomer. The calculated second order rate constant, $\Delta\Delta H^{\ddagger}$, and $\Delta\Delta S^{\ddagger}$ values are respectively 3.9×10^4 mol^{-1} s^{-1}, + 18.1 kJ mol^{-1}, and −94 J mol^{-1} K^{-1} for the Δ-FeALAMP, and 2.4×10^4 mol^{-1} s^{-1}, + 15.1 kJ mol^{-1}, and −109 J mol^{-1} K^{-1} for the Λ-FeALAMP (pH = 7, phosphate buffer, $\mu = 0.1$, 25 °C).

These data show that the observed stereoselectivity is a consequence of a rather large difference in the activation entropy which overcompensates the effect of the activation enthalpy on the free activation energy.

The example described here shows that synthetic reagents may be able to recognize the chirality of the protein surface at the reacting site and may therefore be useful probes for investigations of structural relationships between the reagent and the metalloprotein.

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