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Stereoselective electron transfer between Cu(II) superoxide dismutase and Λ- and Δ-$[Fe(pdta)]^{2-}$

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Abstract

The kinetics of reduction of human Cu(II), Zn(II) superoxide dismutase (SOD) by Λ-, Δ- and racemic-$[Fe(pdta)]^{2-}$ have been studied as a function of pH, ionic strength and temperature. The reaction is observed to be first order with respect to both the concentration of SOD and $[Fe(pdta)]^{2-}$. At 25 °C, pH 6.0 (0.050 MES) and ionic strength 0.03 mol dm^{-3}, the second-order rate constants, k, k_Λ, k_Δ, are 13.1±0.4, 16.6±0.7 and 9.5±0.5 dm^3 mol^{-1} s^{-1} for the racemic mixture, lambda and delta isomers, respectively. At the same temperature and ionic strength and pH 5.0 and 7.0, k for reduction of SOD by the racemic mixture is 20.0±2 and 10.0±0.3 dm^3 mol^{-1} s^{-1}, respectively. At an ionic strength of 0.094 mol dm^{-3} (25 °C and pH 6.0) k for reduction of SOD by the racemic mixture is 8.0±0.2 dm^3 mol^{-1} s^{-1}. The magnitude and sense of the stereoselectivity is observed to be independent of pH and ionic strength over the ranges studied and at 25 °C, k_Λ/k_Δ is 1.7. The rate of reaction at pH 6.0, ionic strength 0.03 mol dm^{-3} was measured as a function of temperature between 5 and 50 °C yielding the activation parameters of 62.5±0.5 and 71.9±0.8 kJ mol^{-1} for $\Delta H^\ddagger$ and −12±2 and 16±3 J mol^{-1} K^{-1} for $\Delta S^\ddagger$ for SOD reduction by the lambda and delta isomers, respectively. These activation parameters are compensatory in that the enantiomeric preference of SOD for the lambda isomer at 25 °C results from a lower activation enthalpy which compensates for a more negative activation entropy relative to reduction by the delta isomer. At sufficiently high temperatures, the stereoselectivity is predicted to change in favor of the delta isomer due to its more positive activation entropy.

Keywords: Electron transfer; Kinetics of reduction; Copper; Zinc; Superoxide dismutase; Iron complexes

1. Introduction

Stereoselectivity in the electron transfer reactions between metalloproteins and chiral coordination compounds has been reported [1–3] for several metalloproteins and recently reviewed [1c]. All of these reactions involve electron transport enzymes and have focused on the nature of binding of the coordination compound to the enzyme when the location of binding of complex on the protein's surface could be deduced [3]. In addition, stereoselective electron transfer reactions of horse cytochrome *c* and spinach plastocyanin and ferredoxin with coordination compounds have been studied as a function of temperature and reactions of all three enzymes display compensation between the entropy and enthalpy changes of activation in that the enantiomer with the lower $\Delta H^\ddagger$ is also observed to have a more negative $\Delta S^\ddagger$ [1]. Consequently, the magnitude of chiral discrimination depends on temperature and at some temperature changes from favoring one enantiomer to favoring the other. This behavior has been interpreted [1] in terms of a higher $\Delta H^\ddagger$ reflecting better chiral recognition and tighter binding of the redox pair and at the same time greater desolvation of the reacting partners and consequently a more positive $\Delta S^\ddagger$.

This study reports observations of stereoselectivity in the reduction of human Cu(II), Zn(II) superoxide dismutase (SOD) by Λ- and Δ-$[Fe(pdta)]^{2-}$ ($pdta^{4-}$ = 1,2-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetate(4−)) as a function of temperature, pH and ionic strength. SOD is the first non-electron transport protein for which stereoselective electron transfer has been reported and consequently the first protein studied in this way for which an active-site channel exists that restricts access of potential substrates to the metal center. Λ- and Δ-$[Fe(pdta)]^{2-}$ were chosen as electron transfer partners for SOD in these studies because anionic complexes are expected to associate more favorably with the cationic active-site channel of SOD [4]. The

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$[Fe(pdta)]^{2-}$ enantiomers are also small enough that they might gain at least partial entry to the active-site channel of the enzyme. It is known for example that hexachloroiridate can reside in the active-site channel very close to the copper [5]. Moreover, it is known [6] that *R*- and *S*-$ptda^{4-}$ stereospecifically form Δ-$[Fe(R\text{-}pdta)]^{2-}$ and Λ-$[Fe(S\text{-}pdta)]^{2-}$, respectively, in solution allowing examination of iron complexes that would otherwise racemize by ligand substitution at rates competitive with the electron transfer reaction making impossible any determination of stereoselectivity in the electron transfer reaction.

2. Experimental

Commercially available materials were usually reagent or analytical reagent grade and were used without further purification, unless noted otherwise. Water used for the preparation of solutions was distilled and further purified by reverse osmosis.

Resolved *R*- and *S*-H_4pdta were prepared and purified by literature methods [7]. Toluene was used in place of benzene for the extraction of the resolved 1,2-diaminopropane [8] from which the tetra acid is prepared. The $[Fe(pdta)]^{2-}$ solutions used for kinetic experiments were prepared by the dissolution of weighed amounts of primary standard grade $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ under anaerobic conditions in a solution containing ~20% molar excess of H_4pdta and buffer adjusted to the desired pH and ionic strength. All solutions were prepared, stored and kinetic measurements made in a COY Type A anaerobic chamber with a gaseous atmosphere of ~95% nitrogen and 5% hydrogen containing bags of palladium catalyst which rapidly convert adventitious oxygen to water. Under these conditions the $[Fe(pdta)]^{2-}$ solutions were stable for several days. Human Cu(II),Zn(II) superoxide dismutase (SOD), prepared by recombinant DNA techniques [9], was generously provided by Chiron Corporation. The enzyme exists in air, and is isolated, in the Cu(II) state [4]. Samples for kinetic experiments were either washed with reaction buffer three times and micro-concentrated using ultra filtration, or lyophilized solid samples were dissolved directly in reaction buffer and used without further purification. Kinetic results from experiments using both methods of SOD preparation gave indistinguishable results. The concentration of enzyme was determined from the mass of the original sample and confirmed by measuring the absorbance of the SOD solution at 674 nm ($\epsilon = 260$ $dm^3\ mol^{-1}\ cm^{-1}$) [10]. Buffers were prepared from the neutral solids and from acetic acid by adjusting the pH with a minimum of dilute HCl or NaOH. Ionic strength maintenance, beyond that provided by the salt-form of the buffer and reactants, was provided by added NaCl.

Kinetic measurements were made by injecting a small volume of concentrated SOD into a cuvette containing the $[Fe(pdta)]^{2-}$ and buffer at thermal equilibrium. Following mixing, the absorbance change, primarily due to the oxidation of the iron complex, was monitored at 320 nm with internal referencing at 500 nm using a Hewlett Packard model 8452 diode array spectrophotometer contained inside the COY anaerobic chamber. The absorbance versus time plots that resulted were fit to a single exponential function using commercial kinetics software from On-Line Instrument Systems (OLIS). All experiments were done with the iron complex in at least a ten-fold stoichiometric excess of the Cu(II) of the SOD.

3. Results

At constant pH, the reduction of SOD by $[Fe(pdta)]^{2-}$ follows a second-order rate law, Eq. (1). The reac-

$$-d[SOD]/dt = k[SOD][[Fe(pdta)]^{2-}] \quad (1)$$

tion was studied with the concentration of the iron complex in pseudo-first-order excess of the SOD. The absorbance increases, observed at 320 nm as SOD was reduced and the iron complex oxidized, were fit well by a single exponential function. Typical data are displayed in Fig. 1. The observed rate constants obtained from these fits at 25 °C, pH 6.0 (0.050 mol dm^{-3} MES) and ionic strength 0.03 mol dm^{-3} are displayed in Fig. 2. The linearity of the plots in Fig. 2 demonstrates the first-order dependence of the reaction rate on the concentration of $[Fe(pdta)]^{2-}$. Data are displayed for the reaction of the racemic mixture of $[Fe(pdta)]^{2-}$ as well as for each resolved enantiomer. The second-order rate constants derived from the slopes of the lines in Fig. 2 are 13.1 ± 0.4, 16.6 ± 0.7 and 9.5 ± 0.5 $dm^3\ mol^{-1}\ s^{-1}$ for the racemic mixture, lambda and delta isomers, respectively. The ratio k_Λ/k_Δ is 1.7.

The rate and stereoselectivity of this reaction has also been studied as a function of pH, ionic strength and temperature. The reaction rate is sensitive to changes in pH with the rate constants decreasing with increasing pH, Table 1. The extent of stereoselectivity is not observed to depend on pH within the experimental uncertainties.

The reaction rate and stereoselectivity were also measured at ionic strength 0.094 mol dm^{-3}, pH 6.0 and 25 °C. The second-order rate constants are 8.0 ± 0.2,

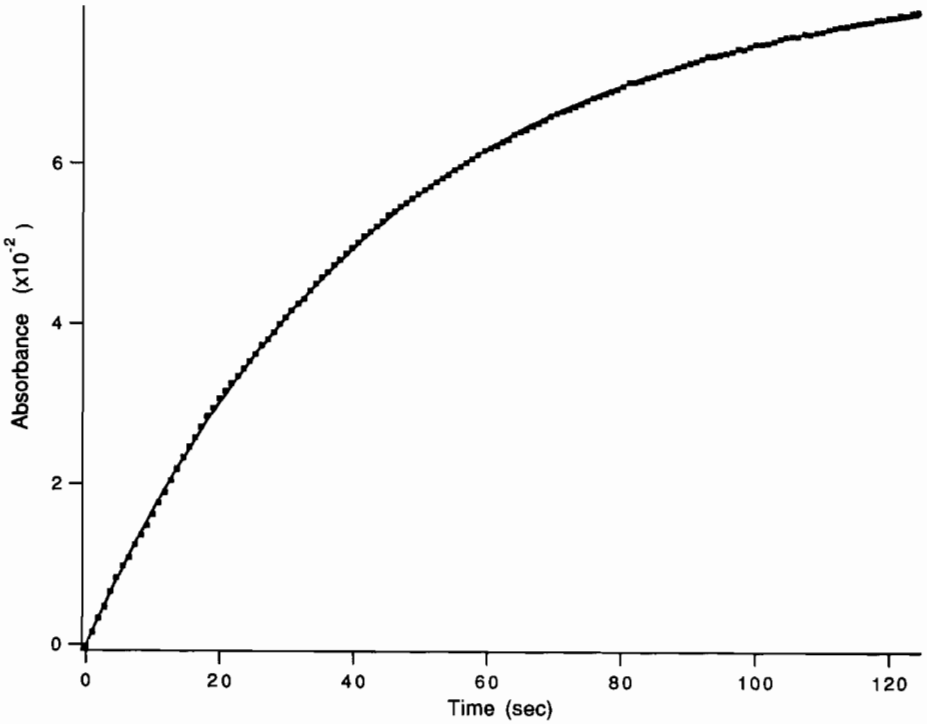

Fig. 1. Typical first-order kinetic data. Absorbance change at 320 nm as SOD is reduced by $[Fe(pdta)]^{2-}$ at 25 °C, ionic strength 0.03 mol dm^{-3} and pH 6.0, solid line, and computer fit to a single exponential, solid squares.

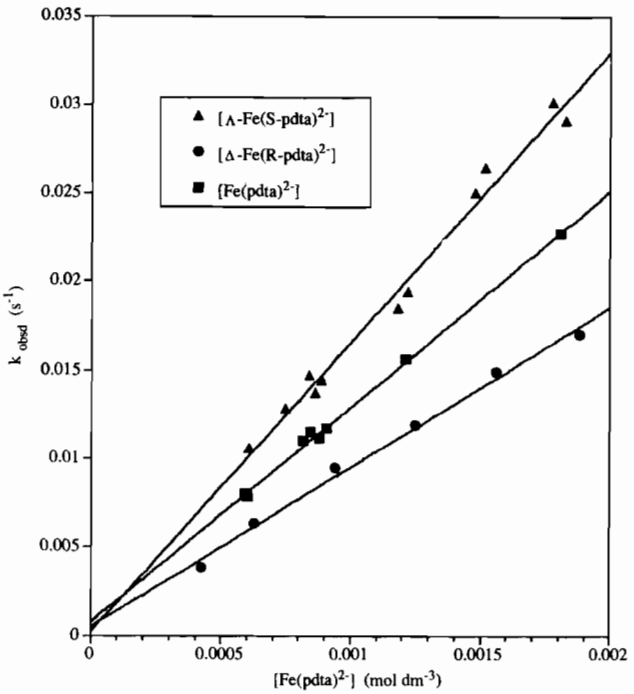

Fig. 2. Observed first-order rate constants for the reduction of SOD by Λ-, Δ- and racemic-$[Fe(pdta)]^{2-}$ at 25 °C, ionic strength 0.03 mol dm^{-3} and pH 6.0 vs. the initial $[Fe(pdta)]^{2-}$ concentration.

10.1 ± 0.5 and 5.9 ± 0.4 dm^3 mol^{-1} s^{-1} for the racemic mixture, lambda and delta isomers, respectively. Stereoselectivity is observed to be independent of ionic strength over the limited range of ionic strength studied here; the ratio k_Λ/k_Δ is 1.7.

The rates of SOD reduction by each enantiomer of the iron complex at pH 6.0, ionic strength 0.03 mol dm^{-3} were measured at five temperatures between 5 and 50 °C. Eyring plots of these data are displayed in Fig. 3. Fits to the lines shown in Fig. 3 yield activation parameters of 62.5 ± 0.5 and 71.9 ± 0.8 kJ mol^{-1} for $\Delta H^\ddagger$ and -12 ± 2 and 16 ± 3 J mol^{-1} K^{-1} for $\Delta S^\ddagger$ for the lambda and delta isomers, respectively. These activation parameters are compensatory in that the enantiomeric preference of SOD for the lambda isomer at 25 °C results from a lower activation enthalpy which compensates for a more negative activation entropy relative to reduction by the delta isomer. At sufficiently high temperatures, the stereoselectivity is predicted to change in favor of the delta isomer due to its more positive activation entropy.

4. Discussion

The observation of stereoselectivity in this reaction is especially interesting because it provides the first example of stereoselective electron transfer utilizing a non-electron transport protein [1c]. It was hoped that choosing an enzyme with a restrictive active-site cavity would provide substantially greater stereoselectivity than observed with electron transport proteins previously

Table 1
Rate constants and stereoselectivity as a function of pH

pH	racemic-[Fe(pdta)]$^{2-}$ (dm^3 mol^{-1} s^{-1})	Λ-[Fe(*S*-pdta)]$^{2-}$ (dm^3 mol^{-1} s^{-1})	Δ-[Fe(*R*-pdta)]$^{2-}$ (dm^3 mol^{-1} s^{-1})	k_Λ/k_Δ
5.0	20.0±2	27.0±2	15.0±2	1.8
6.0	13.0±0.4	16.6±0.7	9.5±0.5	1.7
7.0	10.0±0.3	12.3±0.6	7.3±0.4	1.7

All data obtained at ionic strength 0.03 mol dm^{-3} and 25 °C. Where necessary, sodium chloride was added to maintain ionic strength. The buffers used were: 0.050 mol dm^{-3} acetic acid at pH 5.0, 0.050 mol dm^{-3} MES at pH 6.0, and 0.050 mol dm^{-3} HEPES at pH 7.0 (MES = 2-[*N*-morpholino]ethanesulfonic acid], HEPES = *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]).

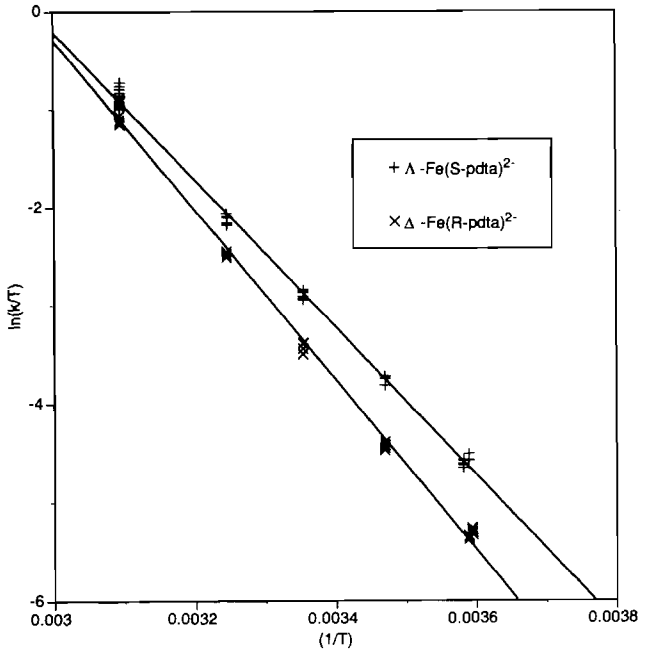

Fig. 3. Eyring plots for the reduction of SOD by Λ- and Δ-[Fe(pdta)]$^{2-}$ at 25 °C, ionic strength 0.03 mol dm^{-3} and pH 6.0.

studied since the chiral cavity might provide more stringent requirements for close approach of the chiral reductant to the redox center. It is conceivable that even complete exclusion of one enantiomer might be possible for such a system. While substantial stereoselectivity is observed, with the lambda isomer of the iron complex being almost twice as reactive as its delta enantiomer at 25 °C, it is somewhat disappointing that more striking selectivity is not observed in this reaction since this magnitude of selectivity is already achieved for several electron transport enzymes [1,3a] where presumably the association of redox partners might be expected to be less stereochemically constrained. It may be that only modest selectivity is achieved in this reaction because the iron complexes used are too large to gain significant penetration into the active-site channel of the enzyme. It is known that this channel is at most a few angstroms [4,5,11] across and would not be expected to accommodate an ion as large as the iron complex without significant rearrangement of groups around the active site. However, it is also known, from the X-ray structure determination [5], that the hexachloroiridate ion is associated with arginine-141 in the active-site cavity of the enzyme within a few angstroms of the copper. If this octahedral coordination compound can penetrate the active-site cavity to this degree, it seems possible that the [Fe(pdta)]$^{2-}$ enantiomers might also gain at least partial access to this cavity. The negative charge on the [Fe(pdta)]$^{2-}$ should facilitate approach of the complex to this site on the protein's surface since the active site is known to be highly positively charged and effective at binding anions [4].

The observed pH dependence of the reaction, Table 1, is not surprising since it is known that the reduction potential for SOD increases as the pH is decreased from pH 9 to 5. This would increase the redox potential for the overall reaction as the pH is lowered and be expected to increase the rate of electron transfer for SOD reduction by [Fe(pdta)]$^{2-}$. The possibility that this pH dependence originates with [Fe(pdta)]$^{2-}$ cannot be excluded since these complexes are labile and could undergo substitution and subsequent acid–base chemistry at a coordinated water molecule. This seems unlikely to be the explanation for the observed effect, however, since protonation of an aquo– or hydroxo–iron–pdta complex would be expected to make oxidation of the complex less favorable and decrease the rate of electron transfer. Consequently, the most plausible explanation for the observed increase in reaction rate as the pH decreases is the effect that decreasing pH is known to have on the SOD reduction potential.

The net charge on SOD at pH 6.0 is negative [4,12] as is the charge on [Fe(pdta)]$^{2-}$, and one might have expected the reaction rate to increase with increasing ionic strength. The opposite effect is observed. However, the active site of SOD has a pronounced positive charge [4] and the observed ionic strength dependence likely reflects the shielding of the negatively charged [Fe(pdta)]$^{2-}$ from this positively-charged active site as the ionic strength is increased. That is, active-site charge on SOD is more important than net charge. While this is not surprising, it does provide evidence that the

$[Fe(pdta)]^{2-}$ is reacting at or near the positively-charged active-site channel of SOD.

The compensatory activation parameters observed for this reaction make this the fourth protein system for which this effect has been observed. Bernauer et al. were the first to report [1] activation parameter compensation in stereoselective electron transfer reactions of metalloproteins and have made similar observations for reactions involving horse cytochrome *c*, and spinach plastocyanin and ferredoxin. Since there are no known exceptions to this behavior and because the four enzymes studied are so different in structure, composition and function, one must begin to regard this behavior as a general phenomenon of these reactions. Bernauer et al. have suggested [1] that compensation results because closer association of reactants (i.e. chiral recognition) produces a larger $\Delta H^{\ddagger}$, due to the greater work required to bring the reactants together, and results in a more positive $\Delta S^{\ddagger}$, due to the greater desolvation of the transition state. Consequently, the complex that benefits from chiral recognition has a higher $\Delta H^{\ddagger}$ and a more positive $\Delta S^{\ddagger}$ than does its enantiomer.

We are reluctant to embrace this as a general explanation for the observed compensatory activation parameters because we see no reason to expect that $\Delta H^{\ddagger}$ in general should be *greater* for the reacting pair that 'fit' together more closely. To the contrary, it seems plausible that for many reactions the experimental $\Delta H^{\ddagger}$ would be *less* for the reactant pair benefitting from chiral recognition because of a more favorable association process for forming the transition state complex. In particular, where prior association of reactants is important, the experimental $\Delta H^{\ddagger}$ is a composite of the enthalpy change for the association equilibrium, ΔH°_{assoc}, and the activation energy for electron transfer, $\Delta H^{\ddagger}_{et}$. A favorable association might readily result in a more negative ΔH°_{assoc} for the enantiomer benefiting from chiral recognition and lead to a *lower* experimental $\Delta H^{\ddagger}$. Moreover, if the enantiomer benefiting from chiral recognition produces an association complex that is better configured for electron transfer than the other enantiomer, $\Delta H^{\ddagger}_{et}$ would be lower as well.

If for some reactions chiral recognition does lead to a lower $\Delta H^{\ddagger}$, as just described, then one must show how this chiral recognition could at the same time lead to a more negative $\Delta S^{\ddagger}$ in order to account for the observed compensatory effect. It is notoriously difficult to predict the sign or magnitude of $\Delta S^{\ddagger}$ for electron transfer reactions, let alone small differences in $\Delta S^{\ddagger}$ for reactions differing only in stereochemistry. While one might expect slightly greater desolvation for the reactant pair with better chiral recognition, as Bernauer has suggested, and this would lead to a more positive $\Delta S^{\ddagger}$, the transition state for the better-fit pair also would be expected to have greater symmetry than the less well-matched enantiomer and this would make $\Delta S^{\ddagger}$ more negative. The relative importance of desolvation versus reactant pair symmetry in the transition state in determining $\Delta S^{\ddagger}$ is impossible to predict. However, if the contribution to $\Delta S^{\ddagger}$ from transition state symmetry is of greater relative importance than desolvation, then the compensatory effect could be rationalized in terms of lower $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the enantiomer displaying chiral recognition.

Unfortunately, limited data exist for this interesting effect and, in particular, little information is available regarding the equilibrium constants and related thermodynamic data for the association reactions that occur prior to electron transfer and contribute to the overall experimental activation parameters observed. For the one metalloprotein stereoselective electron transfer reaction for which the association equilibrium constants are known, no activation parameters are available [3a]. A more complete understanding of this compensatory effect will benefit from additional thermodynamic data for the association reactions of chiral molecules with metalloproteins.

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