KINETIC STUDIES OF THE REDUCTION OF PSEUDOMONAS AERUGINOSA FERRICYTOCHROME c_{551} BY $Fe(EDTA)^{2-}$*

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Summary: Kinetic studies of the reduction of Pseudomonas aeruginosa ferricytochrome c_{551} by $Fe(EDTA)^{2-}$ have been made. The reaction was found to follow a second-order rate law: k 4.2×10^3 M^{-1} s^{-1} [25°, μ 0.1 M, pH 7.0 (phosphate)]; $\Delta H^{\ddagger}$ 3.2 kcal/mol; $\Delta S^{\ddagger}$ -30 cal/mol-deg. The electrostatics-corrected self-exchange rate constant (k_{11}^{corr}) calculated for cytochrome c_{551} based on the $Fe(EDTA)^{2-}$ cross reaction is 2 M^{-1} s^{-1}, as compared to a value of 6 M^{-1} s^{-1} for horse heart cytochrome c. The close correspondence of the two k_{11}^{corr} values is taken as an indication that the two proteins employ very similar electron transfer mechanisms in their reactions with $Fe(EDTA)^{2-}$. It is proposed that this mechanism involves reagent contact, but little protein conformational change, at the partially exposed heme edge.

We have previously studied the kinetics of reduction of horse heart ferricytochrome c by $Fe(EDTA)^{2-}$ (1). The measured second order rate constant of 2.6×10^4 M^{-1} s^{-1} [25°, μ 0.1 M, pH 7.0 (phosphate)] has been used to calculate an electrostatics-corrected, self-exchange rate constant, k_{11}^{corr}, for the protein of 6 M^{-1} s^{-1} (2). This latter value is somewhat smaller than the experimental k_{11} (extrapolated to the same conditions) of 2.8×10^2 M^{-1}, which has led us to propose that $Fe(EDTA)^{2-}$ has difficulty in penetrating the protein surface in the vicinity of the partially exposed heme edge (2).

As our proposal depends on the reliability of a calculation based on a cross reaction between a positively-charged (3) protein and a negatively-charged substrate, we have turned to negatively-charged (4,5)

*Abbreviations: EDTA, ethylenediaminetetraacetate; phen, 1,10-phenanthroline.
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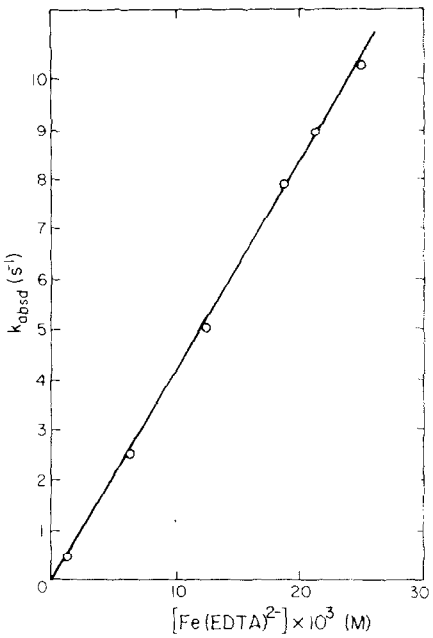

Figure 1. Concentration dependence of k_{obsd} for the reduction of ferricytochrome $\underline{c}_{551}$ by $Fe(EDTA)^{2-}$ [pH 7.0 (phosphate), μ 0.1 M, 25°]

ferricytochrome $\underline{c}_{551}$ from Pseudomonas aeruginosa, whose three-dimensional structure is very similar to that of the horse heart protein (3, 6, 7). Accordingly, we report herein the results of a kinetic study of the reduction of ferricytochrome $\underline{c}_{551}$ by $Fe(EDTA)^{2-}$. The mechanistic meaning of some striking similarities and differences in the reactivities of the two cytochromes $\underline{c}$ with $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$ is considered.

Methods: Cytochrome $\underline{c}_{551}$ from Pseudomonas aeruginosa was purified according to the method of Ambler and Wynn (5) to a ratio A_{551}/A_{280} of 1.2. Reagent grade chemicals and deionized-distilled water were used throughout. The nitrogen gas used for deoxygenation of solutions for kinetic studies was passed through two chromous scrubbing towers to eliminate impurity oxidants. Ferrous EDTA solutions were prepared as previously described (8) with the concentration of EDTA in 20% excess over iron to insure complete formation of the $Fe(EDTA)^{2-}$ complex. The reduction of ferricytochrome $\underline{c}_{551}$ was followed at 551 nm. All data were analyzed as pseudo-first-order in protein with the reducing agent in 100- to 1000-fold excess.

All kinetic experiments were performed on a Durrum Model D-110 stopped-flow spectrometer. Solutions were allowed at least 20 minutes to come to temperature equilibrium prior to mixing.

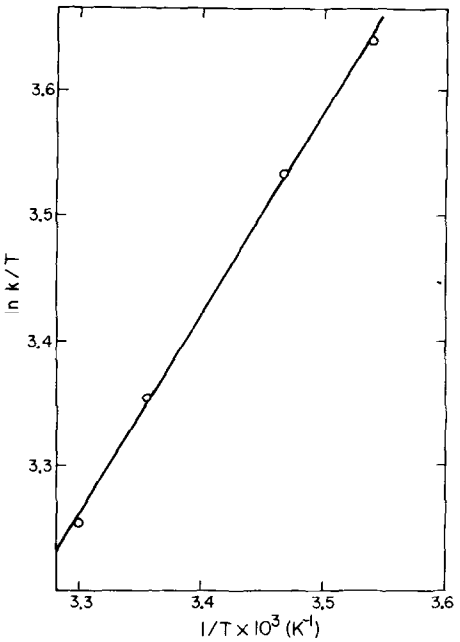

Figure 2. Eyring plot for the reduction of ferricytochrome c by $Fe(EDTA)^{2-}$ [pH 7.0 (phosphate), μ 0.1 M].

Temperature was controlled to ± 0.2° by a Forma Scientific temperature bath. Data were sent to a Tektronix 564B storage oscilloscope and to an A/D converter. The A/D converter was used in conjunction with a PDP-10 computer to evaluate the observed rate constants. A Cary 17 spectrophotometer was used for all absorption spectral measurements and a Brinkman pH 101 instrument was used for all pH determinations.

Results: First-order plots were linear for greater than 90% of the reduction of ferricytochrome c_{551} by $Fe(EDTA)^{2-}$. The dependence of observed rate constants on concentration of reductant at pH 7.0 is shown in Figure 1. The second-order rate constant is 4.2 ± 0.1 × 10^3 M^{-1} s^{-1} [25°, μ 0.1 M, pH 7.0 (phosphate)], and the activation parameters for the reduction of ferricytochrome c_{551} obtained from a standard Eyring plot (Figure 2) are as follows: $\Delta H^{\ddagger}$ 3.2 ± 0.1 kcal/mol and $\Delta S^{\ddagger}$ -30 ± 1 cal/mol-deg.

Discussion: The rate constant for reduction of ferricytochrome c_{551} by $Fe(EDTA)^{2-}$ is nearly a factor of ten smaller than that obtained from the analogous horse heart protein reaction, which is not unexpected

in view of the fact that the former pair of reactants are like-charged. The difference in rate in fact may well be entirely due to electrostatic charge effects, as both horse heart cytochrome c (3) and cytochrome c_{551} (9) have the same reduction potential (E 260 mV). To explore this point further, we have utilized a previously-developed model (2) to calculate the electrostatics-corrected self-exchange rate constant (k_{11}^{corr}) for cytochrome c_{551} based on the cross reaction with $Fe(EDTA)^{2-}$. The result is 2 M^{-1} s^{-1}, as compared to 6 M^{-1} s^{-1} for the corresponding k_{11}^{corr} for the horse heart protein (2). The parameters and work terms as defined before (2) that were used in the cytochrome c_{551}-$Fe(EDTA)^{2-}$ calculation are as follows: $Fe(EDTA)^{-/2-}$, E 120 mV, k_{22} 3×10^4 M^{-1} s^{-1}, R 4 Å (2); cytochrome c_{551}, E 260 mV, R 14.4 Å, Z -2/-3 (9); k_{12} 4.2×10^3 M^{-1} s^{-1}; w_{12} 0.194, w_{21} 0.146, w_{11} 0.079, w_{22} 0.493, $\Delta G_{11}^{*uncorr}$ 16.80, ΔG_{11}^{*corr} 17.03 kcal/mol.

The fact that the k_{11}^{corr} values for cytochrome c_{551} and horse heart cytochrome c based on $Fe(EDTA)^{2-}$ are virtually the same is entirely consistent with a mechanistic model in which the transition state for electron transfer features substrate contact with the protein at a point on the surface near the partially exposed heme edge. If the substrate were not able to penetrate the surface (and induce a conformational change) of the protein, the electrostatics-corrected electron transfer rate would simply be a function of the distance of closest approach to the heme edge. It may be concluded from our kinetic analysis that this closest approach distance is approximately the same in the $Fe(EDTA)^{2-}$ reduction of the two cytochromes. In this connection, it is of interest to note that the X-ray structural studies have indicated that the distance from the surface to the partially exposed heme edge does not vary appreciably in the two proteins (7). Apparently, the hydrophilic nature of most of the surface of $Fe(EDTA)^{2-}$ makes it a particularly poor substrate insofar as penetration into the hydrophobic interior of a protein is concerned (2).

The situation is markedly different in the $Co(phen)_3^{3+}$ reactions with ferrocytochrome c_{551} and horse heart ferrocytochrome c (10). Here the calculated k_{11}^{corr} for the bacterial protein (1.8×10^5 M^{-1} s^{-1}) is over 10^2 times larger than that (7.1×10^2 M^{-1} s^{-1}) for cytochrome c (9). As both of these k_{11}^{corr} values are substantially larger than the corresponding quantities based on the $Fe(EDTA)^{2-}$ reactions, it is likely that there are favorable nonelectrostatic $Co(phen)_3^{3+}$-protein interactions in the transition state for electron transfer. These interactions probably involve the phen rings and hydrophobic protein residues, as proposed previously (2). As cytochrome c_{551} has a shorter polypeptide chain (4,5) than cytochrome c (3) (82 amino-acid residues vs. 104), and that as a result both the composition and flexibility of the chain near the partially exposed heme edge are likely to be altered, it is hardly surprising, given a model including strong nonelectrostatic substrate-protein interactions, that the k_{11}^{corr} values are quite different. It could not have been predicted in advance that the k_{11}^{corr} for cytochrome c_{551} would have been larger than that for cytochrome c, but the fact that it is allows us to conclude that $Co(phen)_3^{3+}$ is able to penetrate and contact the redox center in the bacterial protein quite effectively.

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