Kinetic Studies on 1:1 Electron-Transfer Reactions Involving Blue Copper Proteins.† 4. Reactions of Reduced *Rhus vernicifera* Stellacyanin with $Co(edta)^-$, $Co(C_2O_4)_3^{3-}$, and $Ru(en)_3^{3+}$; Reactivity Patterns for Stellacyanin

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Abstract: Contrary to a previous report, it has been demonstrated that the oxidation of stellacyanin, SCu^I, with $Co(edta)^-$ does not exhibit limiting kinetics. Moreover, the reaction is unaffected by redox-inactive complexes $Cr(CN)_6^{3-}$ (2.0×10^{-3} M), $Co(CN)_6^{3-}$ (2.5×10^{-4} M), and $Cr(edta)(H_2O)^-$ (6.0×10^{-3} M). The kinetics with 3- and 3+ oxidants, $Co(C_2O_4)_3^{3-}$ and $Ru(en)_3^{3+}$, respectively, have also been studied, and in all cases the simple rate law $k[SCu^I][oxidant]$ is found to apply. For stellacyanin, some 16 reactions with inorganic oxidants have now been studied, with no evidence for limiting kinetics stemming from extensive association prior to electron transfer. Interestingly, cytochrome *c*, the only other positively charged electron-transport metalloprotein to have been extensively investigated, likewise provides no examples of limiting kinetics. The reaction with $Co(C_2O_4)_3^{3-}$ gives no dependence on pH within the range 5-10 (four buffers). Effects of pH with $Ru(en)_3^{3+}$ and $Co(phen)_3^{3+}$ (previously reported pK_a of 7.7) as oxidants suggest that these complexes use an alternative functional site on the protein for electron transfer that is close to an uncoordinated histidine. Effects of ionic strength are also considered.

This paper is concerned with an assessment of the redox behavior of stellacyanin (1 Cu atom; M_r ca. 20000) from *Rhus vernicifera*[1,2] using inorganic redox partners. It follows other similar assessments from this group on blue (type 1) single-Cu proteins, plastocyanin[3,4] and azurin.[5] Stellacyanin has a single polypeptide chain of 107 amino acid residues with carbohydrate moieties (contributing ca. 40% to the molecular weight) attached at three different positions.[6] From a variety of evidence[6] including X-ray diffraction[7-9] and EXAFS measurements,[10] it is known that the Cu in plastocyanin and azurin is coordinated to a cysteine, methionine, and two histidines. Stellacyanin has no methionine, and the binding of the Cu must therefore be different. From NMR studies, it has been suggested that a disulfide residue is coordinated in place of methionine.[11] The reduction potential for the stellacyanin couple here referred to as SCu^{II}/SCu^I (184 mV)[12] is significantly less than the ca. 350 mV reported for plastocyanin and azurin.[1,2] A further interesting feature is that stellacyanin has an isoelectric point at 9.86.[12] Plastocyanin and azurin have much lower isoelectric points and are both negatively charged at pH > 5.[1,2]

From previous studies with plastocyanin[3,4] and azurin,[5] evidence has been obtained consistent with the utilization of at least two functional sites for electron transfer on each protein.[13] The different approaches defined are here extended to an oxidation of stellacyanin SCu^I with the 3- and 3+ oxidants $Co(C_2O_4)_3^{3-}$ and $Ru(en)_3^{3+}$. In addition we have not been able to reproduce earlier results[14] for the oxidation of SCu^I with $Co(edta)^-$, and this system has therefore been reinvestigated.

Experimental Section

Protein. Stellacyanin from the Japanese lacquer tree *Rhus vernicifera* was obtained from the acetone powder as supplied by Saito and Co., Tokyo, by the method of Reinhammer.[12] UV-visible absorption spectra peak ratios obtained were $A_{280}/A_{604} = 5.8 \pm 0.1$ (lit. 5.6)[12] and $A_{280}/A_{250} = 3.0$ (lit. 3.0). Only a single protein band was detected on polyacrylamide gel electrophoresis. Stock solutions (ca. 2×10^{-4} M) in 0.2 M Na_2HPO_4 were stored frozen at ca. -15 °C. Reduction was carried out by addition of crystals of either sodium dithionite or ascorbic acid (B. D. H., Analar). The protein was dialyzed for at least 24 h at 0 °C, under nitrogen, against the buffer required for kinetic studies. Kinetic results were independent of the reductant used. Air-free (N_2) conditions were employed in all experiments to avoid slow air oxidation of SCu^I. As a check of purity, rate constants for the oxidation of SCu^I with $Co(phen)_3^{3+}$ were determined and found to be in excellent agreement with those of McArdle et al.[15]

Complexes. Potassium tris(oxalato)cobaltate(III), $K_3[Co(C_2O_4)_3]\cdot 3.5H_2O$, was prepared by the literature method[16] and gave absorbance maxima at 245, 420, and 596 nm, with absorption coefficients (M^{-1} cm^{-1}) 2.22×10^4 (lit. 2.14×10^4),[17] 221 (218), and 167 (165), respectively. Stock solutions were kept in the dark and were generally used within 30 min of preparation.[18] Tris(ethylenediamine)ruthenium(III) bromide, $[Ru(en)_3]Br_3$,[19] was prepared by I_2 oxidation of $[Ru(en)_3][ZnCl_4]$, followed by conversion to the bromide by addition of concentrated HBr to a nearly saturated solution of the iodide. The Ru(II) complex $[Ru(en)_3][ZnCl_4]$ was prepared by the literature method[20] and purified according to Lavallee and Lavallee.[21] The product gave a single peak at 310 nm (ϵ 359 M^{-1} cm^{-1} (lit. 360 M^{-1} cm^{-1}))[19] and satisfactory analyses for C, H, and N. Sodium (1,2-diaminoethanetetraacetato)cobaltate(III), $Na[Co(edta)]\cdot 4H_2O$, was prepared by the method of Dwyer et al.[22] and gave absorbance maxima at 535 nm (ϵ 320 M^{-1} cm^{-1}) and 381 nm (ϵ 216 M^{-1} cm^{-1}), in good agreement with the literature values of 536 nm (ϵ 321 M^{-1} cm^{-1}) and 380 nm (ϵ 227 M^{-1} cm^{-1}).[23] Aquo(1,2-diaminoethane-

† No reprints available.

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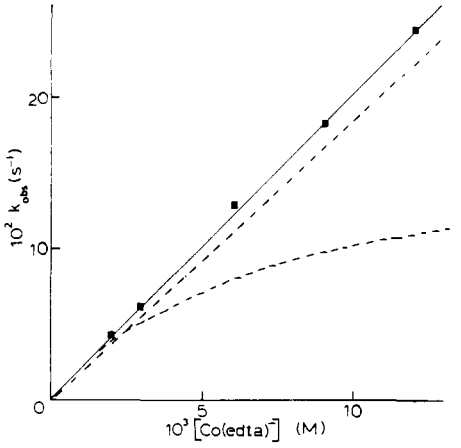

Figure 1. Dependence of first-order rate constants k_{obsd} on $[Co(edta)^-]$ for the oxidation of stellacyanin, SCu^I, with $Co(edta)^-$, pH 7.0 (phosphate): I = 0.5 M (phosphate). The broken lines are for data reported in ref 14 and 34, I = 0.5 M (phosphate) with (-·-) and without (- -) addition of $Cr(CN)_6^{3-}$ (2.5–5.0 × 10^{-4} M).

tetraacetato)chromium(III), [Cr(Hedta)OH_2], was prepared as described by Hamm[24] and gave a spectrum in 0.1 M $HClO_4$ with maxima at 546 nm (ε 148 M^{-1} cm^{-1}) and 386 nm (ε 107 M^{-1} cm^{-1}). The coordinated H_2O has an acid dissociation pK_a = 7.5.[24] Potassium (*trans*-1,2-diaminocyclohexanetetraacetato)manganate(III), K[Mn(cydta)]·2.5H_2O, was prepared by a literature method[25] and gave a spectrum at pH's in the range 2–6 consisting of a single broad band at 510 nm (ε 345 M^{-1} cm^{-1}) as before.[26] The complex has a coordinated H_2O with acid dissociation pK_a = 8.1.[26] Potassium hexacyanocobaltate(III), $K_3[Co(CN)_6]$, was prepared by published methods[27] and gave the literature spectrum peaks at 259 nm (ε 140 M^{-1} cm^{-1}) and 312 nm (ε 196 M^{-1} cm^{-1}).[28] A commercial sample (Research Organic/Inorganic Chemical Co.) of potassium hexacyanochromate(III), $K_3[Cr(CN)_6]$, was recrystallized from warm water by the addition of ethanol before use to give peaks at λ 307 nm (ε 60.5 M^{-1} cm^{-1}) and 377 nm (ε 859 M^{-1} cm^{-1}). Potassium hexacyanoferrate(III), $K_3[Fe(CN)_6]$, (B. D. H., Analar) was used without further purification, peak 420 nm (ε 1010 M^{-1} cm^{-1}).

Buffers. Solutions (10^{-2} M) of the following, with the addition of HCl (B. D. H., Analar) as required, were used: NaH_2PO_4/Na_2HPO_4 (B. D. H. Analar), pH 7.0–7.5; sodium cacodylate $Na[(CH_3)_2AsO_2]$ (B. D. H., Laboratory Reagent), pH 5.0–7.5; tris(hydroxymethyl)aminomethane (Trizma) here referred to as Tris (Sigma Chemicals), pH 7.0–9.0. Solutions of sodium bicarbonate (Fisons, Analar) and NaOH (Wilkinson-Vickers, Analar) were used for experiments at pH 10. The pH of solutions were checked by means of a Radiometer (PHM 62) instrument fitted with a combined electrode.

Kinetic Studies. The ionic strength (I) was adjusted to the appropriate value (usually 0.10 M) with NaCl, unless otherwise stated. The kinetics were monitored on a Dionex stopped-flow spectrophotometer, the output from which was either photographed from an oscilloscope or stored digitally with a Datalab DL901 transient recorder. The transient recorder was interfaced to a Commodore PET 2001-16K desk-top computer.[29] A simple program permitted display of ln $(A_\infty - A_t)$ against time plots and rate constants. All absorbance changes were consistent with a 1:1 stoichiometry, e.g., eq 1. The oxidant was in at least 30-fold excess of the

$$SCu^I + Co^{III} \rightarrow SCu^{II} + Co^{II} \quad (1)$$

protein except as stated. Reactions were monitored at the visible absorption maximum of SCu^{II} at 604 nm (lit. ε's, 3820 and 4080 M^{-1} cm^{-1})[30] except for the runs at the highest $[Co(edta)^-]$ for which it was necessary to shift to 620 nm because of the high background absorbance of the oxidant. The ln (A_∞/A_t) against time plots were linear for at least three half-lives. First-order rate constants were obtained from the slopes.

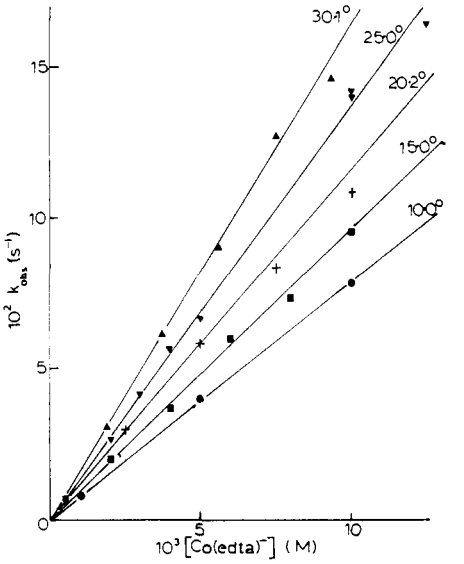

Figure 2. Dependence of k_{obsd} on oxidant concentration for the reaction of $Co(edta)^-$ with SCu^I at pH 7.0 (phosphate), I = 0.5 M (NaCl).

The complex $Ru(en)_3^{3+}$ is reported to be sensitive to air oxidation at pH greater than 2. We have found it stable at pH 3 for several hours, but at higher pH's the complex decomposes in minutes even under air-free conditions. Therefore all reactions of this oxidant were carried out by preparing the complex in 10^{-3} M HCl solutions, with a sufficient amount of buffer in the protein solution to give the required concentration on mixing. At pH 10, the decomposition products appear to attack the protein and result in a two-stage process involving formation and decay of the Cu^{II} absorbance. The precision of individual rate constants was less satisfactory under these conditions, and a full pH dependence was therefore not investigated.

Treatment of Data. Activation parameters $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were obtained from a simultaneous fit of k_{obsd} vs. [oxidant] data to the Eyring equation, using a standard (unweighted) nonlinear least-squares program modified for use on a Commodore PET.

Results

Oxidation of SCu^I with $Co(edta)^-$. No evidence was obtained for limiting kinetics at 25 °C, pH 7.0, I = 0.50 M (phosphate) (Table I).[31] Figure 1 illustrates the linear dependence of first-order rate constants k_{obsd} on oxidant concentration. A comparison is also made with previous data[14] (Figure 1). The simple rate law (eq 2) applies and defines second-order rate constants k (20.1 M^{-1} s^{-1} at 25 °C). The new data is in good agreement with the set of experiments previously carried out in the presence of redox inactive $Cr(CN)_6^{3-}$, Figure 1. Results included in Table II,[31] I = 0.50 M (NaCl), confirm that the presence of $Cr(CN)_6^{3-}$ (1.0–2.0) × 10^{-4} M), or alternatively redox inactive $Co(CN)_6^{3-}$ (2.5 × 10^{-4}), has no effect on rate constants. Similarly, at pH 6.9 and 7.0, I = 0.50 M (phos), the redox-inactive complex Cr(edta)$(H_2O)^-$ ((4.0–6.0) × 10^{-3} M) has no effect with $Co(edta)^-$ at a concentration 2.0 × 10^{-3} M (Table I). Had the earlier data held, Cr(edta)$(H_2O)^-$ might have been expected to produce (by blocking) a retardation effect. From the results in Table II at I = 0.50 M (NaCl) (Figure 2), k (25 °C) = 13.6 M^{-1} s^{-1} and activation parameters are $\Delta H^\ddagger$ = 5.6 ± 0.1 kcal mol^{-1} and $\Delta S^\ddagger$ = −34.0 ± 0.5 cal K^{-1} mol^{-1}. These parameters are in good agreement with values $\Delta H^\ddagger$ = 5.5 ± 0.2 kcal mol^{-1} $\Delta S^\ddagger$ = −34.0 ± 2.0 cal K^{-1} mol^{-1} reported by Holwerda and Clemmer[32] at I = 0.10 M (NaCl). We have been able to reproduce (our values 4–10% higher) rate constants reported under these latter conditions.

$$\text{rate} = k[SCu^I)][Co^{III}] \quad (2)$$

Oxidation of SCu^I with $Co(C_2O_4)_3^{3-}$. First-order rate constants k_{obsd} (Table III[31]) give a linear dependence on oxidant concen-

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Table VII. Summary of Rate Constants k and Activation Parameters for the Oxidation of Stellacyanin SCu^{I}

oxidant	k, M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}	conditions: pH	conditions: I, M	ref
$Co(C_2O_4)_3^{3-}$	856	4.5	−30	7.0	0.10 (NaCl)	this work[a]
$Co(edta)^-$	13.9	5.6	−34	7.0	0.50 (phos)	this work
	25.7	5.5	−34	7.2	0.10 (NaCl)	32
$Co(pdta)^-$	17.9	8.5	−24	7.0	0.50 (phos)	34
$Co(cydta)^-$	17.0	8.7	−24	7.0	0.50 (phos)	34
cis-(N)-Co(nta)(gly)$^-$	34.5	3.8	−39	7.0	0.50 (phos)	32
cis(N)-$Co(ida)_2^-$	3.6	2.8	−46	7.0	0.50 (phos)	32
$Ru(en)_3^{3+}$	3.4×10^4	5.4	−19	7.0	0.10 (NaCl)	this work
$Co(phen)_3^{3+}$	1.8×10^5	6.0	−13	7.0	0.10 (NaCl)	15
$Co(5,6\text{-}Me_2phen)_3^{3+}$	1.9×10^4	9.5	−7	7.0	0.10 (NaCl)	15
$Co(4,7\text{-}DPSphen)_3^{3-}$	2.3×10^6	5.9	−10	7.0	0.10 (NaCl)	15
$Ru(NH_3)_5py^{3+}$	1.9×10^5	6.7	−12	6.5	0.10 (Na_2SO_4)	36

[a] A value $k = 730$ M^{-1} s^{-1} (25 °C) has previously been reported, $I = 0.50$ M (phos), see: Holwerda, R. A. et al. *J. Am. Chem. Soc.* 1980, *102*, 1142.

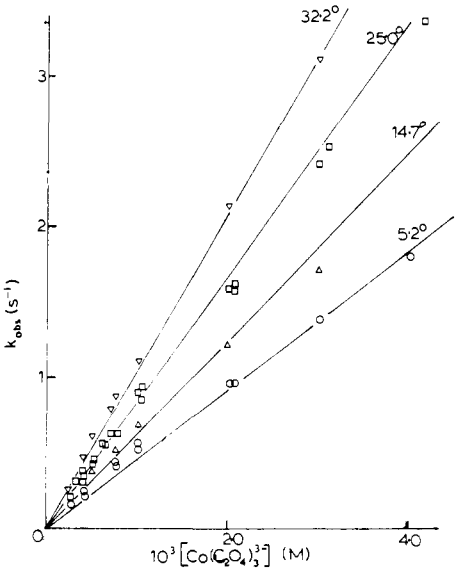

Figure 3. Dependence of k_{obsd} on oxidant concentration for the reaction of $Co(C_2O_4)_3^{3-}$ with SCu^{I} at pH 7.0 (phosphate), $I = 0.10$ M (NaCl).

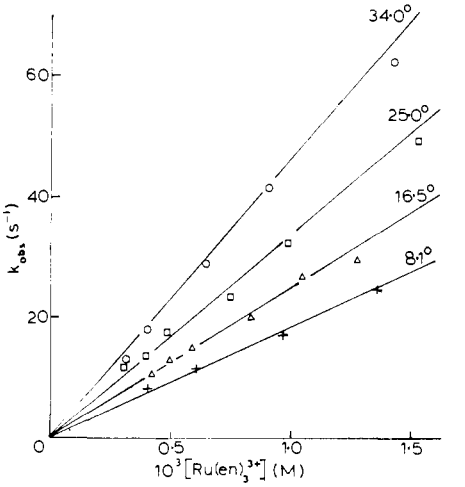

Figure 4. Dependence of k_{obsd} on oxidant concentration for the reaction of $Ru(en)_3^{3+}$ with SCu^{I} at pH 7.0 (phosphate), $I = 0.10$ M (NaCl).

tration at pH 7.0, $I = 0.10$ M (NaCl) (Figure 3). The simple rate law (eq 2) applies therefore with $k = 874$ M^{-1} s^{-1} at 25 °C. Activation parameters obtained are $\Delta H^{\ddagger} = 4.5 \pm 0.14$ kcal mol^{-1} and $\Delta S^{\ddagger} = -30.3 \pm 0.5$ cal K^{-1} mol^{-1}. Redox-inactive $Zr(C_2O_4)_4^{4-}$ (2.5×10^{-3} M) has no effect on rate constants, and there is no evidence therefore for significant association of 3- or 4- oxalato complexes with SCu^{I}. Second-order rate constants k are unaffected by pH 5.0–10.0 (four buffers) (Table IV).[31] The variation of k with ionic strength (0.005–0.035 M) was investigated (Table V).[31]

Oxidation of SCu^{I} with $Ru(en)_3^{3+}$. At pH 7.0 ($I = 0.10$ M (NaCl)), first-order rate constants k_{obsd} (Table VI)[31] give a linear dependence on oxidant concentration (Figure 4). The second-order rate constant at 25 °C is 3.4×10^4 M^{-1} s^{-1}. Activation parameters are $\Delta H^{\ddagger} = 5.4 \pm 0.2$ kcal mol^{-1} and $\Delta S^{\ddagger} = -19 \pm 0.7$ cal K^{-1} mol^{-1}. At pH 10 (carbonate) ($I = 0.10$ M), the second-order rate constant is $12.0 \pm 2.0 \times 10^4$ M^{-1} s^{-1} (six runs), which is some 3.5 higher than the value at pH 7.0.

Oxidation of SCu^{I} with $Mn(cydta)(H_2O)^-$ and $Fe(CN)_6^{3-}$. Both these reactions were too fast to follow at 25 °C by the stopped-flow technique with oxidant concentrations as low as $(1.0\text{–}2.0) \times 10^{-4}$ M and SCu^{I} at a value 1.0×10^{-5} M (pH 7.0 (phosphate), $I = 0.10$ M (NaCl)).

Discussion

Kinetic studies from this laboratory on 1:1 electron-transfer reactions of metalloproteins with inorganic complexes have been concerned with an understanding of precursor complex formation and electron transfer when limiting kinetics are observed. The reaction of SCu^{I} with $Co(edta)^-$ has been somewhat unusual in this context because unlike other systems reported as displaying limiting kinetics, it does not involve reactants with a high charge product. It accordingly attracted our attention as a system meriting further investigation.

We have not been able to reproduce the earlier results[14] indicating limiting kinetics and find that the reaction exhibits second-order kinetic behavior under all conditions investigated, which includes $I = 0.50$ M (phosphate) (Figure 1), $I = 0.50$ M (NaCl) (Figure 2), and $I = 0.10$ M (NaCl) (Table VII). An ionic strength of 0.50 M has only been adopted here to enable comparisons with previous studies. Studies at $I = 0.10$ M are generally preferred because protein complex association constants are larger and more likely to be detectable when ionic atmospheres around each reactant are less extensively developed.

The reaction of SCu^{II} with $Co(edta)^-$ as oxidant gives similar behavior therefore to studies with $Co(pdta)^-$ and $Co(cydta)^-$,[33,34] and discussion relating to different reactivity stemming from the presence of hydrophobic groups on the latter are no longer supported by experimental results.[35] At $I = 0.10$ M (NaCl), Holwerda and Clemmer[32] have found that limiting kinetics do not apply. In their discussion they reiterate previous results from ref 14 at $I = 0.50$ M (phosphate).

(33) The abbreviations pdta and cydta are for the ligands propylenediaminetetraacetate and *trans*-1,2-diaminocyclohexanetetraacetate.

(34) Yoneda, G. S.; Mitchel, G. L.; Blackmer, G. L.; Holwerda, R. A. *Bioinorg. Chem.* **1978**, *8*, 369.

(35) It is still possible that differences in activation parameters, e.g., somewhat higher $\Delta H^{\ddagger}$ values for the reactions of SCu^{I} with $Co(pdta)^-$ and $Co(cydta)^-$ as oxidants, are directly attributable to the hydrophobic nature of ligands on the oxidants, see Table VII. We thank Professor Holwerda for bringing this point to our attention.

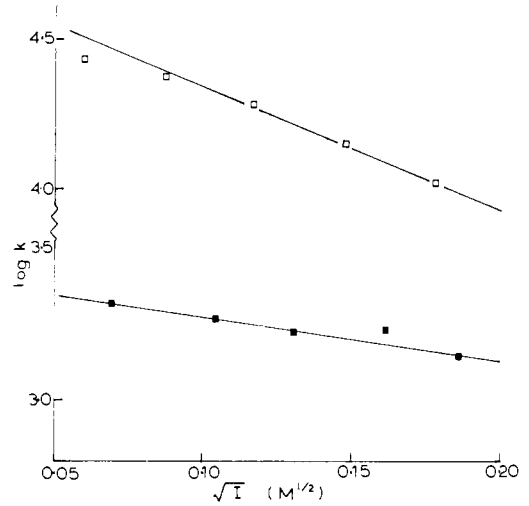

Figure 5. Comparison of the effect of ionic strength, graphed as $I^{1/2}$, on second-order rate constants for the oxidations of stellacyanin, SCu^I, with $Co(C_2O_4)_3^{3-}$ (■), and of plastocyanin PCu^I with $Co(phen)_3^{3+}$ (□) (from ref 3).

Rate constants[14] reported for the $Co(edta)^-$ oxidation in the presence of redox inactive $Cr(CN)_6^{3-}$ have also been checked. Results are now in agreement with those in the absence of $Cr(CN)_6^{3-}$, thus indicating no effect of $Cr(CN)_6^{3-}$. Likewise, redox-inactive $Co(CN)_6^{3-}$ and $Cr(edta)(H_2O)^-$ have no effect on rate constants with $Co(edta)^-$ as oxidant.

In order to help assess better the electrostatic component in reactions of stellacyanin with inorganic complexes, the further reactions of SCu^I with the 3- and 3+ oxidants $Co(C_2O_4)_3^{3-}$ and $Ru(en)_3^{3+}$ have been studied. Rate constants were double checked, and neither of these systems provides evidence for limiting kinetics. It is estimated that K for association prior to electron transfer is $<65\ M^{-1}$ in both instances. Moreover, redox-inactive $Zr(C_2O_4)_4^{4-}$ (2.5×10^{-3} M) has no effect as a potential blocking agent for $Co(C_2O_4)_3^{3-}$, and association of this 4- complex with the protein is likewise not influential.

The situation with stellacyanin is therefore that from eleven full studies reported herein and elsewhere,[15,34,36] and five other studies at 25 °C only,[32] with charges ranging from 3- to 3+, no cases of limiting kinetics are observed. Cytochrome *c* is the only other metalloprotein with an overall positive charge (8+ and 9+ for the two oxidation states of horse heart cytochrome *c*) to have been extensively studied with inorganic redox partners.[37] Interestingly, with the demonstration that the reaction of $Fe(CN)_6^{4-}$ with cytochrome *c*(III) gives simple kinetic behavior,[38] recently confirmed by Ohno and Cusanovish,[39] there are no examples here either for limiting kinetics. These results for stellacyanin and cytochrome *c* are a feature of considerable interest to us. It might appear that the net positive charge stemming largely from lysine amino acid residues is not able to induce strong associations, possibly because the lysines do not give sufficiently dense positive patches on the proteins. Negative patches have been identified on plastocyanin,[8] and also on 2Fe-2S ferredoxins,[40,41] where it has been suggested these define functional sites at which electron transfer takes place.

We also find of interest results for the oxidation of SCu^I and cytochrome *c*(II) with $Co(4,7\text{-DPS phen})_3^{3-}$.[15] This large complex with diffuse negative charge gives favorable association constants (K, M^{-1}), some of the strongest yet detected, in its reactions with the negatively charged proteins plastocyanin (4600),[4] azurin (2750),[5] and the HIPIP Fe/S protein (3420).[26] Some form of association of the aromatic rings with protein amino acid residues has been suggested. If this is indeed the explanation, it is a feature that is not observed with stellacyanin, SCu^I, or cytochrome *c*(II), neither of which exhibits limiting kinetics with this oxidant.

From a count of charges on individual amino acids at pH 7 (assuming no protons are shared), an approximate overall charge on SCu^I of 7+ is obtained. This compares with the estimate of ca. 7- for plastocyanin. Equations relating the variation of rate constant with I to charge of the reactants are difficult to apply in a rigorous fashion when large protein reactants are involved (see, e.g., ref 3). The reactions of SCu^I with $Co(C_2O_4)_3^{3-}$ and of plastocyanin PCu^I with $Co(phen)_3^{3+}$ are of interest in this context since identical charge products are involved. Certainly the slopes of plots of log k against $I^{1/2}$ (Figure 5) are in the direction expected for the charges involved. The observation that the slope is approximately twice as big for the smaller PCu^I (M_r 10 500) may be related to charge density on the two proteins. McArdle et al.[15] have reported data for the oxidation of SCu^I with $Co(phen)_3^{3+}$ that suggest that rate constants do not vary with ionic strength in the range 0.05–0.50 M at pH 7.0. Cummins and Gray[36] have also reported that rate constants for the oxidation of SCu^I with $Ru(NH_3)_5(py)^{3+}$ actually decrease (not increase) with increasing ionic strength in the range 0.05–0.92 M (pH 6.5). These observations suggest that ionic strength effects for stellacyanin have a minor influence, an observation that possibly relates to the presence of the carbohydrate,[6] particularly if the latter shields large sections of the protein surface.

The influence of pH on the reactions of PCu^I[3,4] and ACu^I[5] have been studied previously. These investigations illustrate that protonation occurring at or near to a functional site on the proteins for electron transfer are influential on rate constants. If with another reactant a different effect of pH is observed, then the implication is that a different functional site on the protein is used. The $Co(C_2O_4)_3^{3-}$ oxidation of SCu^I was studied over the range pH 5–10 without any apparent influence on rate constants (four buffers). We find this lack of response over such a wide range quite remarkable in view of the estimated charge of 7+, the fact that stellacyanin has an isoelectric point of 9.86,[12] and also the fact that, like azurin, stellacyanin has two uncoordinated histidine residues.[6] From a small (<2-fold) effect of pH on rate constants for the reaction of SCu^I with $Co(phen)_3^{3+}$, McArdle et al.[15] have obtained a pK_a of 7.7 ± 0.2. A histidine pK_a of 7.35 has moreover been reported from NMR studies.[11] With X-ray structural information, this could help define the location of the functional site used by $Co(phen)_3^{3+}$ but not $Co(C_2O_4)_3^{3-}$. Although some difficulties were experienced in obtaining sufficiently precise data for the $Ru(en)_3^{3+}$ oxidation at pH's >7, a 3.5-fold increase in rate constants was indicated in going to pH 10. The pH effects observed suggest that $Co(phen)_3^{3+}$ and $Ru(en)_3^{3+}$ may use the same functional site on SCu^I for electron transfer. However, owing to the instability of the $Ru(en)_3^{3+}$ at high pH and medium changes, this suggestion must remain tentative, and further confirmation is required.

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Registry No. Cu, 7440-50-8; $Co(C_2O_4)_3^{3-}$, 15053-34-6; $Co(edta)^-$, 15136-66-0; $Ru(en)_3^{3+}$, 21393-87-3.

Supplementary Material Available: A listing of rate constants (Tables I–VI) (8 pages). Ordering information is given on any current masthead page.

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