Kinetic Studies on 1 :1 Electron-transfer Reactions involving Blue Copper Proteins. Part 5.† Reactions of Parsley Plastocyanin and *Pseudomonas aeruginosa* Azurin with the Negatively Charged Oxidants $[(NC)_5FeCNCo(CN)_5]^{5-}$ and $[Fe(CN)_6]^{3-}$ ‡

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The oxidations of plastocyanin, PCu(I), and azurin, ACu(I), with $[(NC)_5FeCNCo(CN)_5]^{5-}$ (0.46 V) and $[Fe(CN)_6]^{3-}$ (0.41 V) have been studied by the stopped-flow method at pH 7.0, I = 0.10 M (NaCl) and 0.22 M (phosphate), with the oxidant in large (>10-fold) excess (concentrations up to 4×10^{-4} M). All four reactions conform to a rate law first order in oxidant and reductant concentrations, with no evidence for rate constants tending to a limiting value as a function of increasing concentration of oxidant. In the case of the ACu(I) + $[Fe(CN)_6]^{3-}$ reaction ($K < 100$ M^{-1}) this is contrary to an earlier report. Enthalpies of activation, $\Delta H^{\ddagger}$ (kcal mol^{-1}), are however negative (−2.9 to −6.0) and support a two-stage process involving association (K) prior to electron transfer (k_{et}) and/or changes involving the degree of solvation of the protein. Replacement of NaCl (0.094 M) by LiCl and KCl produces no effect on rate constants for the PCu(I) + $[Fe(CN)_6]^{3-}$ reaction. Re-examination of previous data for the $[Fe(CN)_6]^{4-}$ + PCu(II) reaction suggests that here also limiting kinetics are probably not effective. Other metalloprotein–complex reactions which display limiting kinetics are considered and rationalized in terms of electrostatics as the dominant factor. For the two reactions of $[(NC)_5FeCNCo(CN)_5]^{5-}$, similar effects of pH are observed to those previously reported with $[Fe(CN)_6]^{3-}$ as oxidant. Thus on decreasing the pH from 7 to <5 the redox reactivity of PCu(I) decrease sharply whereas rate constants for the ACu(I) reaction increase.

Plastocyanin (M 10 500) and azurin (M 13 500) are single (type 1) copper proteins having specific functions in electron-transport processes.[1-4] The Cu is in a distorted tetrahedral co-ordination site,[5] which is hydrophobic in nature and not accessible to solvent. The redox cycle involves the copper(I) (colourless) and copper(II) (blue) states.

A number of studies on electron-transfer reactions of metalloproteins (P) with inorganic complexes (C) have now been reported as displaying limiting kinetics [equation (1)],

$$k_{obs.} = \frac{Kk_{et}(C)}{1 + K(C)} \quad (1)$$

where $k_{obs.}$ is the first-order rate constant obtained with reactant in large excess.[6-10] The simplest interpretation is in terms of equations (2) and (3) where the inclusion of (2)

$$P + C \overset{K}{\rightleftharpoons} P,C \quad (2)$$

$$P,C \xrightarrow{k_{et}} \text{products} \quad (3)$$

implicates what might be termed a long-duration collision. This is of interest because it represents a possible means by which low-probability (long-distance?) electron transfer might be achieved. At present there is no clear rationale as to when K might be large and when limiting kinetics might therefore be expected. Thus the high value of K(610 M^{-1}) previously reported from temperature-jump studies for the oxidation of azurin, ACu(I), with $[Fe(CN)_6]^{3-}$,[11] and the value K = 110 M^{-1} for the $[Fe(CN)_6]^{4-}$ reduction of plastocyanin,[6] PCu(II), are unusual in that both pairs of reactants are negatively charged, and favourable association due to charge alone would not be expected unless positively charged regions on the proteins were involved.

With these results in mind it was decided to investigate the oxidations of PCu(I) (estimated charge 10−) and ACu(I) with the more highly charged $[(NC)_5FeCNCo(CN)_5]^{5-}$ oxidant. Limiting kinetics were not detected in either case and the reactions with $[Fe(CN)_6]^{3-}$ as oxidant were therefore re-examined. A self-consistent picture has emerged which requires some modification of the earlier position.

Experimental

Proteins.—Plastocyanin was isolated from the chloroplast of parsley leaves and purified to absorbance peak ratio for PCu(II) of A_{278}/A_{597} = 1.7 as described previously.[12] Azurin from *Pseudomonas aeruginosa*, peak ratio for ACu(II) of A_{280}/A_{625} = 1.67, was obtained from Microbiological Products, Porton. Absorption coefficients were assumed to be 4 500 M^{-1} cm^{-1} for PCu(II) at the 597 nm peak,[6] and 4 800 M^{-1} cm^{-1} for ACu(II) at the 625 nm peak.[13]

Complexes.—Potassium hexacyanoferrate(III), $K_3[Fe(CN)_6]$ (B.D.H. AnalaR), peaks at 300 (1 600) and 420 nm (1 010 M^{-1} cm^{-1}), and potassium hexacyanoferrate(II), $K_4[Fe(CN)_6]\cdot 3H_2O$ (Hopkin and Williams, AnalaR), peak at 300 nm (330 M^{-1} cm^{-1}), were used without further purification. Solutions of $[Fe(CN)_6]^{4-}$ were used soon after preparation, *i.e.* within 30 min, or alternatively were stored under N_2.

The required μ-cyano-[pentacyanoferrate(III)][pentacyanocobaltate(III)](5−) complex was isolated as the barium salt, $Ba_5[(CN)_5FeCNCo(CN)_5]_2\cdot 6H_2O$, using the following procedure. The 6− complex $Ba_3[(CN)_5FeCNCo(CN)_5]\cdot 16H_2O$ was first prepared as described by Haim and Wilmarth.[14] To oxidise to the 5− complex a quantity of the 6− complex (1 g) was first dissolved in water (20 ml). Solid I_2 (10—20% excess) was added, and the solution heated to 45 °C with stirring. After *ca.* 10 min at 45 °C oxidation was complete, the solution cooled, and any excess of (solid) I_2 filtered off. Ethanol (40 ml) was added. The upper layer (water–ethanol) and any yellow precipitate were removed by decantation and water (10 ml) added to the oily layer. A further 20 ml of

† Part 4 is ref. 18.
‡ *Non-S.I. units employed:* M = mol dm^{-3}, cal = 4.184 J.

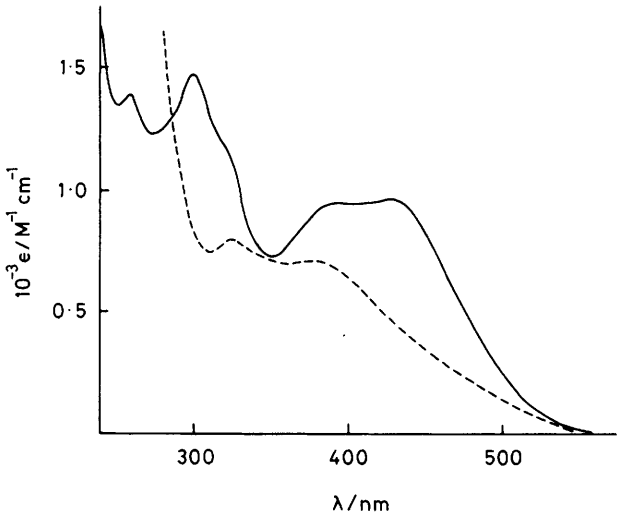

Figure 1. U.v. visible spectra of $[(NC)_5FeCNCo(CN)_5]^{5-}$ (——) and $[(NC)_5FeCNCo(CN)_5]^{6-}$ (— — —) at pH 7

ethanol was added and the aqueous layer (containing I_2 and I^-) again discarded (centrifugation is helpful in separating the layers). The oily layer was evaporated to dryness on a rotary evaporator, or by leaving in a desiccator containing P_2O_5. The spectrum of the 5− product was in agreement with the literature [14] (peak at 300 nm, ε 1 480 M^{-1} cm^{-1}), assuming the solid to be the hexahydrate. Quantitative conversion into the 6− complex was possible by reduction with sulphite. Relevant spectra are shown in Figure 1. No change in the 5− spectrum was observed with pH in the range 3—7.

Buffers.—Two buffers were used. The pH of 10^{-2} M sodium cacodylate, $Na[(CH_3)_2AsO_2]$ (B.D.H. Lab. Reagent), was adjusted in the range 5.0—7.5 as required by addition of HCl. Sodium phosphate (Na_2HPO_4 + NaH_2PO_4) at 10^{-2} M was also adjusted to buffer at pH 7.0. Ionic strengths were adjusted with NaCl to I = 0.10 M. For runs with 10^{-1} M phosphate at pH 7 the ionic strength was 0.22 M without any addition of NaCl. For a series of runs in which the pH was systematically varied in some cases using the pH-jump method (*i.e.* two reactant solutions at different pH values), *i.e.* the protein was held at a constant pH, the pH was varied at the time of mixing with solutions of complex at different pH values.

Reduction Potentials.—At 25 °C, I = 0.10 M (NaCl), and pH 7.2 [tris(hydroxymethyl)methylamine–HCl] the $[Fe(CN)_6]^{3-,4-}$ couple has a reduction potential of 0.41 V.[15] The potential of the $[(NC)_5FeCNCo(CN)_5]^{5-,6-}$ couple was determined by cyclic voltammetry (scan rate 10—100 mV s^{-1}) under identical conditions using a platinum disc working electrode, a platinum wire secondary electrode, and standard calomel electrode ($E^\ominus$ = 0.244 V) as reference. The $E^\ominus$ obtained was 0.46 V (all values quoted *vs.* normal hydrogen electrode, n.h.e.). Reduction potentials for the proteins PCu(II)/PCu(I) (0.37 V at pH 7) and ACu(II)/ACu(I) (0.35 V at pH 6) show some variation with pH.[3]

Stoicheiometry.—The $[(NC)_5FeCNCo(CN)_5]^{5-,6-}$ couple involving the formal oxidation state change in equation (4) is the only change possible for the reactants specified. Much stronger reductants are generally required to generate Co^{II},

$$Fe^{III},Co^{III} + e^- \rightleftharpoons Fe^{II},Co^{III} \quad (4)$$

Table 1. The temperature dependence of first-order rate constants for the oxidation of parsley plastocyanin, PCu(I) (5×10^{-6} M), with $[(NC)_5FeCNCo(CN)_5]^{5-}$ at pH 7.0 (sodium cacodylate–HCl) and I 0.10 M (NaCl)

$10^4[(NC)_5FeCNCo(CN)_5^{5-}]$/M	$k_{obs.}$/s^{-1}			
	10.0	17.9	25.0	30.8 °C
0.76	3.44	3.09		
0.79				2.33
1.50	6.4	5.6	5.15	
1.55				4.6
2.24	9.3	8.9	7.6	
2.29				7.1
3.0	12.5	11.5	10.2	9.5
3.7	15.1	14.2	12.6	
3.8			12.6	11.6
4.1		15.9		
4.5			15.1	14.3

and studies carried out were consistent with 1 : 1 stoicheiometries (5) and (6). Reactions involving $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ likewise involve one-electron changes.

$$PCu(I) + Fe^{III},Co^{III} \longrightarrow PCu(II) + Fe^{II},Co^{III} \quad (5)$$

$$ACu(I) + Fe^{III},Co^{III} \longrightarrow ACu(II) + Fe^{II},Co^{III} \quad (6)$$

Kinetics.—All reactions were studied using a Dionex D-110 stopped-flow spectrophotometer with the inorganic complex in >10-fold excess over the protein. Reactions were monitored at the PCu(II) and ACu(II) peak positions at 597 and 625 nm respectively. The output was either photographed from an oscilloscope or stored digitally using a Datalab DL901 transient recorder. The transient recorder was interfaced to a Commodore PET 2001-16K desk-top computer. A simple program permitted display of $\ln(A - A_\infty)$ against time plots and rate constants. An unweighted non-linear least-squares program was used to compute activation parameters.

Results

First-order rate constants, $k_{obs.}$ (Table 1), for the reaction of PCu(I) with $[(NC)_5FeCNCo(CN)_5]^{5-}$ give a linear dependence on the concentration of the 5− reactant up to 4×10^{-4} M, Figure 2. At 25 °C, pH 7.0, the second-order rate constant is $k = 3.4 \times 10^4$ M^{-1} s^{-1}. From the temperature dependence of k activation parameters $\Delta H^\ddagger = -2.9 \pm 0.1$ kcal mol^{-1} and $\Delta S^\ddagger = -47.5 \pm 0.4$ cal K^{-1} mol^{-1} were obtained. The dependence of k on pH (Table 2) is of the kind observed in earlier studies of PCu(I) with $[Co(phen)_3]^{3+}$ (phen = 1,10-phenanthroline) and $[Fe(CN)_6]^{3-}$, Figure 3. The reaction sequence (7)—(9) (oxid = oxidant) gives a dependence of the kind (10)

$$PCu(I) + H^+ \overset{K_P}{\rightleftharpoons} H^+PCu(I) \quad (7)$$

$$PCu(I) + oxid \xrightarrow{k_o} products \quad (8)$$

$$H^+PCu(I) + oxid \xrightarrow{k_H} products \quad (9)$$

$$k_{obs.} = k_H + \frac{(k_o - k_H)}{1 + K_P[H^+]} \quad (10)$$

with reaction of the protonated form $H^+PCu(I)$ at a low level, $k_H = (0.49 \pm 0.11) \times 10^4$ M^{-1} s^{-1}. The acid dissociation constant for HPCu(I) (reciprocal of K_P) gives a pK_a of 5.97 ± 0.11.

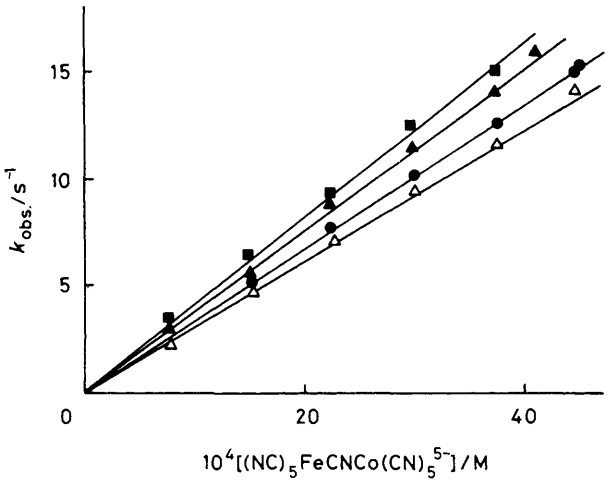

Figure 2. The dependence of first-order rate constants for the oxidation of PCu(I) with $[(NC)_5FeCNCo(CN)_5]^{5-}$ on temperature and concentration of oxidant at pH 7.0 (sodium cacodylate–HCl) and $I = 0.10$ M (NaCl). Temperature = 10.0 (■), 17.9 (▲), 25.0 (●), and 30.8 °C (△)

Table 2. The pH dependence of rate constants at 25 °C for the oxidation of parsley plastocyanin, PCu(I) (*ca.* 3×10^{-6} M), with $[(NC)_5FeCNCo(CN)_5]^{5-}$ (2×10^{-4} M) using cacodylate–HCl buffer, $I = 0.10$ M (NaCl)

pH	7.0	6.63	6.18	5.77	5.40	5.24
$10^{-4}k/M^{-1}\ s^{-1}$	3.38	2.71	2.39	1.61	1.20	0.97
pH	5.10	4.94	4.80			
$10^{-4}k/M^{-1}\ s^{-1}$	0.94	0.65	0.62			

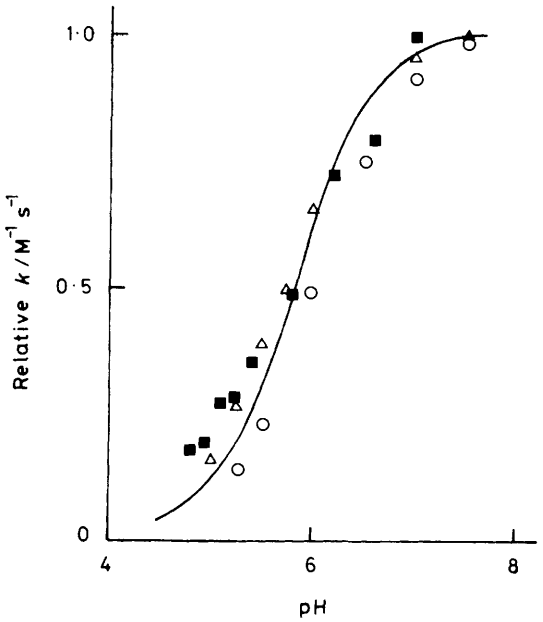

Figure 3. The variation of relative rate constants (rate constant at pH 7.0 taken as 1.0) with pH for the $[(NC)_5FeCNCo(CN)_5]^{5-}$ oxidation of PCu(I) (■) compared to similar data for the reactions with $[Fe(CN)_6]^{3-}$ (△) and $[Co(phen)_3]^{3+}$ (○) as oxidants; $I = 0.10$ M (NaCl)

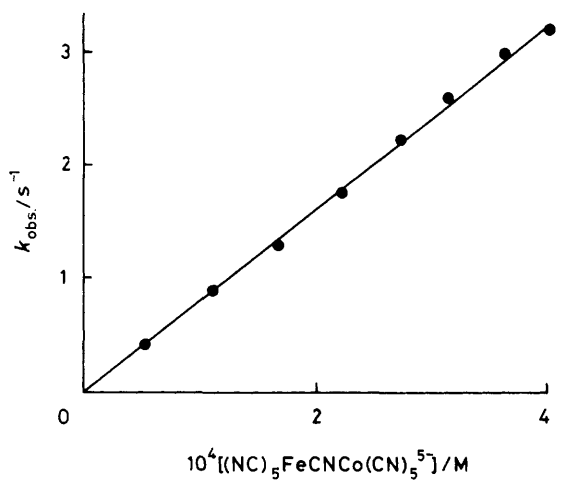

Figure 4. The dependence of first-order rate constants (25 °C) for the oxidation of ACu(I) with $[(NC)_5FeCNCo(CN)_5]^{5-}$ on the concentration of oxidant at pH 7.0 (0.10 M phosphate) and $I = 0.22$ M (phosphate)

Table 3. First-order rate constants (25 °C) for the oxidation of *P. aeruginosa* azurin, ACu(I) (*ca.* 3×10^{-6} M), with $[(NC)_5FeCNCo(CN)_5]^{5-}$ at pH = 7.0 (0.1 M phosphate) and $I = 0.22$ M

$10^4[(NC)_5FeCNCo(CN)_5^{5-}]/M$	$k_{obs.}/s^{-1}$
0.54	0.39
1.11	0.87
1.64	1.28
2.21	1.75
2.72	2.24
3.13	2.60
3.6	2.99
4.0	3.19

Rate constants, $k_{obs.}$, for the oxidation of ACu(I) with $[(NC)_5FeCNCo(CN)_5]^{5-}$ also show a linear dependence on the concentration of 5— complex, Figure 4. These rate constants (Table 3) were determined at 25 °C, $I = 0.22$ M (phosphate), the latter condition being that used by Goldberg and Pecht [11] for their ACu(I) + $[Fe(CN)_6]^{3-}$ study. From the K and k_{et} parameters presented in the latter the curve in Figure 5 has been calculated. We were unable to reproduce these data by the stopped-flow method at $I = 0.33$ or 0.10 M, all studies at pH 7.0 (Table 4). At $I = 0.22$ M runs with protein from different work-ups were in excellent agreement. In view of the extensive data obtained and the simpler kinetic treatment required for the stopped-flow study we conclude that limiting kinetics do not apply in the range studied.

At 25 °C, $I = 0.10$ M (NaCl), and pH 5.8, the second-order rate constant for the ACu(I) + $[(NC)_5FeCNCo(CN)_5]^{5-}$ reaction is $k = 1.24 \times 10^4\ M^{-1}\ s^{-1}$. The activation parameters corresponding to k obtained from the temperature dependence (Table 5) are $\Delta H^{\ddagger} = -6.0 \pm 0.2$ kcal mol^{-1} and $\Delta S^{\ddagger} = -59.8 \pm 0.6$ cal K^{-1} mol^{-1}. A pH of 5.8 was selected because, from a study of the pH profile of the ACu(I) + $[Fe(CN)_6]^{3-}$ reaction,[8] a single protonated ACu(I) species is effective under this condition while at pH 7 significant amounts of protonated and unprotonated forms are present. However, on further investigation of the 5— complex oxidation, Table 6, it was found that $k_{obs.}$ does not in fact level out at pH < 5.8. We are uncertain as to the origin of this behaviour, which again was found to be reproducible.

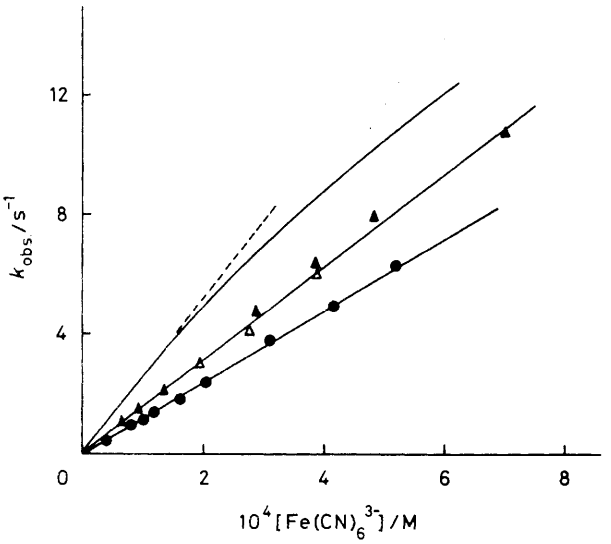

Figure 5. The dependence of first-order rate constants (25 °C) for the oxidation of ACu(I) with $[Fe(CN)_6]^{3-}$ at pH 7.0. The upper curve as predicted from previous data of Goldberg and Pecht [11] is compared with results obtained by stopped-flow at pH 7.0 (sodium cacodylate–HCl) and I = 0.10 M (NaCl) (●), and pH 7.0 (0.10 M phosphate) and I = 0.22 M (phosphate) (▲, first series of runs; △, second series of runs)

Table 4. First-order rate constants (25 °C) for the oxidation of *P. aeruginosa* azurin, ACu(I) (*ca.* 1 × 10^{-5} M), with $[Fe(CN)_6]^{3-}$

Conditions	$10^4[Fe(CN)_6^{3-}]$/M	$k_{obs.}/s^{-1}$
(*a*) pH 7.0 (phosphate), I = 0.22 M	0.70	1.06
	1.40	2.13
	1.96	3.03
	2.80	4.07
	2.92	4.74
	3.88	5.93
	3.89	6.32
	4.86	7.88
	7.00	10.6
(*b*) pH 7.0 (cacodylate), I = 0.10 M (NaCl)	0.41	0.46
	0.83	0.95
	1.03	1.18
	1.24	1.40
	1.65	1.86
	2.06	2.37
	3.13	3.76
	4.18	4.91
	5.22	6.25
(*c*) pH 5.5 (cacodylate), I = 0.10 M (NaCl)	0.42	0.65
	0.84	1.31
	1.05	1.50
	1.26	1.94
	1.68	2.63
	2.10	3.25

It has been observed that metal ions such as K^+ are able to catalyse [16] the electron-exchange reaction of $[Fe(CN)_6]^{3-,4-}$. The effect of replacing NaCl by first LiCl and then KCl in adjusting the ionic strength to 0.10 M was therefore investigated briefly. For the conditions chosen, [NaCl] = 0.094 M, *etc.*, no effect on rate constants was observed.

Table 5. The temperature dependence of first-order rate constants for the oxidation of *P. aeruginosa* azurin, ACu(I) (*ca.* 4 × 10^{-6} M), with $[(NC)_5FeCNCo(CN)_5]^{5-}$ at pH 5.8 (cacodylate–HCl) and I = 0.10 M (NaCl)

	$k_{obs.}/s^{-1}$			
$10^4[(NC)_5FeCNCo(CN)_5^{5-}]/M$	10.3	18.3	25.0	30.5 °C
0.91	1.72	1.28	1.08	0.87
1.03			1.12	
1.81	3.7	2.65	2.20	1.58
2.09			2.56	
2.71		4.0	3.3	2.63
3.0			3.8	
3.6	7.1	5.6	4.7	3.4
3.9			5.3	
4.4	8.8	7.1	6.1	4.5
5.4	10.8			

Table 6. The pH dependence of rate constants at 25 °C for the oxidation of *P. aeruginosa* azurin, ACu(I) (*ca.* 5 × 10^{-6} M), with $[(NC)_5FeCNCo(CN)_5]^{5-}$ using tris(hydroxymethyl)methylamine–HCl (pH 7) and cacodylate–HCl buffers, I = 0.10 M (NaCl)

pH	8.97	8.68	8.60	7.28	6.87	6.23
$10^{-4}k/M^{-1}\ s^{-1}$	0.18	0.19	0.22	0.55	0.72	1.00
pH	6.00	5.80	5.70	5.70	5.30	5.19
$10^{-4}k/M^{-1}\ s^{-1}$	1.10	1.28	1.40	1.45	1.59	1.67
pH	4.80	4.80	4.76			
$10^{-4}k/M^{-1}\ s^{-1}$	2.77	2.86	2.66			

Finally a check was carried out on the previously studied $[Fe(CN)_6]^{4-}$ + PCu(II) reaction.[6] This reaction is thermodynamically unfavourable and only proceeds in the desired direction by using a large excess of $[Fe(CN)_6]^{4-}$. In a recent reinvestigation of the $[Fe(CN)_6]^{4-}$ reduction of cytochrome c(III) we have demonstrated that anomalous limiting kinetics can appear if the reaction does not proceed to >90% completion and a rigorous equilibrium kinetic treatment is not applied.[15] In fact some of the conditions used for the $[Fe(CN)_6]^{4-}$ + PCu(II) reaction appear to be similarly in error. Unfortunately, in repeat studies, we have not been able to apply the rigorous kinetic treatment required to correct these rate constants due to some autoreduction of PCu(II) and difficulties in ascertaining PCu(II) and PCu(I) concentrations with sufficient accuracy. We do however feel that there are good reasons for doubting the applicability of limiting kinetics, and that the previous report [6] of such behaviour probably should be disregarded.

Discussion

From the data presented for both the $[(CN)_5Fe(CN)Co(CN)_5]^{5-}$ and $[Fe(CN)_6]^{3-}$ oxidations of PCu(I) and ACu(I) we conclude that limiting kinetics do not apply with $K < 200\ M^{-1}$ in all cases, and $<100\ M^{-1}$ for the $[Fe(CN)_6]^{3-}$ oxidation of ACu(I). The curved plot in Figure 5 calculated from Goldberg and Pecht's data [11] for the ACu(I) + $[Fe(CN)_6]^{3-}$ reaction gives 25% deviation from linear behaviour at 7 × 10^{-4} M $[Fe(CN)_6]^{3-}$ whereas our results deviate by at the most 2%. As indicated the same conclusion probably applies also to the $[Fe(CN)_6]^{4-}$ + PCu(II) study. Therefore the apparent anomaly that large and measurable K values are observed for reactants of the same overall charge type is removed. However we note that the PCu(I) and ACu(I) reactions all give negative $\Delta H^{\ddagger}$ values (Table 7). Since second-

Table 7. Summary of rate constants (25 °C) and activation parameters for the $[(NC)_5FeCNCo(CN)_5]^{5-}$ and $[Fe(CN)_6]^{3-}$ oxidations of parsley plastocyanin, PCu(I), and *P. aeruginosa* azurin, ACu(I), at pH 7.0 (cacodylate–HCl) and I = 0.10 M (NaCl)

Reaction	$10^{-4}k$/ M^{-1} s^{-1}	$\Delta H^{\ddagger}$/ kcal mol^{-1}	$\Delta S^{\ddagger}$/ cal K^{-1} mol^{-1}
PCu(I) + $[(NC)_5FeCNCo(CN)_5]^{5-}$	3.4	−2.9	−48
PCu(I) + $[Fe(CN)_6]^{3-}$ [a]	9.4	−3.3	−47
ACu(I) + $[(NC)_5FeCNCo(CN)_5]^{5-}$ (pH 5.8)	1.24	−6.0	−60
(pH 7.0) [b]	0.81		
ACu(I) + $[Fe(CN)_6]^{3-}$ (pH 7.0) [b]	2.7	−4.1 [c]	−52

[a] I = 0.108 M (NaCl). [b] I = 0.22 M (phosphate). [c] As reported in ref. 11 at pH 7.0.

order rate constants k are likely to remain composite and equal to Kk_{et} as defined in equations (2) and (3) (with K at a low level), it is possible for a negative ΔH (for K) to override a positive $\Delta H^{\ddagger}_{et}$ (for k_{et}).

Negative ΔH and ΔS parameters for K are perfectly reasonable in terms of increased ordering of solvent attendant on the association (K) of two negatively charged redox partners. Although there are no examples of negatively charged inorganic complexes giving measurable association, the converse situation of two oppositely charged complexes interacting and giving positive ΔH (small) and ΔS terms is now fairly well documented.[17] Similar values have also been reported for the association of negatively charged proteins with positively charged complexes.[6,9]

In addition to the conclusions presented here we have previously demonstrated that limiting kinetics do not hold for the $[Fe(CN)_6]^{4-}$ reduction of cytochrome c(III),[15] and for the $[Co(edta)]^{-}$ (edta = ethylenediaminetetra-acetate) oxidation of stellacyanin, SCu(I).[18] Other examples of limiting kinetics which remain involve the reactions of negatively charged [2Fe–2S] and 2[4Fe–4S] ferredoxins with 3+, 4+, and 5+ complexes.[9,10] The reaction of PCu(I) with $[Co(phen)_3]^{3+}$ is a milder effect which is substantiated by competitive-inhibition studies (involving association) with a range of 3+, 4+, and 5+ redox-inactive complexes.[19] In all these cases the reactants of higher charge interact more strongly with the protein and electrostatic forces appear to be dominant. There remains one exception which cannot be explained in a similar fashion and remains anomalous. Thus the reactions of the cobalt(III) complex $[CoL_3]^{3-}$, where L^{2-} = 4,7-bis(sulphonatophenyl)-1,10-phenanthroline, with PCu(I),[7] ACu(I),[8] and the high-potential Fe/S protein [20] (all of which carry negative charges) give well established limiting kinetics. The behaviour of this complex may be unusual due to the diffuse charge and high aromaticity. One suggestion [3] which has been made is that the rate-determining step may not involve electron transfer. With this one exception it is now possible to rationalise the occurrence of limiting kinetics in reactions of proteins with complexes in terms of favourable overall charge interactions.

It should also be mentioned here that equations (2) and (3) do not provide a unique interpretation of the rate equation (1) as has already been indicated.[3,5] An alternative which has been considered is the ' dead-end ' mechanism, where association of C occurs at another site on the protein to give non-reactive P,C. Some sort of conformational change is envisaged to account for the switch-off in protein reactivity. Although such a mechanism is applicable in the context of protein protonation, no evidence [5,9] has yet been obtained for substitution-inert inorganic complexes giving such an effect. The stance which we have adopted is that discussion should proceed in terms of equations (2) and (3) until evidence for the alternative is obtained. It is however worth making the point that if this alternative mechanism were applicable the same numerical values of K for association of the reactants (and discussion in terms of electrostatics) would apply.

The pH dependences reported for the reactions of PCu(I) and ACu(I) with $[(NC)_5FeCNCo(CN)_5]^{5-}$ are of the same kind as observed previously with $[Fe(CN)_6]^{3-}$ as oxidant. Thus PCu(I) becomes inactive on decreasing the pH from 7 to 5, due to protonation which results in dissociation of His87 from the Cu^{1}. With ACu(I), protonation at the unco-ordinated His35 is believed to increase reactivity with the 5- and 3-oxidants, an effect which implicates a locality close to His35 as the site on ACu(I) at which electron transfer occurs. The additional effect at pH $<$5.8 for the 5− oxidant is not readily explained, but is not crucial to the main theme of this paper.

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