Kinetic Studies on 1 :1 Electron-transfer Reactions involving Blue Copper Proteins. Part 7.[1] Effects of pH and Redox-inactive $[Pt(NH_3)_6]^{4+}$ on Reactions of Parsley Plastocyanin with Different Inorganic Redox Partners †

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The effect of pH on the reduction of parsley plastocyanin, PCu(II), by a positively charged complex, $[Ru(NH_3)_5(py)]^{2+}$ (py = pyridine), has been studied for the first time. Protonation at the negative patch of the protein, which includes residues 42—45, is proposed as an explanation of the 60% (maximum) decrease in rate constant, pK_a 5.0. Inactivation of PCu(I) by H^+ is apparent with the complexes $[Co(bipy)_2(O_2CMe)_2]^+$ (bipy = 2,2'-bipyridine) and $[Co(dipic)_2]^-$[dipic = dipicolinate (pyridine-2,6-dicarboxylate)] as oxidants, as noted previously for $[Co(phen)_3]^{3+}$(phen = 1,10-phenanthroline), $[Co(bipy)_3]^{3+}$, and $[Fe(CN)_6]^{3-}$ (pK_a 5.7—6.1) and assigned as an H^+-induced change effective at the copper active site. The strong competitive inhibition exhibited by redox-inactive $[Pt(NH_3)_6]^{4+}$ on the PCu(I) + $[Co(phen)_3]^{3+}$ reaction is tested further at pH 5.8 using other oxidants, $[Co(bipy)_3]^{3+}$, $[Ru(NH_3)_5(py)]^{3+}$, $[Co(bipy)_2(O_2CMe)_2]^+$, and $[Co(dipic)_2]^-$. Partial inhibition of all three 3+ complexes is observed (53% maximum effect at high inhibitor concentrations), and of the 1+ complex (25% decrease) consistent with these complexes interacting at the negative patch on the protein incorporating Tyr 83. The small *ca.* 8% acceleration observed with $[Co(dipic)_2]^-$ does not lead to a clear-cut interpretation. The reduction of PCu(II) by $[Ru(NH_3)_5(py)]^{2+}$ is inhibited by $[Pt(NH_3)_6]^{4+}$ (40% decrease) clearly designating the Tyr 83 locality as the dominant site for reaction, and not as previously supposed the His 87 site.

Factors affecting electron-transfer reactions of the blue copper proteins are under consideration in this series of papers. In addition to the active site chemistry,[2] identification of the site(s) on the protein surface used for electron transfer (and hence the distance over which electrons are transferred) is of interest. Competitive inhibition,[1] effects of pH,[3] and chemical modification [4] of the protein are all relevant in this context. Protonation of an amino-acid residue, insofar as it affects the reactivity at a nearby site, is the most ready (and natural) modification which can be achieved. Such affects are considered in this paper. The previous search for redox-inactive inorganic complexes which exhibit competitive inhibition (*i.e.* blocking) of the $[Co(phen)_3]^{3+}$ oxidation of parsley plastocyanin, PCu(I),[1] has indicated that $[Pt(NH_3)_6]^{4+}$ associates strongly with the protein, and is the most effective inhibitor so far studied. An assessment of this inhibitor with different oxidants, and one reductant, is now considered.

Preferential association of hexa-ammine complexes close to Tyr 83, detected by the n.m.r. line-broadening effect of paramagnetic $[Cr(NH_3)_6]^{3+}$,[5,6] and other studies already described,[1,7] has led to the designation of a broad region of highly conserved protein surface, incorporating Tyr 83 and the negative patch 42—45, as a locality favourable to association with positively charged complexes. There seems every likelihood that such an area with close (*ca.* 10 Å) approach to the copper active site is also relevant to other electron-transfer reactions of plastocyanin. In principle, experiments using a selective and powerful competitive inhibitor should make it possible to designate whether one or both of the physiological partners of plastocyanin use this same site. Thus our aim is to test further the effects of pH and competitive inhibition, and in subsequent studies apply such approaches to a consideration of the reactions of plastocyanin with cytochrome f and P700.

To avoid any influence of the conjugate-base $[Pt(NH_3)_5(NH_2)]^{3+}$, the various effects of $[Pt(NH_3)_6]^{4+}$ (pK_a 7.1) were studied at pH 5.8.

† *Non-S.I. unit employed:* M = mol dm^{-3}.

Experimental

Preparation of Protein.—Plastocyanin was isolated from parsley leaves [8] and handled as previously described.[1,3] An absorbance maximum at 597 nm (ε 4 500 M^{-1} cm^{-1}) for the oxidised protein PCu(II) was used to monitor concentrations. To obtain PCu(I) a few crystals of sodium dithionite (BDH, GPR grade), sufficient to remove the blue colour and representing an excess of reductant, were added. Solutions were dialysed against appropriate buffer (with at least two changes) for at least 30 h at *ca.* 20 °C. It was not possible to use pH values as low as 4.3 because PCu(II) denatures.

Preparation of Complexes.—These were obtained and purified to known spectra (λ/nm, ε/M^{-1} cm^{-1}) by procedures already described: hexa-ammineplatinum(IV) chloride, $[Pt(NH_3)_6]Cl_4\cdot H_2O$, 260 (129); [9] tris(2,2'-bipyridine)cobalt(III) perchlorate, $[Co(bipy)_3][ClO_4]_3\cdot 3H_2O$, 306 (34 100), 317 (30 700), and 450 (68.4); [10] penta-amminepyridineruthenium(III) perchlorate, $[Ru(NH_3)_5(py)][ClO_4]_3$, 247 (4 500), 253 (4 570), and 262 (4 540); [11] penta-amminepyridineruthenium(II) perchlorate $[Ru(NH_3)_5(py)][ClO_4]_2$, 407 (7 800) [12] (Found: C, 12.9; H, 4.15; N, 17.8. Calc. for $C_5H_{20}Cl_2N_6O_8Ru$: C, 12.9; H, 4.3; N, 18.1%), single band on Sephadex SPC25 column; diacetatobis(2,2'-bipyridine)cobalt(III) perchlorate, $[Co(bipy)_2(O_2CMe)_2]ClO_4$, 509 (120); [13] and ammonium bis(pyridine-2,6-dicarboxylato)cobaltate(III), $NH_4[Co(dipic)_2]$, 510 (630).[14] Relevant (known) reduction potentials ($E^\ominus$) are listed in Table 1. We were unable to determine $E^\ominus$ for the $[Co(bipy)_2(O_2CMe)_2]^{+,0}$ couple due to non-reversibility.

Buffers.—Tris(hydroxymethyl)aminoethane ('Tris') (Sigma Chemicals) was used in a mixed buffer with maleate both at 0.005 M and adjusted to the required pH with NaOH to give solutions at pH 7.0—8.0. For the pH range 5.6—6.0 the buffer used was 0.010 M 2-(*N*-morpholino)ethanesulphonic acid (mes) (Sigma Chemicals), log K_B = 6.1 at 25 °C, with NaOH added. Acetate (0.010 M) was used for pH < 5.6. All pH values were checked using a Radiometer (PHM 62) pH-meter

Table 1. Reduction potentials relevant to the present studies

Complex	$E^{\ominus}$/mV	Ref.
PCu(II)–PCu(I)	370	*a*
$[Co(bipy)_3]^{3+,2+}$	370	*b*
$[Co(phen)_3]^{3+,2+}$	370	*b*
$[Ru(NH_3)_5(py)]^{3+,2+}$	273	12
$[Co(dipic)_2]^{-,2-}$	400	14
$[Fe(CN)_6]^{3-,4-}$	410	*c*

[a] A. G. Lappin, 'Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1981, vol. 13, p. 27. [b] E. Paglia and C. Sirani, *Gazz. Chim. Ital.*, 1957, **81**, 1125. [c] See, for example, J. Butler, D. M. Davies, and A. G. Sykes, *J. Inorg. Biochem.*, 1981, **15**, 41.

Table 2. The variation of first-order rate constants, $k_{obs.}$ (25 °C), with oxidant concentration for the $[Co(bipy)_2(O_2CMe)_2]^+$ and $[Co(dipic)_2]^-$ oxidation of PCu(I) at pH 7.5 (Tris–HCl) and I = 0.10 M (NaCl)

Oxidant	$10^3[Co^{III}]$/M	$10^2k_{obs.}/s^{-1}$
$[Co(bipy)_2(O_2CMe)_2]^+$	0.52	0.85
	1.12	1.90
	1.97	3.3
	2.14	3.8
	3.50	5.9
$[Co(dipic)_2]^-$	0.14	6.7
	0.25	12.0
	0.51	25.1
	0.61	30
	1.17	58
	1.60	78

fitted with a combined electrode or a Russell (CWR/322) glass electrode.

Kinetics.—The ionic strength of run solutions was adjusted to 0.10 M with NaCl (BDH, AnalaR). A Dionex D-110 stopped-flow spectrophotometer was used to monitor absorbance changes at the PCu(II) peak at 597 nm. A large (>10-fold) excess of inorganic redox partner and inhibitor was used. Rate constants were obtained using a Datalab DL901 transient recorder and Commodore PET 2001-16K desk-top computer.[15] Plots of absorbance (A) changes $\ln(A_\infty - A_t)$ against time were linear to at least four half-lives. First-order rate constants, $k_{obs.}$, were obtained from the slopes. All absorbance changes were consistent with 1 : 1 stoicheiometries, equations (1) and (2). It was possible to study the

$$PCu(I) + Co^{III} \longrightarrow PCu(II) + Co^{II} \quad (1)$$

$$PCu(II) + Ru^{II} \longrightarrow PCu(I) + Ru^{III} \quad (2)$$

reverse of (2) by having the ruthenium(III) complex in large excess, and such as to ensure >95% conversion in the required direction. Where first-order dependences on the inorganic redox partners are applicable, $k_{obs.}$ values could be converted directly into second-order rate constants k_{exp}.

Kinetic schemes and rate laws applying in the previous study with $[Co(phen)_3]^{3+}$ as oxidant (redox partners designated R, and inhibitor as B) were tested for. Thus the reaction sequence (3)—(6) gives expression (7). A term $K[R]$ in the denominator of equation (7) is small and can be ignored (<1% contribution) for all the studies described. Values of second-order rate constants k ($=Kk_{et}$) are as previously reported,[3,7] or as determined in this paper.

Table 3. The dependence of rate constants (25 °C) for the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II) (1.0×10^{-5} M) on concentration of reductant and pH at I = 0.10 M (NaCl)

pH	$10^4[Ru(NH_3)_5(py)^{2+}]$/M	$10^{-1}k_{obs.}/s^{-1}$	$10^{-5}k_{exp}/M^{-1}\ s^{-1}$
4.8 [a]	2.17	5.6	2.58
5.0 [a]	1.00	2.75	2.75
	1.90	5.3	2.77
	3.55	9.8	2.76
	5.30	14.5	2.74
5.2 [a]	2.17	6.9	3.2
5.6 [b]	2.17	8.0	3.7
6.0 [b]	2.18	8.5	3.9
7.0 [c]	2.18	9.1	4.2
7.6 [c]	1.09	4.7	4.3
	2.10	8.5	4.1
	2.17	9.3	4.2
	4.2	17.8	4.2
	5.1	20.7	4.1
	6.3	26.4	4.2
8.0 [c]	2.18	9.3	4.3

[a] Acetate. [b] mes. [c] Tris–maleate.

$$PCu(I) + R \underset{}{\overset{K}{\rightleftharpoons}} PCu(I),R \quad (3)$$

$$PCu(I),R \xrightarrow{k_{et}} products \quad (4)$$

$$PCu(I) + B \underset{}{\overset{K_B}{\rightleftharpoons}} PCu(I),B \quad (5)$$

$$PCu(I),B + R \xrightarrow{k_B} products \quad (6)$$

$$k_{exp} = \frac{Kk_{et} + k_BK_B[B]}{1 + K_B[B]} \quad (7)$$

Treatment of Data.—An unweighted non-linear least-squares program was used in all cases.

Results

Rate Law Dependences.—These were investigated for the $[Co(bipy)_2(O_2CMe)_2]^+$ and $[Co(dipic)_2]^-$ oxidations of PCu(I) at pH 7.5, in the absence of inhibitor, Table 2. The dependence (8) is strictly adhered to over the range of

$$k_{obs.} = k_{exp}[Co^{III}] \quad (8)$$

$[Co^{III}]$ studied, with no evidence for less than first-order dependence on oxidant concentration. Limits of K as defined in equation (3) are <30 M^{-1} for both reactions. Similarly, although tested for over a somewhat lower range of concentrations, an equation corresponding to (8) applies in the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II) at pH 5.0 and 7.6, Table 3.

Effects of pH.—Rate constants at pH 7.5 (Table 2) and pH 5.8 (Table 4) with no Pt^{IV} added clearly indicate that both the $[Co(bipy)_2(O_2CMe)_2]^+$ and $[Co(dipic)_2]^-$ oxidations of PCu(I) exhibit sharp decreases in k_{exp} with decreasing pH. Ratios of k_{exp} at pH 7.5 and 5.8 are 1.8 for $[Co(bipy)_2(O_2CMe)_2]^+$ and 1.75 for $[Co(dipic)_2]^-$, which are very similar (and it is concluded of the same origin) as those previously reported, *e.g.* for $[Co(phen)_3]^{3+}$ and $[Co(bipy)_3]^{3+}$ (both 2.0).[3] For the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II) a

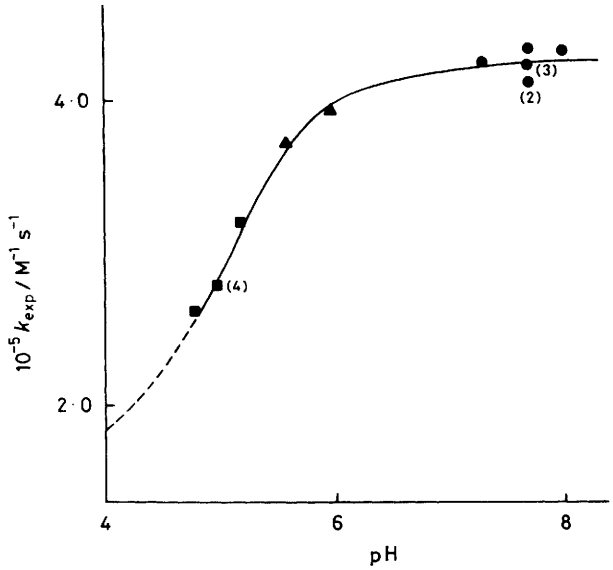

Figure 1. The effect of pH on rate constants k_{exp} (25 °C) for the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II) (1×10^{-5} M) at $I = 0.10$ M (NaCl). Buffers used were acetate (■), mes (▲), and Tris–maleate (●)

different shape of pH profile is apparent, Figure 1, and the corresponding ratio is 1.08, indicating little effect over this range. Equation (9) gives a good fit of the full dependence of

$$k_{exp} = \frac{k + k_H K_H[H^+]}{1 + K_H[H^+]} \quad (9)$$

k_{exp} on $[H^+]$, Table 3, with the various constants as defined in (10)—(12). Values of $k = (4.2 \pm 0.02) \times 10^5$ M^{-1} s^{-1}, $k_H =$

$$PCu(II) + H^+ \overset{K_H}{\rightleftharpoons} H^+PCu(II) \quad (10)$$

$$PCu(II) + Ru^{II} \xrightarrow{k} \text{products} \quad (11)$$

$$H^+PCu(II) + Ru^{II} \xrightarrow{k_H} \text{products} \quad (12)$$

$(1.52 \pm 0.34) \times 10^5$ M^{-1} s^{-1}, and $K_H = (1.0 \pm 0.6) \times 10^5$ M^{-1} (corresponding to an acid dissociation, pK_a 5.0 ± 0.2) were obtained. Three separate preparations of $[Ru(NH_3)_5(py)]^{2+}$ gave spectra (and analyses) in agreement with the literature, and self-consistent rate constants which approach the limits determinable by the stopped-flow apparatus. These rate constants should replace previous values,[3] which we have been unable to reproduce with sufficient precision.

Effects of $[Pt(NH_3)_6]^{4+}$.—Rate constants k_{exp} indicating the effect of competitive inhibition by $[Pt(NH_3)_6]^{4+}$ are shown in Table 4. A comparison of the relative influences of $[Co(bipy)_3]^{3+}$, $[Ru(NH_3)_5(py)]^{3+}$, $[Co(bipy)_2(O_2CMe)_2]^+$, and $[Co(dipic)_2]^-$ with those for $[Co(phen)_3]^{3+}$ is illustrated in Figure 2. Partial inhibition consistent with equation (7) is indicated for the positively charged oxidants. Moreover $[Pt(NH_3)_6]^{4+}$ inhibits the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II), Table 5. The relative effects for $[Ru(NH_3)_5(py)]^{2+}$ and $[Ru(NH_3)_5(py)]^{3+}$ are shown in Figure 3. Only three data points were obtained for $[Ru(NH_3)_5(py)]^{3+}$, and these are perhaps less reliable because of the unfavourable thermodynamics and the need to work at high oxidant concentrations to ensure that the reaction proceeds to >95% completion.

Table 4. The effect of redox-inactive $[Pt(NH_3)_6]^{4+}$ on second-order rate constants, k_{exp} (25 °C), for the oxidation of PCu(I) (1×10^{-5} M) at pH 5.8 (mes) and $I = 0.10$ M (NaCl)

Oxidant	$10^4[Pt(NH_3)_6^{4+}]$/ M	$10^{-2}k_{exp}$/ M^{-1} s^{-1}
$[Co(bipy)_3]^{3+}$	0	3.14
(5.7×10^{-4} M)	0.34	2.49
	0.85	2.18
	1.70	1.97
	2.56	1.83
$[Ru(NH_3)_5(py)]^{3+}$	0	420
(2.5×10^{-3} M)	0.81	330
	1.62	292
	2.00	272
$[Co(bipy)_2(O_2CMe)_2]^+$	0	0.094
(2.4×10^{-3} M)	0.32	0.085
	0.65	0.082
	1.30	0.077
	1.94	0.075
	2.58	0.073
$[Co(dipic)_2]^-$	0	2.87
(3.6×10^{-4} M)	0.30	2.98
	0.59	3.00
	0.64	3.02
	1.18	3.03
	1.28	3.13
	2.40	3.06
	2.56	3.22

Table 5. The effect of redox-inactive $[Pt(NH_3)_6]^{4+}$ on the $[Ru(NH_3)_5(py)]^{2+}$ (2.08×10^{-4} M) reduction of plastocyanin PCu(II) (1×10^{-5} M) at pH 5.8 (mes) and $I = 0.10$ M (NaCl)

$10^4[Pt(NH_3)_6^{4+}]$/M	$10^{-5}k_{exp}$/M^{-1} s^{-1}
0	3.8
0.29	3.4
0.48	2.9
0.96	2.8
1.44	2.5
2.16	2.34
2.89	2.49
4.04	2.35

Inhibition of the 2+ reductant is less than the effect noted for the 3+ oxidants in Figure 2. The best fit of equation (7) to k_{exp} values gives individual K_B and k_B values as listed in Table 6. Data for the 3+ oxidants combined give $K_B =$ $17\,400 \pm 4\,000$ M^{-1} and $k_B/k = 0.53 \pm 0.03$. A small 8% acceleration effect was observed for the reaction with $[Co(dipic)_2]^-$ as oxidant and $[Pt(NH_3)_6]^{4+}$ as inhibitor.

Discussion

The pH profile of rate constants k for the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II), Figure 1, is the first using a positively charged reductant for PCu(II). Previously it has been noted that with $[Fe(CN)_6]^{4-}$ as reductant there is a 20% increase in rate constants over the range pH 7.0—4.5, with no tendency to level out or give a fit to equation (9).[1,16] Such a mild effect for reactants of *ca.* 8− (protein) and 4− charge probably reflects more distant protonation(s) on the protein. Line-broadening n.m.r. experiments [5,6] with redox-inactive $[Cr(CN)_6]^{3-}$ have indicated a high degree of specificity for association of this complex near to His 87 (see Figure 6 in Part 6),[1] where the exposed imidazole edge of His 87 represents the closest approach (6 Å) of the Cu to the protein surface.[2] It would be

Table 6. Summary of association constants (K_B) and rate constants k and k_B for the reaction of plastocyanin with different redox partners at 25 °C, pH 5.8 (mes), and I = 0.10 M (NaCl). The rate constant k_B is for the reaction of protein associated with $[Pt(NH_3)_6]^{4+}$ and the redox partner. Percentage errors are in parentheses

Redox partner	K_B/M^{-1}	k [a]/M^{-1} s^{-1}	k_B/M^{-1} s^{-1}
$[Co(phen)_3]^{3+}$	16 400 (12)	1 400 (6)	720 (4)
$[Co(bipy)_3]^{3+}$	20 600 (10)	314 (4)	160 (3)
$[Ru(NH_3)_5(py)]^{3+}$	(16 000) [b]	4.2×10^4 (12)	(2.3×10^4) [b]
$[Ru(NH_3)_5(py)]^{2+}$	17 200 (24)	3.8×10^5 (2)	2.1×10^5 (7)
$[Co(bipy)_2(O_2CMe)_2]^+$	16 200 (12)	9.4 (4)	6.9 (2)
$[Co(dipic)_2]^-$	*c*	287 (3)	*ca.* 306

[a] Corresponds to Kk_{et} in equations (3) and (4). [b] Only three data points. High concentrations of oxidant required for reaction to proceed to >95% reaction. [c] Effect too small to enable K_B to be determined accurately.

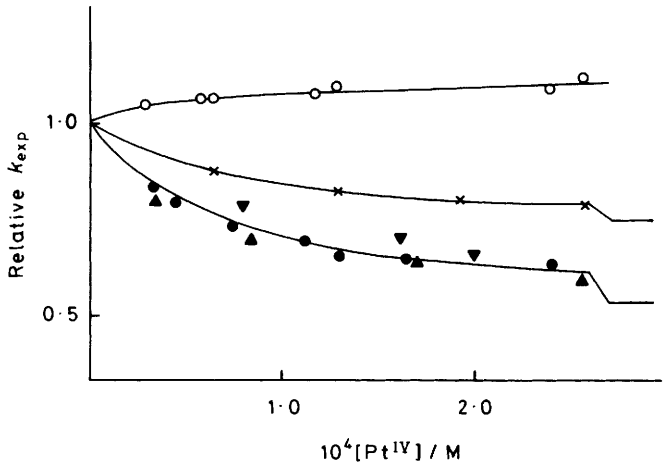

Figure 2. The effect of $[Pt(NH_3)_6]^{4+}$ on second-order rate constants k_{exp} (25 °C) for oxidation of PCu(I) at pH 5.8 (mes) and I = 0.10 M (NaCl). Rate constants (on a relative scale) are with oxidants $[Co(phen)_3]^{3+}$ (●) (previous study [3]), $[Co(bipy)_3]^{3+}$ (▲), $[Ru(NH_3)_5(py)]^{3+}$ (▼), $[Co(bipy)_2(O_2CMe)_2]^+$ (×), and $[Co(dipic)_2]^-$ (○)

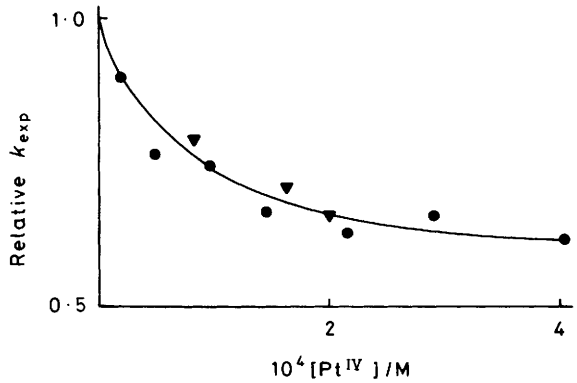

Figure 3. The effect of $[Pt(NH_3)_6]^{4+}$ on second-order rate constants k_{exp} (25 °C) (relative scale) for the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II) (●), and $[Ru(NH_3)_5(py)]^{3+}$ oxidation of PCu(I) (▼) at pH 5.8 and I = 0.10 M (NaCl)

surprising if the $[Fe(CN)_6]^{3-,4-}$ couple did not interact favourably here also. Present sequencing information [2,17] indicates no acidic residues (the nearest is 59 at a distance of *ca.* 9 Å) or basic residues at this site. By repeating diffraction studies for PCu(II) crystals prepared at different pH values, Freeman [2] has demonstrated that the Cu(II) active site remains unchanged and independent of pH within the range relevant to the present studies. Therefore, the pH effect observed for the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II), pK_a = 5.0, is the result of protonation at an acidic residue close to the site at which $[Ru(NH_3)_5(py)]^{2+}$ interacts with the protein. Since $[Pt(NH_3)_6]^{4+}$ inhibits the reduction, the most likely site for protonation is the negative patch incorporating residues 42—45. Other reductants (including proteins) using the same site as $[Ru(NH_3)_5(py)]^{2+}$ would be expected to display the same pK_a of 5.0. The conclusion that the Tyr 83 locality is the dominant binding site for reaction of PCu(II) with $[Ru(NH_3)_5(py)]^{2+}$ (which applies also to the reverse reaction) is contrary to an earlier assignment and related discussion concerning penetration and overlap of the pyridine ligand with the imidazole of His 87.[12,18]

Effects of pH on the $[Co(bipy)_2(O_2CMe)_2]^+$ and $[Co(dipic)_2]^-$ oxidation of PCu(I) have been noted. These studies clearly conform to those already reported with $[Co(phen)_3]^{3+}$, $[Co(bipy)_3]^{3+}$, and $[Fe(CN)_6]^{3-}$ as oxidants,[3] and are consistent with formation of a redox-inactive protonated form of PCu(I), pK_a in the range 5.7—6.1. Protonation at the active site with dissociation of the imidazole of His 87 to give a three-co-ordinate and planar Cu^I will account for these similar pK_a values for oxidants using different binding sites. However, as has been indicated elsewhere,[1] this simple interpretation may need modifying to include the possibility that protonation occurs at the negative patch with transmission of the effect to the copper active site.

Besides pH effects, the other aim in the present studies was to investigate the competitive inhibition by $[Pt(NH_3)_6]^{4+}$ on different redox partners. A range of oxidants from $[Co(phen)_3]^{3+}$, $[Co(bipy)_3]^{3+}$, $[Ru(NH_3)_5(py)]^{3+}$, and $[Co(bipy)_2(O_2CMe)_2]^+$ through to negatively charged $[Co(dipic)_2]^-$ and $[Fe(CN)_6]^{3-}$ have now been studied. Different effects can contribute in the last two cases, which are more difficult to quantify therefore.[1] The responses of all four positively charged complexes are consistent with reaction at the site on the protein including Tyr 83 and the negative patch which is only partially blocked by $[Pt(NH_3)_6]^{4+}$. The partial blocking is accounted for by the region being sufficiently large to associate with two complexes at a time. Thus the 3+ complexes are more strongly influenced by the presence of the 4+ inhibitor (53% effect) than is the 1+ complex (25% effect). It is also noted that $[Pt(NH_3)_6]^{4+}$ inhibits the $[Ru(NH_3)_5(py)]^{2+}$ reduction of PCu(II) (40% effect), consistent with electrostatic effects and little or no influence of the oxidation state of the Cu.

If charge is the only consideration then the reaction with $[Co(dipic)_2]^-$ as oxidant would be expected to take place at the His 87 site. For a 1— charge however it is not clear whether this will be the only influence. The reasons for the specificity of $[Fe(CN)_6]^{3-}$ for the His 87 locality is not clearly under-

stood. A favourable factor is likely to be the positive charge at (or close to) the copper active site. The $[Pt(NH_3)_6]^{4+}$ studies are in this case ambiguous since the enhancement in rate could be an influence of overall charge at the His 87 site when $[Pt(NH_3)_6]^{4+}$ is associated at the Tyr 83/42—45 site, or alternatively the result of some enhancement in reactivity at the Tyr 83/42—45 site whether or not reaction in the absence of $[Pt(NH_3)_6]^{4+}$ is at this site. It is interesting that the effect is so small compared with, for example, the three-fold effect of redox-inactive $[Cr(NH_3)_6]^{3+}$ on the $[Co(edta)]^-$ (edta = ethylenediaminetetra-acetate) oxidation of [2Fe-2S] ferredoxin.[19] An essential difference is that a smaller binding site appears to be relevant in the case of the [2Fe-2S] protein, and $[Cr(NH_3)_6]^{3+}$ gives complete blocking of the reaction with positively charged oxidants. Because of the small size of the effect with $[Co(dipic)_2]^-$ we are inclined to the view that this oxidant uses the His 87 site on PCu(I). Further evidence is however required; for example, one could look at the n.m.r. line-broadening effect of $[Cr(dipic)_2]^-$ on PCu(I).

These studies clearly show that positively charged oxidants have a preference for the Tyr 83/42—45 site. The complex $[Pt(NH_3)_6]^{4+}$ shows general applicability as a competitive inhibitor for this site.

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