Kinetic Studies on 1:1 Electron-transfer Reactions involving Blue Copper Proteins. Part 12.† Reactions of Spinach Plastocyanin with Inorganic Redox Partners‡

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Reactions of spinach plastocyanin have been studied at 25 °C, I = 0.10 M (NaCl), and a comparison is made with the previously studied parsley plastocyanin, which has 31 different residues in a single polypeptide chain of 99 amino-acid residues. Active-site protonations leading to a switch-off in reactivity of PCu(I) are notably different, as indicated by the acid dissociation constants pK_a for spinach (4.9 average) and parsley (5.5). Protonation of PCu(II) at the east-face binding site, which incorporates Tyr 83, gives similar pK_a' values of 5.6 (spinach) and 5.8 (parsley). A number of other features are similar, including rate constants at pH 7.5 with $[Fe(CN)_6]^{3-}$, $[Co(phen)_3]^{3+}$ (phen = 1,10-phenanthroline), and $[Co(dipic)_2]^-$ (dipic = dipicolinate) as oxidants for PCu(I), and $[Ru(NH_3)_5(py)]^{2+}$ (py = pyridine) and $[Co(terpy)_2]^{2+}$ (terpy = 2,2′:6′2″-terpyridyl) as reductants for PCu(II), which are all within ±33% of those for parsley plastocyanin, suggesting conservation of binding sites, reduction potential, and mechanism of electron transfer. With $[Fe(CN)_6]^{3-}$ as oxidant, reaction is assigned to the His 87 (north) site, whereas with $[Co(phen)_3]^{3+}$ reaction occurs at two (or more) binding sites with (from the effect of H^+) *ca.* 70% of reaction at the Tyr 83 site. The oxidant $[Ru(NH_3)_5(CH_3CN)]^{3+}$ has been used for the first time. Blocking with redox inactive $[(NH_3)_5Co(NH_2)Co(NH_3)_5]^{5+}$ (K_B = 6 300 M^{-1}, pH 7.5) suggests that 91% of this reaction is at the Tyr 83 site. From similar blocking experiments with $[Co(phen)_3]^{3+}$ as oxidant the corresponding value is 80% (61% for parsley). Only mild association and blocking is observed with Mg^{2+} ($K \approx 38$ M^{-1}).

The effects of chemical modification on reactivity is a subject receiving increasing attention in the study of electron-transport metalloproteins.[1-4] At the same time it should not be overlooked that there are natural changes in metalloproteins, of an evolutionary kind, in progressing from (for example) blue-green algae to higher plant sources, which also merit investigation. This series of papers[5] on single Cu (type 1) metalloproteins[6,7] has been concerned in the main with the reactivity of plastocyanin (M 10 500) from parsley leaves. It has been concluded that conserved negatively charged localities either side of the Tyr 83 at 42—45 (in particular), and 59—61 on the east face of the (normal view) molecule may both be involved.[7] Recent amino-acid sequence formation[7,8] has, however, shown parsley to have unexpected sequence variations as compared to other higher plant plastocyanins. Features to note are the deletions at 57 and 58, and loss of negative charge at the normally conserved 59—61 negative patch (only 59 is acidic).[8] There is need therefore to study another plant source in order to determine what effect these changes have on reactivity, and we have selected spinach plastocyanin for this purpose.

It is possible to condense this report because the experimental approach and rate laws duplicate earlier studies.[7] The oxidant $[Ru(NH_3)_5(CH_3CN)]^{3+}$ as a positively charged oxidant with no aromatic ligands is included for the first time. The effectiveness of Mg^{2+} as a blocker is also investigated, because of its relevance to photosynthetic electron transport.

Some of the work described has been referred to in a recent communication.[9]

† Part 11, D. Beoku-Betts, S. K. Chapman, C. V. Knox, and A. G. Sykes, *Inorg. Chem.*, 1985, **24**, 1677.

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

Experimental

Spinach plastocyanin was isolated from fresh leaves by the procedure described by Ellefson *et al.*,[10] and purified to an absorbance (A) peak ratio of $A_{278}/A_{597} = 1.3 \pm 0.1$. The protein was dialysed to the required pH and ionic strength except for runs at pH < 5.3, when the pH of protein solutions was adjusted at the time of stopped-flow mixing. All other handling procedures were as previously described in ref. 5 and other papers in this series.

Complexes used were as previously reported in this series of papers.[7] The procedure for the preparation of bis(2,2′:6′,2″-terpyridyl)cobalt(II) perchlorate, $[Co(terpy)_2][ClO_4]_2 \cdot H_2O$, has been described.[11] Final crystallisation was achieved at 2 °C by addition of small amounts of saturated $NaClO_4$ over several days. Spectra [λ/nm (ε/M^{-1} cm^{-1})] obtained, 445 (1 620) and 505 (1 415), were in good agreement with literature values, 445 (1 581) and 505 (1 374).[11] Solutions of $[Co(terpy)_2]^{2+}$ were kept under a N_2 atmosphere. The standard reduction potential is 270 mV. Acetonitrilepenta-ammineruthenium(III) perchlorate, $[Ru(NH_3)_5(CH_3CN)][ClO_4]_3$, was prepared by the procedure described by Clarke and Ford.[12] Spectra [λ/nm (ε/M^{-1} cm^{-1})] obtained, 295 (593) and 380 (138), were in good agreement with literature values, 295 (575) and 380 (135).[12] A standard reduction potential of 462 mV has been reported.[13] The complex is unstable at pH 7.5 (Tris–HCl) [Tris = tris(hydroxymethyl)methylamine], I = 0.10 M (NaCl); $t_{\frac{1}{2}}$ ~20 min at 25 °C. At pH 5 (acetate buffer) the half-life is ~10 h. The pH-jump method was used to attain working (stopped-flow) pH values in the vicinity of pH 7.5. In this procedure solutions of complex contributing 1 mM acetate buffer (pH 5) were mixed with solutions of protein contributing 40 mM Tris–HCl, to give a final pH of 7.5. Solutions of the ruthenium(III) complex were kept under argon and were covered with aluminium foil.

Reactions were studied by the stopped-flow method with the inorganic complex in ≥10-fold excess. First-order rate constants, $k_{obs.}$, were obtained from the slope of plots of absorbance changes $\ln\Delta A$ against time, which were linear for greater than 3—4 half-lives. The oxidation of plastocyanin PCu(I) with $[Co(phen)_3]^{3+}$ (phen = 1,10-phenanthroline) has previously been shown to exhibit a non-linear dependence on oxidant concentration.[5] In other reactions for which linear dependences have been demonstrated $k_{obs.}$ was converted directly to second-order rate constants $k_{exp.}$.

Unweighted linear and non-linear least-squares fitting procedures were used.

Results

pH *Effects with Three Oxidants.*—Rate constants (Table 1) for $[Fe(CN)_6]^{3-}$ are in accord with the reaction sequence (1)—(3), which gives the expression (4),[14] where P is the protein, in this case PCu(I), and C the complex.

$$H^+P \rightleftharpoons H^+ + P \quad (1)$$

$$P + C \xrightarrow{k_0} \text{products} \quad (2)$$

$$H^+P + C \xrightarrow{k_H} \text{products} \quad (3)$$

$$k_{exp.} = \frac{k_0K_a + k_H[H^+]}{K_a + [H^+]} \quad (4)$$

In this and other studies of PCu(I), k_H has been found to make random ±8% contributions, which do not appear significant, and k_H is therefore fixed at zero in our computations. From a fit of data for $[Fe(CN)_6]^{3-}$, $k_0 = (8.0 \pm 0.1) \times 10^4$ M^{-1} s^{-1}, and $pK_a = 4.78 \pm 0.04$, in satisfactory agreement with the n.m.r. pK_a of 4.9.[15] Rate constants for $[Co(phen)_3]^{3+}$ (Table 2) give $k_0 = (2.5 \pm 0.3) \times 10^3$ M^{-1} s^{-1} and apparent $pK_a = 5.55 \pm 0.03$, and for $[Co(dipic)_2]^-$ [dipic = dipicolinate (pyridine-2,6-dicarboxylate)] (Table 3), $k_0 = (4.0 \pm 0.05) \times 10^2$ M^{-1} s^{-1} and $pK_a = 5.01 \pm 0.02$. Rate constants in ref. 5 appear to be in error by a factor of 2. In a modified reaction scheme[9] the higher apparent pK_a for $[Co(phen)_3]^{3+}$ is believed to be due to a combination of independent acid dissociations at the active site (K_a), and binding site (K_a'), which gives rise to equation (5), where k_1 and

$$k_{exp.} = \frac{k_1K_aK_a' + k_2K_a'[H^+]}{K_aK_a' + K_a'[H^+] + K_a[H^+] + [H^+]^2} \quad (5)$$

k_2 represent the upper and lower limits of curve (*b*) in the Figure. With pK_a fixed at the n.m.r. value (4.9) and the rate constant $k_1 = 2\,500$ M^{-1} s^{-1} at pH 7.5, a value of pK_a'(red.) for PCu(I) is 5.6 ± 0.2, and $k_2 = 750 \pm 70$ M^{-1} s^{-1} for maximum reaction at this site (Figure). If protonation near to the Tyr 83 residue completely inhibits reaction then the treatment suggests 70% of the reaction is at this site.

pH *Effects with Two Reductants.*—Active-site protonation is not observed for PCu(II), and the interpretation implicates pK_a' only. With $[Ru(NH_3)_5(py)]^{2+}$ (py = pyridine) as reductant (Table 4), the scheme as in (1)—(3) and equation (4) gives pK_a'(oxid.) = 5.33 ± 0.07, $k_0 = (5.8 \pm 0.08) \times 10^5$ M^{-1} s^{-1}, and $k_H = (3.1 \pm 0.08) \times 10^5$ M^{-1} s^{-1}. With $[Co(terpy)_2]^{2+}$ as reductant (Table 5), pK_a'(oxid.) = 5.34 ± 0.05, $k_0 = (8.2 \pm 0.05) \times 10^4$ M^{-1} s^{-1}, and $k_H = (4.4 \pm 0.11) \times 10^4$ M^{-1} s^{-1}. Values of pK_a'(oxid.) obtained here for PCu(II) are in excellent agreement, but differ from pK_a'(red.) for the PCu(I) state.

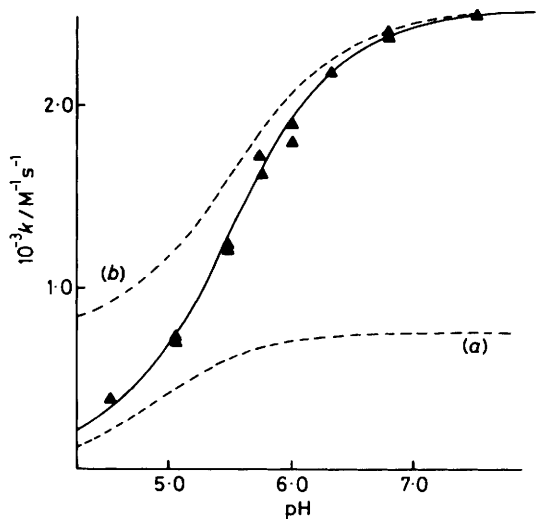

Figure. The variation of rate constants $k_{exp.}$ (25 °C) for the oxidation of spinach PCu(I) by $[Co(phen)_3]^{3+}$, I = 0.10 M (NaCl), with pH, and simulated curves (– – –) indicating the relative influences of pK_a as determined by n.m.r. [curve (*a*), protonation at the active site], and pK_a' [curve (*b*), protonation at the Tyr 83 binding site]

Table 1. Dependence of rate constants (25.0 °C) for the $[Fe(CN)_6]^{3-}$ oxidation of spinach plastocyanin PCu(I) (~1 × 10^{-5} M) on concentration of oxidant and pH, I = 0.10 M (NaCl)

pH	$10^4[Fe^{III}]$/M	$10^{-4}k_{exp.}$/M^{-1} s^{-1}
3.99[a]	2.3	1.11
	3.5	1.14
4.18[a]	3.0	2.26
4.37[a]	2.8	2.67
4.72[a]	2.4	3.6
4.90[a]	2.3	4.7
	5.2	4.6
5.25[a]	2.8	5.7
	4.4	5.6
	5.7	6.0
5.80[b]	2.4	6.9
	4.2	6.9
6.20[b]	3.4	7.6
	4.6	7.5
6.75[b]	1.28	8.2
	4.0	7.9
7.08[c]	1.57	8.1
	3.58	8.5
7.55[c]	2.83	8.5
	3.97	8.4

[a] Acetate. [b] 2-(*N*-Morpholino)ethanesulphonic acid (mes). [c] Tris.

Effects of Redox Inactive Complexes(B).—Reactions of PCu(I) with $[Co(phen)_3]^{3+}$ and $[Ru(NH_3)_5(CH_3CN)]^{3+}$ as oxidants were studied. A linear dependence of first-order rate constants $k_{obs.}$ (Table 6) against $[Ru(NH_3)_5(CH_3CN)^{3+}]$ is observed, giving $k = (1.82 \pm 0.04) \times 10^5$ M^{-1} s^{-1}. It was not possible to test for a non-linear dependence on this 3+ oxidant at higher concentrations because of the rapidity of the reaction. With the addition of increasing amounts of redox inactive $[(NH_3)_5Co(NH_2)Co(NH_3)_5]^{5+}$ rate constants at pH 7.5 decrease as indicated in Table 7. The reaction scheme (6)—(8)

$$PCu(I) + B \rightleftharpoons PCu(I),B \quad (6)$$

$$PCu(I) + \text{oxidant} \xrightarrow{k} \text{products} \quad (7)$$

Table 2. Dependence of rate constants (25.0 °C) for the $[Co(phen)_3]^{3+}$ oxidation of spinach plastocyanin PCu(I) ($\sim 1 \times 10^{-5}$ M) on concentration of oxidant and pH, I = 0.10 M (NaCl)

pH	$10^4[Co^{III}]$/M	$10^{-3}k_{exp.}$/M^{-1} s^{-1}
4.54[a]	2.9	0.36
	4.6	0.37
5.05[a]	2.8	0.71
	4.6	0.72
5.30[b]	4.7	1.01
5.48[b]	2.3	1.18
	3.4	1.19
5.75[b]	2.8	1.60
	4.5	1.71
6.00[b]	3.2	1.78
	4.4	1.89
6.30[b]	3.5	2.20
6.80[b]	1.1	2.39
	2.8	2.38
7.50[c]	4.4	2.44

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

Table 3. Dependence of rate constants (25.0 °C) for the $[Co(dipic)_2]^-$ oxidation of spinach plastocyanin PCu(I) ($\sim 5 \times 10^{-6}$ M) on concentration of oxidant and pH, I = 0.10 M (NaCl)

pH	$10^3[Co^{III}]$/M	$10^{-2}k_{exp.}$/M^{-1} s^{-1}
4.55[a]	0.84	1.20
	1.03	1.21
	1.57	1.24
4.84[a]	0.38	1.53
5.04[a]	0.41	2.12
	0.45	2.07
	0.83	2.02
5.23[a]	0.44	2.36
	0.54	2.24
5.51[b]	0.41	3.09
	0.71	3.12
5.82[b]	0.56	3.5
	0.82	3.5
6.25[b]	0.63	3.7
	0.95	3.8
6.89[b]	0.77	4.0
7.30[c]	0.75	4.0
	1.15	4.0

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

$$\text{PCu(I),B} + \text{oxidant} \xrightarrow{k_B} \text{products} \quad (8)$$

$$\frac{k_{obs.}}{[\text{oxidant}]} = \frac{k + K_B k_B[B]}{1 + K_B[B]} \quad (9)$$

gives expression (9),[16] where $(1 + K_B[B])$ is assumed $\gg K$[oxidant] at the oxidant concentrations employed. For the reaction of $[Co(phen)_3]^{3+}$, $K_B = (6.8 \pm 1.0) \times 10^3$ M^{-1} and $k_B = 630 \pm 57$ M^{-1} s^{-1}. With $[Ru(NH_3)_5(CH_3CN)]^{3+}$ as oxidant, $K_B = (6.3 \pm 0.1) \times 10^3$ M^{-1} and $k_B = (1.5 \pm 0.03) \times 10^4$ M^{-1} s^{-1}.

The effect of Mg^{2+} (up to 1.0×10^2 M) on the $[Co(phen)_3]^{3+}$ oxidation was similarly investigated (Table 8). Only a small effect was observed. Assuming 80% maximum blocking at the Tyr 83 binding site $K_B \approx 38$ M^{-1}.

Discussion

A comparison of the reactivities of spinach and parsley plastocyanin PCu(I) has revealed a difference in active-site protonation, with pK_a values of 4.8 (spinach) and 5.5 (parsley) as determined by the dependence of rate constants on pH with $[Fe(CN)_6]^{3-}$ as oxidant.[5,7,9] With $[Co(dipic)_2]^-$ as oxidant a similar pK_a of 5.0 (spinach) is obtained. From n.m.r. measurements pK_a values of 4.9 (spinach)[15] and 5.7 (parsley)[9] are observed for the process involving protonation (and dissociation) of one of the co-ordinated histidines (His 87),[17] and the agreement with kinetically determined values indicates that this same process is involved. From comparisons made,[9] it would appear that the active site pK_a obtained for parsley lies out of line with those for other plastocyanins.

The reaction of PCu(I) with $[Co(phen)_3]^{3+}$ is also sensitive to active-site protonation and switch-off in reactivity. However from the rate constant variation with pH bigger (apparent) pK_a values, which are 5.6 for spinach and 6.1 for parsley, are obtained. Two acid dissociation processes are indicated in the treatment previously referred to,[9] one for active-site protonation (K_a), which is assumed to be the same as obtained in the $[Fe(CN)_6]^{3-}$ and n.m.r. studies, and the other for the Tyr 83 (east face) binding site protonation (K_a'). Values of pK_a' for PCu(I), referred to here as pK_a'(red.), are 5.6 (spinach) and 5.8 (parsley),[9] and are of similar magnitude.

Fourteen higher plant plastocyanins have now been sequenced.[7] A comparison of the sequences for spinach and parsley has revealed that 31 of 99 spinach amino-acid residues

Table 4. Dependence of rate constants (25.0 °C) for the $[Ru(NH_3)_5(py)]^{2+}$ reduction of spinach plastocyanin PCu(II) ($\sim 5 \times 10^{-5}$ M) on concentration of reductant and pH, I = 0.10 M (NaCl)

pH	$10^3[Ru^{II}]$/M	$10^{-5}k_{exp.}$/M^{-1} s^{-1}
4.25[a]	0.71	3.3
	1.64	3.3
4.72[a]	0.88	3.6
	1.43	3.6
5.33[b]	0.73	4.3
	1.54	4.4
5.84[b]	0.74	5.3
	1.47	5.2
6.53[b]	0.67	5.6
	1.39	5.5

[a] Acetate. [b] mes.

Table 5. Dependence of rate constants (25.0 °C) for the $[Co(terpy)_2]^{2+}$ reduction of spinach plastocyanin PCu(II) ($\sim 5 \times 10^{-6}$ M) on concentration of reductant and pH, I = 0.10 M (NaCl)

pH	$10^4[Co^{II}]$/M	$10^{-4}k_{exp.}$/M^{-1} s^{-1}
4.55[a]	0.80	4.7
	2.02	4.9
	3.49	5.0
4.83[a]	1.14	5.3
	2.45	5.4
5.20[a]	1.32	6.1
	2.38	6.1
5.58[b]	1.35	6.6
	2.61	6.7
6.02[b]	1.85	7.5
	3.00	7.5
6.37[b]	1.11	7.7
	1.56	7.9
6.95[b]	1.40	7.9
	2.84	8.1
7.50[c]	1.17	8.1
	1.67	8.2
	2.43	8.2

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

Table 6. Dependence of first-order rate constants (25.0 °C) for the $[Ru(NH_3)_5(CH_3CN)]^{3+}$ oxidation of spinach plastocyanin PCu(I) ($\sim 1 \times 10^{-5}$ M) on oxidant concentration, pH 7.50 (Tris–HCl), I = 0.10 M (NaCl)

$10^4[Ru^{III}]$/M	1.30	1.70	2.22	2.50	3.8	5.2	6.9
$k_{obs.}/s^{-1}$	19.3	30.3	37	43	67	89	118

Table 7. The effect of redox inactive $[(NH_3)_5Co(NH_2)Co(NH_3)_5]^{5+}$ on rate constants (25 °C) for the oxidation of spinach plastocyanin PCu(I) ($\sim 1 \times 10^{-5}$ M) by $[Co(phen)_3]^{3+}$ (4.5×10^{-4} M) and $[Ru(NH_3)_5(CH_3CN)]^{3+}$ (1.70×10^{-4} M) at pH 7.5 (Tris–HCl), I = 0.10 M (NaCl)

Oxidant $[Co(phen)_3]^{3+}$

$10^3[Co^{III}]$/M	0	0.103	0.216	0.37	0.82	1.04	1.86	2.60
$k_{obs.}/s^{-1}$	1.14	0.74	0.65	0.54	0.44	0.40	0.33	0.30

Oxidant $[Ru(NH_3)_5(CH_3CN)]^{3+}$

$10^3[Co^{III}]$/M	0	0.42	0.93	1.57	3.30
$10^{-1}k_{obs.}/s^{-1}$	3.03	1.03	0.67	0.52	0.40

Table 8. The effect of Mg^{2+} on rate constants (25 °C) for the oxidation of spinach plastocyanin PCu(I) ($\sim 1 \times 10^{-5}$ M) by $[Co(phen)_3]^{3+}$ (2.0×10^{-4} M) at pH 7.5 (Tris), I = 0.10 M (NaCl)

$10^2[Mg^{2+}]$/M	0	0.10	0.25	0.43	0.72	1.0
$k_{obs.}/s^{-1}$	0.50	0.47	0.46	0.44	0.41	0.40

Table 9. Summary of rate constants (25 °C) valid at pH 7.5 for the reactions of spinach and parsley plastocyanins, I = 0.10 M (NaCl)

Reaction	k_0(spinach)/ $M^{-1}\,s^{-1}$	k_0(parsley)/ $M^{-1}\,s^{-1}$	Ratio
PCu(I) + $[Fe(CN)_6]^{3-}$	8.0×10^4	9.4×10^4	0.90
PCu(I) + $[Co(dipic)_2]^-$	4.0×10^2	4.9×10^2	0.83
PCu(I) + $[Co(phen)_3]^{3+}$	2.5×10^3	3.0×10^3	0.83
$[Ru(NH_3)_5(py)]^{2+}$ + PCu(II)	5.8×10^5	4.2×10^5	1.33
$[Co(terpy)_2]^{2+}$ + PCu(II)	8.2×10^4	7.3×10^4	1.12

are different.[7] The corresponding number of changes for spinach and French bean plastocyanin (the reactivity of which has also been studied[9]) is more typically 16.[7] In the case of parsley there are deletions at positions 57 and 58, a feature not previously observed in higher plant plastocyanins, but also observed for plastocyanins from green algae. Also, the highly conserved 59—61 acidic patch located close to Tyr 83 (and on the other side of Tyr 83 from the 42—45 negative patch) is no longer retained in parsley, the sequence of which reads, instead, Gln-Pro-Glu.[8] If the two deletions are disregarded then Glu 61 aligns with the conserved Glu 59 of spinach, and some functionality might be implied. The occurrence of a Pro residue, and the bend introduced in the polypeptide chain, could however introduce some variation in structure. Interestingly, in spite of the quite extensive overall number of sequence changes, there is conservation of charge (from the amino-acid composition and presumed to apply at pH ~7) on spinach (−9), parsley (−8), and French bean (−9) plastocyanins.

It is tempting to assign the differences in active-site pK_a values for spinach and parsley PCu(I) to the changes in sequence in the 57—61 locality, particularly as the α-carbon of residue 59 (for example) is relatively close, at ~10 Å from the Cu. Whether the deletions at 57 and 58 are directly responsible can be tested by studying the reactivity of plastocyanin from a fully sequenced green algal source such as *Scenedesmus obliquus*, and this we plan to do. The key question concerns the ease of access of a proton to the Cu active site. The pK_a values indicate that this is easier for parsley plastocyanin. We have noted previously that other single blue Cu proteins do not exhibit a similar switch-off in reactivity of the Cu^I state, and that this effect appears unique to the plastocyanins.[18] More structural information is required to comment further.

Also important is the similarity in rate constants (±33%), and binding site pK_a' values for spinach and parsley plastocyanins, suggesting that conservation of charge at the 3− level at the 59—61 patch is *not* important. For the positively charged oxidant $[Co(phen)_3]^{3+}$, blocking experiments indicate a greater proportion of reaction occurs at the spinach Tyr 83 site, 80% as opposed to 61% in the case of parsley. From the effects of protonation the treatment in equation (5) indicates that 70% of the $[Co(phen)_3]^{3+}$ reaction is at the Tyr 83 site. The treatment here probably gives a less precise estimate than experiments with the 5+ blocker $[(NH_3)_5Co(NH_2)Co(NH_3)_5]^{5+}$. For the two reductants, proton inhibition suggests only 46% of reaction is at the Tyr 83 site as compared to 51% for parsley. This different pattern for PCu(I) and PCu(II) is consistent with conformational changes at the Tyr 83 site (see below). The different ratios in Table 9 for oxidants (< 1) and reductants (> 1) should also be noted.

The binding site pK_a' of 5.3 for reductants $[Ru(NH_3)_5(py)]^{2+}$ and $[Co(terpy)_2]^{2+}$ with spinach PCu(II) referred to as pK_a'(oxid.) compares with 5.6 for spinach PCu(I) with $[Co(phen)_3]^{3+}$ as oxidant. This difference in pK_a', while not as big as that observed for parsley (5.0 and 5.8 respectively), clearly indicates an effect of the Cu oxidation state on protonation at the Tyr 83 site, which is > 10 Å distant. At this distance the effect of oxidation state of the Cu is most likely transmitted as a conformational change.[19] Evidence for conformational changes has also been obtained from fluorescence measurements.[20]

The oxidant $[Ru(NH_3)_5(CH_3CN)]^{3+}$ has not previously been used in this type of study. It was selected to determine whether the aromatic ligands 1,10-phenanthroline, 2,2′-bipyridine, and pyridine, present in positively charged complexes so far used, is crucial to reaction occurring at the east face. Attractive though the idea of stacking at the (exposed) Tyr 83 ring is, such a process has yet to be confirmed. Reaction with the acetonitrile complex is blocked by redox inactive $[(NH_3)_5Co(NH_2)Co(NH_3)_5]^{5+}$ consistent with 91% reaction occurring at the Tyr 83 site. In other words reaction at the east face is more extensive than with $[Co(phen)_3]^{3+}$. It is of course possible that the methyl group of the CH_3CN ligand might interact favourably with the Tyr 83 ring. However we note that in the case of the 5+ dicobalt complex and other ammine complexes used as blockers, which have no organic component, association at the Tyr 83 site remains extensive. It would appear therefore that such interactions at the Tyr 83 site are primarily electrostatic in origin.

Finally it has been demonstrated here that Mg^{2+} produces only a mild blocking effect at the Tyr 83 site, association constant $K \approx 38\ M^{-1}$. It is known that Mg^{2+} is effective in photosynthetic electron transport from plastocyanin PCu(I) to the $P700^+$ components of photosystem I.[21] The results obtained suggest that association of Mg^{2+} with $P700^+$ may be relevant.

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