Effect of Oxidation State of Plastocyanin on the Remote Binding Site pK_a†

Joseph McGinnis, John D. Sinclair-Day, and A. Geoffrey Sykes*
Department of Inorganic Chemistry, The University, Newcastle upon Tyne NE1 7RU

The variation of rate constants for the $[Co(phen)_3]^{2+}$ [phen = 1,10-phenanthroline), $[Co(terpy)_2]^{2+}$ (terpy = 2,2';6',2"-terpyridine), and $[Ru(NH_3)_5(py)]^{2+}$ (py = pyridine) reductions of parsley plastocyanin PCu(II) with pH indicate an acid dissociation pK_a at the remote east face of 5.05, whereas for the oxidant $[Co(phen)_3]^{3+}$ with PCu(I) the value is 5.8. Implications of this change and the relevance to the reaction with cytochrome f are considered.

Effects of pH on the reactivity of the single (type 1) copper protein plastocyanin (M 10 500; E^0 370 mV) from parsley leaves are considered in this paper.[1] In previous work it has been demonstrated that at pH 7.5 an oxidant such as $[Co(phen)_3]^{3+}$ (phen = 1,10-phenanthroline) (370 mV) reacts ~ 61% at the Tyr 83 binding site (the so-called east face) of the molecule.[2] It is presumed that the rest of the reaction is at (or close to) the His 87 (north) site, which represents the closest possible approach (6 Å) from the surface of the protein to the Cu active site.[3] Rate constants for the reaction of $[Co(phen)_3]^{3+}$ at the Tyr 83 site decrease with pH (pK_a 5.8). Association of redox inactive $[Pt(NH_3)_6]^{4+}$ is also inhibited by protonation (pK_a 5.8).[4] With $[Ru(NH_3)_5(py)]^{2+}$ (py = pyridine) as reductant for PCu(II), rate constants for reaction solely at the Tyr 83 site also decrease with pH, but the pK_a is now 5.0.[5] Because of the extensive distribution of negative charge at the east face,[1,3] it cannot be assumed that $[Co(phen)_3]^{3+}$ and $[Ru(NH_3)_5(py)]^{2+}$ react at precisely the same locality, and are influenced by the same acid dissociation process. It has been possible to obtain further relevant information by studying the $[Co(phen)_3]^{2+}$ reduction of PCu(II), equation (1). Microscopic reversibility requires that

$$[Co(phen)_3]^{2+} + PCu(II) \longrightarrow [Co(phen)_3]^{3+} + PCu(I) \quad (1)$$

$[Co(phen)_3]^{2+}$ and $[Co(phen)_3]^{3+}$ react at an identical site (or sites) on plastocyanin.

Results and Discussion

Experimental details were as previously described.[1,4,5] The pH-jump method was used with the protein dialysed into 0.10 M NaCl at pH 7.5 (1 mM Tris–HCl) [Tris = tris(hydroxymethyl)-aminoethane], and solutions of complex made up in 40 mM buffer [acetate, 2-(*N*-morpholino)ethanesulphonic acid (mes), and Tris] at the required pH. Because of the potential lability of $[Co(phen)_3]^{2+}$ a 6:1 ratio of 1,10-phenanthroline to Co^{II} was used to retain the complex in the tris-chelated form. A reaction between phen and PCu(II) was observed, but this is at least an order of magnitude slower than the redox process. The variation of second-order rate constants $k_{exp.}$ with pH is indicated in Table 1 and the effect illustrated on a relative scale in the Figure. These values give a good fit to equation (2),

$$k_{exp.} = \frac{k_o K_a + k_H[H^+]}{K_a + [H^+]} \quad (2)$$

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

Table 1. The variation of second-order rate constants for the reduction of parsley PCu(II) (~1 × 10^{-5} M) with pH at 25 °C and I = 0.10 M (NaCl)*

Reductant $[Co(phen)_3]^{2+}$ at (1.2—2.6) × 10^{-4} M

pH	4.43	4.95	5.22	5.53	6.59	7.40
$10^{-3}k_{exp.}$/M^{-1} s^{-1}	1.380	1.665	1.96	2.13	2.44	2.48

Reductant $[Co(terpy)_2]^{2+}$ at (0.6—3.7) × 10^{-4} M

pH	4.25	4.54	4.80	5.10	5.30	5.48
$10^{-4}k_{exp.}$/M^{-1} s^{-1}	4.17	4.39	4.95	5.72	5.96	6.38
pH	5.71	6.02	6.30	6.75	7.50	
$10^{-4}k_{exp.}$/M^{-1} s^{-1}	6.73	6.87	7.15	7.34	7.46	

Reductant $[Ru(NH_3)_5(py)]^{2+}$ at (1.3—3.8) × 10^{-4} M

pH	3.96	4.31	4.42	4.47	4.73	4.92
$10^{-5}k_{exp.}$/M^{-1} s^{-1}	2.25	2.47	2.66	2.74	2.96	3.05
pH	5.25	5.48	5.70	6.50	7.28	
$10^{-5}k_{exp.}$/M^{-1} s^{-1}	3.50	3.72	4.00	4.38	4.45	

* Buffers used: acetate, pH 4.2—5.5; mes, pH 5.3—6.8; Tris, pH > 7.

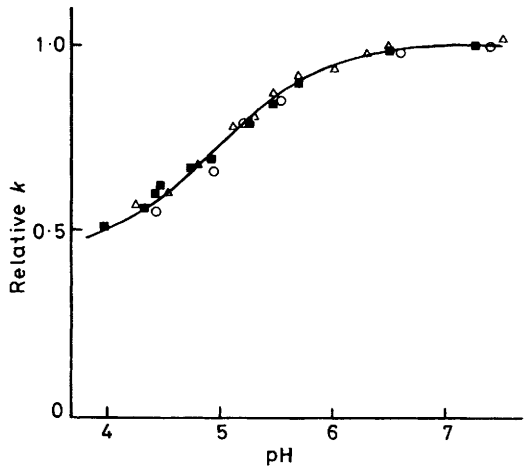

Figure. Variation of rate constants (on a relative scale) with pH for the reduction of parsley plastocyanin PCu(II) with $[Co(phen)_3]^{2+}$ (○), $[Co(terpy)_2]^{2+}$ (△), and $[Ru(NH_3)_5(py)]^{2+}$ (■) at 25 °C and I = 0.10 M (NaCl)

where the various constants are as defined in equations (3)—(5).

$$HPCu(II) \overset{K_a}{\rightleftharpoons} H^+ + PCu(II) \quad (3)$$

$$Co^{II} + HPCu(II) \xrightarrow{k_H} \quad (4)$$

$$Co^{II} + PCu(II) \xrightarrow{k_o} \quad (5)$$

Table 2. Summary of pK_a, k_H, and k_o values for the reduction of parsley PCu(II) at 25 °C and I = 0.10 M (NaCl)

Reductant	pK_a	$k_H/M^{-1}\ s^{-1}$	$k_o/M^{-1}\ s^{-1}$
$[Co(phen)_3]^{2+}$	5.08	1.11×10^3	2.49×10^3
$[Co(terpy)_2]^{2+}$	5.02	3.56×10^4	7.32×10^4
$[Ru(NH_3)_5(py)]^{2+}$	5.07	2.19×10^5	4.44×10^5

A non-linear least-squares treatment gives $pK_a = 5.08 \pm 0.06$, $k_H = (1.11 \pm 0.06) \times 10^3\ M^{-1}\ s^{-1}$, and $k_o = (2.49 \pm 0.03) \times 10^3\ M^{-1}\ s^{-1}$.

Further to substantiate this study, we have used $[Co(terpy)_2]^{2+}$ (terpy is the tridentate ligand 2,2′:6′,2″-terpyridine) as a reductant (E^0 260 mV) for PCu(II), Table 1. These results are also illustrated in the Figure. From a fit to equation (2), $pK_a = 5.02 \pm 0.05$, $k_H = (3.6 \pm 0.02) \times 10^4\ M^{-1}\ s^{-1}$, and $k_o = (7.3 \pm 0.01) \times 10^4\ M^{-1}\ s^{-1}$. We have also sought better to define the pK_a and amplitude of the effect of pH, with $[Ru(NH_3)_5(py)]^{2+}$ as reductant, Table 1. The new results give $pK_a = 5.07 \pm 0.05$, $k_H = (2.19 \pm 0.06) \times 10^5\ M^{-1}\ s^{-1}$, and $k_o = (4.4 \pm 0.1) \times 10^5\ M^{-1}\ s^{-1}$ (the previous pK_a was 5.0, with $k_H = 1.52 \times 10^5\ M^{-1}\ s^{-1}$).

The results obtained are collected in Table 2. Clearly all three reductants are influenced by the same pK_a of 5.05 ± 0.03. Since moreover $[Co(phen)_3]^{2+}$ and $[Co(phen)_3]^{3+}$ must use the same site, it can be concluded that all three reductants react at this same site, and that there are no variations with the different ligands. Ratios $(k_o - k_H)/k_o$ indicating the effectiveness of protonation are for $[Co(phen)_3]^{2+}$ (55%), $[Co(terpy)_2]^{2+}$ (51%), and $[Ru(NH_3)_5(py)]^{2+}$ (51%), which compare with the value for $[Co(phen)_3]^{3+}$ (58%).[2] The latter is about the same as the maximum effectiveness of the redox inactive complex $[(NH_3)_5CoNH_2Co(NH_3)_5]^{5+}$ on the $[Co(phen)_3]^{3+}$ reaction at pH 7.6 (61%),[4] suggesting that a proton and blocking complex induce the same net effect.[6] It has been concluded that this is a measure of the reaction taking place at the Tyr 83 east face site. The remaining ~ 40% reaction is believed to occur at an alternative site (or sites), the most likely candidate being the His 87 site.

A somewhat different situation pertains in the protein–protein reactions of PCu(II) with cytochrome c(II)[6] and cytochrome f(II),[7] when effects of protonation and blocking are much more extensive and approaching 100%, indicating much greater specificity for the east face. Values of pK_a for PCu(II) are 4.90 and 5.07 respectively from these studies.

It is concluded that for parsley plastocyanin, the Tyr 83 binding site pK_a of 5.8 for PCu(I) is shifted to 5.05 for PCu(II). One possible explanation of a pK_a of 5.8 is that two carboxylates share a proton, whereas one of 5.0 may stem from protonation at a single carboxylate only. If the carboxylates in question are at the 42—45 patch, then it seems at first unlikely that the change in charge on the Cu can be influential at 18 Å distance. However, much depends on the size of the dielectric constant within the protein, about which little is known. A conformation change which affects the charge distribution at the Tyr 83 site is an alternative explanation. The His 37 residue, which is co-ordinated to the Cu, is linked directly to the 42—45 patch by a chain of highly conserved amino-acid residues. Close proximity of the Tyr 83 residue to the co-ordinated Cys 84 may also be important. Fluorescence experiments on the nitro modified Tyr 83 derivative[8] have indicated sensitivity of the Tyr 83 residue to oxidation state of the Cu. Crystal structure information for poplar plastocyanin gives no evidence for changes at the east face as the oxidation state of the Cu changes. However, crystals were grown from 2.7 M $[NH_4]_2SO_4$,[3] and fluorescence experiments appear to demonstrate that this level of $[NH_4]_2SO_4$ excludes such changes at the east face.[8]

The sensitivity of protonation to oxidation state of the Cu reported here is no doubt important in the function of the protein. The natural photosynthetic electron-transport partners plastocyanin and cytochrome f are believed to have complementary surfaces which leads to efficient association prior to electron transfer. One problem is how dissociation of the product pair can occur following electron transfer. A conformation change after electron transfer bringing about a change in pK_a at the binding site is clearly one way in which this could be achieved.

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