Acid Dissociation Constants for Plastocyanin in the Cu^{I} State

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Acid dissociation constants for four plastocyanins in the PCu^{I} state determined by n.m.r. are compared with those determined by a kinetic method using $[Fe(CN)_6]^{3-}$ and $[Co(phen)_3]^{3+}$ (phen = 1,10-phenanthroline) as oxidants.

Plastocyanin is a single polypeptide of 99 amino acids (M.Wt. 10 500) containing a single Cu active site with characteristic type 1 properties including intense blue colour of the PCu^{II} state, $\lambda_{max.}$ 597 nm, $\varepsilon = 4500\,M^{-1}\,cm^{-1}$.[1] It is involved in electron transport between photosystems II and I of the chloroplast in higher plants and blue-green algae: E^0 (*in vitro*) for the PCu^{II}/PCu^{I} couple is ~360 mV. The Cu is co-ordinated to His37, Cys84, His87, Met92, (N, S, N, S donor atoms, respectively) in a distorted tetrahedral arrangement,[2] as illustrated for PCu^{I} in structure (**1**).[3] The switch-off in redox reactivity for PCu^{I} on decreasing the pH from 7.5 with

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inorganic complexes as oxidant has been noted.[4] This has been associated with protonation of His87,[5] although there has been a lack of quantitative agreement between kinetic and n.m.r. information. From crystallography it has been established that protonation gives a planar 3-co-ordinate structure, (**2**).[3]

The acid dissociation constant K_a for active site protonation at (A) is defined as in equation (1), and corresponds to

$$HP_A^+ \overset{K_a}{\rightleftharpoons} H^+ + P \quad (1)$$

protonation (and dissociation) of His87. Previously, n.m.r. pK_a values for PCu^I from spinach (4.9)[6] and the alga *Anabaena variabilis* (5.1)[6] have been in sharp disagreement with those obtained by the kinetic method (a procedure involving determination of rate constants at different [H$^+$] values)[7] for parsley PCu^I with $[Co(phen)_3]^{3+}$ (phen = 1,10-phenanthroline) (6.1) and $[Fe(CN)_6]^{3-}$ (5.7; more recent value 5.5), and for the reaction of spinach PCu^I with $[Co(phen)_3]^{3+}$ (5.7).[4] No satisfactory explanation of this discrepancy has been forthcoming.

Further n.m.r. and kinetic studies on PCu^I from parsley,[4] spinach,[4] French bean,[8,9] and *A. variabilis*,[6,10,11] which we report, have successfully resolved this anomaly. Isolation and handling procedures were as in work referenced. Table 1 summarises the results obtained. By inspection there is now some agreement of pK_a values determined by n.m.r. and kinetic studies with $[Fe(CN)_6]^{3-}$ as oxidant, indicating that the oxidation by $[Fe(CN)_6]^{3-}$ is strongly influenced by active site protonation. However, apparent pK_a values from studies with $[Co(phen)_3]^{3+}$ as oxidant are 0.4—0.8 units higher than those with $[Fe(CN)_6]^{3-}$. Also pK_a values for the reaction of parsley PCu^I with $[Fe(CN)_6]^{3-}$ and $[Co(phen)_3]^{3+}$ as well as from n.m.r. studies are significantly higher than those for PCu^I from the other sources.

The higher pK_a values with $[Co(phen)_3]^{3+}$ are considered first. These can be accounted for by a second protonation (K_a') effective at or near the binding site (B) used by $[Co(phen)_3]^{3+}$. Assuming the two protonations to be completely independent the modified scheme is shown in equations (2)—(4). Protonation at (A) gives redox-inactive protein. From equations (2)—(4) the expression (5) can be

$$\begin{array}{ccc} H_2P_{AB}^{2+} & \overset{K_a'}{\rightleftharpoons} & HP_A^+ \\ & {\scriptstyle +H^+} & \\ K_a \uparrow\downarrow + H^+ & & \uparrow\downarrow K_a \\ HP_B^+ & \overset{K_a'}{\rightleftharpoons} & P + H^+ \end{array} \quad (2)$$

$$P + \text{oxidant} \xrightarrow{k_1} \text{products} \quad (3)$$

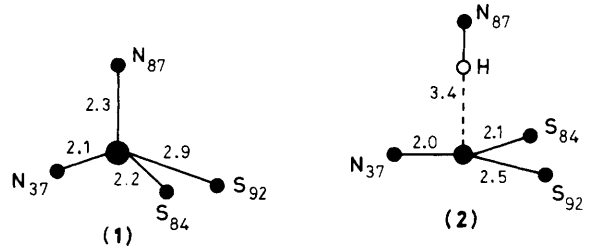
$$HP_B^+ + \text{oxidant} \xrightarrow{k_2} \text{products} \quad (4)$$

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

derived. The procedure which we have employed is to fit the experimental data using equation (5), with K_a fixed at the value determined by n.m.r. studies. Figure 1 indicates a best fit of experimental points for parsley PCu^I + $[Co(phen)_3]^{3+}$, with the lower curve for protonation at (A) (K_a), and the upper curve for protonation at (B) (K_a'). For reactions with $[Co(phen)_3]^{3+}$ the contribution from protonation at (B) is a major contributing factor, whereas active site protonation is the predominant influence on $[Fe(CN)_6]^{3-}$ oxidation. Values of pK_a' were obtained for parsley (5.8), spinach (5.6), French bean (5.7), and *A. variabilis* (5.7).

The behaviour observed is therefore consistent with different binding sites for $[Fe(CN)_6]^{3-}$ and $[Co(phen)_3]^{3+}$, which from earlier n.m.r. studies have been designated as close to His87 (sometimes referred to as the north site) and Tyr83 (the east site) respectively. Values of pK_a' close to 5.7 are high for protonation at a single carboxylate residue, but could be accounted for by proton sharing between two adjacent carboxylates. There are a number of carboxylates close to Tyr83, most notably (for plant plastocyanins) the highly conserved patch 42—45. Protonation of such residues will decrease their affinity for positively charged $[Co(phen)_3]^{3+}$ in the association step prior to electron transfer. The response of *A. variabilis* plastocyanin is of interest since there are fewer negatively charged residues and only 42 of 42—45 is a carboxylate. We note however the close proximity of Asp42 and Glu85 which are both adjacent to Tyr83, and could provide an adequate site both for association of $[Co(phen)_3]^{3+}$ and for protonation.

With regard to the high pK_a value for active site protonation of parsley plastocyanin, preliminary sequencing information[12] has indicated some striking differences compared with other higher plant plastocyanins, most notably the deletion of residues 57 and 58, which are some 10—12 Å from the active



Bond lengths in Å for PCu^I before protonation, structure (**1**), and after protonation, structure (**2**).

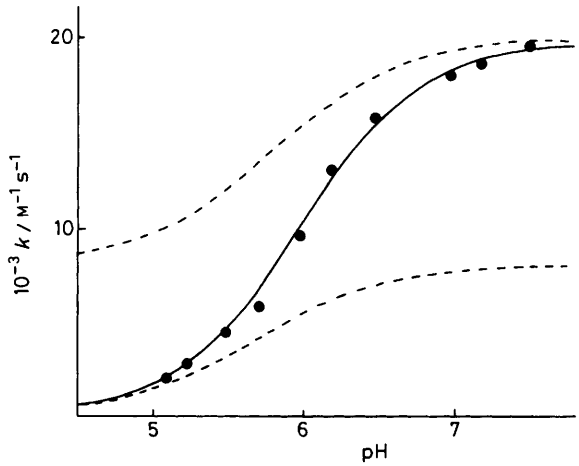


Figure 1. The variation of rate constants k (25 °C) for the oxidation of parsley PCu^I by $[Co(phen)_3]^{3+}$, I = 0.10 M (NaCl), with pH and simulated curves (broken lines) indicating the relative influences of pK_a as determined by n.m.r. [lower curve; protonation at (A)] and pK_a' [upper curve; protonation at (B)]. The solid curve indicates the fit to equation (5).

Table 1. A comparison of acid dissociation constants (25 °C) for plastocyanin PCu^{I} from different sources as determined by n.m.r. and kinetics, $I = 0.10$ M (NaCl). Experimentally determined values listed for $[Co(phen)_3]^{3+}$ are a composite of pK_a and pK_a' components.

Source of plastocyanin	N.m.r.	$[Fe(CN)_6]^{3-}$	$[Co(phen)_6]^{3+}$
Parsley	5.7[a]	5.5	6.1
Spinach	4.9[a]	4.9	5.7
French bean	4.85[a]	4.6	5.5
A. variabilis	5.1[b]	4.8	5.5

[a] Conditions 0.10 M phosphate, and 40 °C. Accuracy $\leq \pm 0.10$. [b] 0.02 M phosphate buffer.

site. Of the 17 other plastocyanins fully sequenced only plastocyanin from the algal source *Chlorella* is known to have this same feature.[13]

Interestingly, all other blue type 1 proteins investigated including azurin,[14] stellacyanin,[15] umecyanin,[16] and rusticyanin,[17] do not display active site protonation as observed for plastocyanin. An important question which is raised is the relevance protonation might have to the function of plastocyanin in the chloroplast, where protons as well as electrons have to be transferred.

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