Stopped-flow Kinetic Studies on Electron-transfer Reactions of Blue Copper Proteins. Evidence for an Initial Association in Reactions of Plastocyanin with Inorganic Complexes

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Summary Protein–complex association has been detected prior to electron-transfer in reactions involving parsley plastocyanin Cu^I and Cu^{II}, and has a significant influence on the overall electron-transfer process.

Plastocyanins and azurins are blue copper (type 1) proteins which function as electron-transfer mediators by making use of Cu^I (colourless) and Cu^{II} (blue) oxidation states.[1,2] They contain 1 Cu atom per protein (mol. wt. 10,700 and 13,900, respectively), which is probably in a distorted tetrahedral ligand environment.[1] The latter is believed to be highly appropriate for efficient outer-sphere electron-transfer.[3]

Data obtained for the tris(1,10-phenanthroline)cobalt(III) oxidation of plastocyanin, PCu^I, from parsley[4] are in excellent agreement with the recent results of Gray *et al.*[5] for the $[Co(phen)_3]^{3+}$ oxidation of both bean PCu^I, and *Pseudomonas aeruginosa* azurin, ACu^I (Table 1). Other results for the $[Fe(CN)_6]^{3-}$ oxidation of PCu^I and ACu^I, and $[Fe(CN)_6]^{4-}$ reduction of PCu^{II} and ACu^{II}† are included in Table 1. Although the kinetic parameters are in close agreement for reactions of the two proteins with a common reagent, a wide range of activation parameters ($\Delta H^\ddagger$ and $\Delta S^\ddagger$) is obtained which is difficult to explain.

By using higher reactant concentrations $[Co(phen)_3{}^{3+}] = (0{\cdot}1—4{\cdot}0) \times 10^{-3}$ M, as compared to $(0{\cdot}1—1{\cdot}2) \times 10^{-3}$ M used by Gray *et al.*,[5] a non-linear dependence of first-order rate constants k_{obs} on oxidant is obtained, see Figure. Plots of $(k_{obs})^{-1}$ against $[Co(phen)_3{}^{3+}]^{-1}$ are linear, which is consistent with the reaction sequence in equations (1) and (2), and a dependence as in equation (3). Values of K and k_{et}

Table 1. A summary of kinetic data for the overall 1 : 1 redox process.

Reactants	k(25 °C)/ l mol^{-1} s^{-1}	$\Delta H^\ddagger$ /kcal mol^{-1}	$\Delta S^\ddagger$ /cal K^{-1} mol^{-1}
$PCu^I + [Co(phen)_3]^{3+}$ [a]	$2{\cdot}9 \times 10^3$	14·3	6
$PCu^I + [Co(phen)_3]^{3+}$ [b,c]	$4{\cdot}9 \times 10^3$	14·0	5
$ACu^I + [Co(phen)_3]^{3+}$ [c]	$3{\cdot}2 \times 10^3$	14·3	5
$PCu^I + [Fe(CN)_6]^{3-}$ [a]	$9{\cdot}4 \times 10^4$	−3·3	−47
$ACu^I + [Fe(CN)_6]^{3-}$ [d]	$2{\cdot}7 \times 10^4$	−4·1	−52
$[Fe(CN)_6]^{4-} + PCu^{II}$ [a]	$1{\cdot}9 \times 10^4$	6·3	−17·5
$[Fe(CN)_6]^{4-} + ACu^{II}$ [d]	345	5·9	−27·1

[a] Parsley plastocyanin; $I = 0{\cdot}10$ M (NaCl), 10^{-3} M Na^+ phosphate buffer (this work). [b] Bean plastocyanin; $I = 0{\cdot}1$ M (NaCl), Na^+ phosphate buffer. [c] Ref. 5. [d] $I = 0{\cdot}22$ M from K^+ phosphate buffer. (ref. 7)

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† The reduction potential of the PCu^{II}–PCu^I couple is *ca.* 360 mV (see ref. 6) and for the $[Fe(CN)_6]^{3-/4-}$ couple is 410 mV (I. M. Kolthoff and W. J. Tomsicek, *J. Phys. Chem.*, 1935, **39**, 945). By having the $[Fe(CN)_6]^{3-}$ or $[Fe(CN)_6]^{4-}$ reactant in large excess it was possible to study the forward and reverse reactions separately by the stopped-flow technique. The temperature-jump technique was used to obtain data in ref. 7.

TABLE 2.
Summary of the protein–complex association constants, rate constants for the electron transfer step, and the corresponding enthalpic and entropic terms at 25 °C.

Reactants	K /l mol^{-1}	ΔH° /kcal mol^{-1}	ΔS° /cal K^{-1} mol^{-1}	k_{et} /s^{-1}	$\Delta H^\ddagger_{et}$ /kcal mol^{-1}	$\Delta S^\ddagger_{et}$ /cal K^{-1} mol^{-1}
$PCu^I + [Co(phen)_3]^{3+}$ [a]	167	10	45	17·9	4·3	−39
$ACu^I + [Fe(CN)_6]^{3-}$ [b]	610	−7·7	−13·1	45	3·6	−38·9
$[Fe(CN)_6]^{4-} + PCu^{II}$ [a]	110	−5·1	−7·8	170	11·4	−9·7
$[Fe(CN)_6]^{4-} + ACu^{II}$ [b]	54	−5·5	−10·5	6·4	11·4	−16·6

[a] Parsley plastocyanin; $I = 0{\cdot}10$ M (NaCl), 10^{-2} M Na^+ phosphate buffer (this work). [b] $I = 0{\cdot}22$ M from K^+ phosphate buffer (ref. 7).

$$PCu^I + [Co(phen)_3]^{3+} \overset{K}{\rightleftharpoons} PCu^I,[Co(phen)_3]^{3+} \qquad (1)$$

$$PCu^I,[Co(phen)_3]^{3+} \xrightarrow{k_{et}} PCu^{II} + [Co(phen)_3]^{2+} \qquad (2)$$

$$k_{obs} = \{k_{et}K[Co(phen)_3{}^{3+}]\}/\{1 + K[Co(phen)_3{}^{3+}]\} \qquad (3)$$

were obtained from a least-squares treatment of equation (3). Similarly, by using a wide range of $[Fe(CN)_6{}^{4-}] = (0{\cdot}3$—$2{\cdot}0) \times 10^{-3}$ M in the reduction of PCu^{II}, a non-linear dependence of k_{obs} on reductant was observed. The negative $\Delta H^\ddagger$ for $[Fe(CN)_6]^{3-}$ oxidation of PCu^I, Table 1, in itself carries an implication of a prior association such as in equation (1).[8] A summary of K and k_{et} data is given in Table 2.

Meaningful trends can now be distinguished. Thus it can be seen that ΔH° and ΔS° for protein–complex association are very much dependent on the type of complex used. For the association step (preceding electron transfer) in the reaction of $[Fe(CN)_6]^{4-}$ with $[Co(NH_3)_5py]^{3+}$ (py = pyridine), it has been reported that (at 25 °C) $K = 2100$ l mol^{-1}, ΔH° *ca.* 0 kcal mol^{-1} and $\Delta S^\circ = 15$ cal K^{-1} mol^{-1}.[9] We suggest that a significant factor contributing to ΔS° (Table 2) is the charge. For reactions of opposite charge, charge neutralisation occurs in going from the reactants to the transition state, water of solvation is released, and ΔS° is positive, whereas for reactions of like charge an increase in solvation occurs and ΔS° is negative. On this basis the effective charge on both proteins is negative.‡ Isoelectric points for plastocyanin (*ca.* 4·3)[2] and azurins (4·9)[7] indicate that the overall charge on the proteins is negative under the conditions of the reaction, but for the present we are not able to conclude whether it is this charge or that of a localised binding site which is effective. The numerically large values of ΔH° suggest specific complex–protein interactions; *e.g.* H-bonding in the case of cyanide complexes, and interaction of the phenanthroline ligand with organic groups on the protein are possibilities.[10] In the case of $[Fe(CN)_6]^{4-}$ reactions the favourable ΔH° outweighs the unfavourable ΔS° for similarly charged reactants and results in a significant K value. A further feature is the close agreement of $\Delta H^\ddagger_{et}$ and $\Delta S^\ddagger_{et}$ values, firstly for reactions of Cu^I and secondly for the Cu^{II} proteins. The large negative values of $\Delta S^\ddagger_{et}$ for both $[Co(phen)_3]^{3+}$ and $[Fe(CN)_6]^{3-}$ oxidations of Cu^I suggest conformational (or possibly geometric) changes involving the Cu^I protein.[11] For two oppositely charged reactants values of $\Delta S^\ddagger_{et}$ of around zero or small and positive (consistent with charge neutralisation) might have been expected.[9]

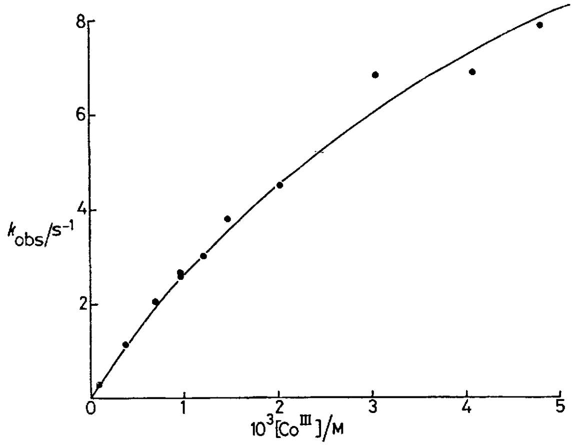

FIGURE. Dependence of first-order rate constants, k_{obs} at 25 °C on $[Co(phen)_3{}^{3+}]$ for the oxidation of parsley PCu^I at pH 7·5 (Na^+ phosphate buffer), $I = 0{\cdot}10$M (NaCl).

From the data presented it is clear that factors influencing electron transfer cannot be assessed solely by consideration of overall parameters (Table 1) as has been attempted in much work to date. It is interesting to note that the reaction of PCu^I with cytochrome f, its natural redox partner, occurs with an overall positive entropy of activation, presumably owing to interaction of oppositely charged groups.[6]

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‡ The converse was suggested in ref. 7 although no clear reasoning was presented. It is hoped that further studies on stellacyanin (isoelectric point 9·86, B. Reinhammar, *Biochim. Biophys. Acta*, 1970, **205**, 35) at present in progress will give information on this point.

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[9] A. J. Miralles, R. E. Armstrong, and A. Haim, *J. Amer. Chem. Soc.*, 1977, **99**, 1416; see also further examples in L. A. A. Oliveira, H. E. Toma, and E. Geisbrecht, *Inorg. Chim. Acta*, 1977, **22**, 269.
[10] Such effects are often apparant as stacking effects, *e.g.* G. R. Cayley and D. W. Margerum, *J.C.S. Chem. Comm.*, 1974, 1002; E. Frieden, *J. Chem. Educ.*, 1975, **52**, 754; P. R. Mitchell and H. Sigel, *Angew. Chem.*, 1976, **15**, 548.
[11] It has been suggested previously by E. Stellwagen and R. G. Shulman, *J. Mol. Biol.*, 1973, **80**, 559 that $\Delta S^\ddagger_{et} = -31{\cdot}4$ cal K^{-1} mol^{-1} for the oxidation of cytochrome c(II) with $[Fe(CN)_6]^{3-}$ is consistent with a conformational change.