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# CENTENARY LECTURE*

# Long-range Electron-transfer in Blue Copper Proteins

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## 1 Introduction

It is widely recognized that blue copper proteins function as electron-transfer agents in biological systems.[1-10] Plastocyanin, for example, which is one of the most thoroughly studied of all the blue copper proteins, is a key component of the electron-transfer apparatus in the chloroplasts of green leaves. In its oxidized form, here referred to as the $Cu^{2+}$ state, it is intensely blue, because of 600 nm absorption attributable to charge transfer from a cysteine sulphur to the copper. The review by Sykes,[9] based on his Tilden Lecture, is an excellent place to read about the structure and properties of plastocyanin.

Because it is important to know what factors control the rate of transfer of an electron through a protein interior, bioinorganic chemists have investigated reactions involving plastocyanin and other blue copper proteins with redox-active inorganic complexes as artificial substrates. In the early experiments, the kinetics of many of these copper-protein–inorganic-reagent electron-transfer reactions were found to be second order, and bimolecular mechanisms were proposed.[4,5]

In 1977, Segal and Sykes made an important advance when they discovered that the kinetics of oxidation of reduced spinach or parsley plastocyanin, $Pl(Cu^{+})$, by $Co(phen)_3^{3+}$ exhibited signs of saturation behaviour, thereby suggesting that rate-limiting ($\sim 20\ s^{-1}$) electron-transfer might occur within a relatively stable $Pl(Cu^{+}):Co(phen)_3^{3+}$ precursor complex.[11,12] N.m.r. spectroscopic studies pinpointed the probable site of $Co(phen)_3^{3+}$ binding, indicating that the redox

* Delivered at a Symposium of the Dalton Division of the Royal Society of Chemistry, Scientific Societies' Lecture Theatre, London, on 14th March, 1985.

[1] R. Malkin and B. G. Malmström, *Adv. Enzymol.*, 1970, **33**, 177.
[2] R. J. P. Williams, *Inorg. Chim. Acta Rev.*, 1971, **5**, 137.
[3] J. A. Fee, *Struct. Bonding (Berlin)*, 1975, **23**, 1.
[4] R. A. Holwerda, S. Wherland, and H. B. Gray, *Ann. Rev. Biophys. Bioeng.*, 1976, **5**, 363.
[5] S. Wherland and H. B. Gray in 'Biological Aspects of Inorganic Chemistry,' ed. A. W. Addison, W. R. Cullen, D. Dolphin, and B. R. James, Wiley, New York, 1977, p. 289.
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[7] 'Copper Proteins', ed. T. G. Spiro, Wiley, New York, 1981.
[8] 'Copper Proteins and Copper Enzymes', ed. R. Lontie, CRC Press, 1984, Vols. 1—3.
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[10] A. G. Lappin in 'Metal Ions in Biological Systems', ed. H. Sigel, Dekker, New York, 1981, Vol. 13, Chap. 2, p. 15.
[11] M. G. Segal and A. G. Sykes, *J. Chem. Soc., Chem. Commun.*, 1977, 764.
[12] M. G. Segal and A. G. Sykes, *J. Am. Chem. Soc.*, 1978, **100**, 4585.

centres ($Cu^+$ and $Co^{3+}$) are separated by over 10 Å in the precursor complex.[13–15] But what is the $Cu^+$ to $Co^{3+}$ distance at the instant of electron-transfer? And what is the distance dependence of the electron-transfer rate?

## 2 Ruthenium-modified Proteins

I felt several years ago that to address such questions would require a new type of experiment, one in which the redox-active metal complex is tightly bonded to the protein in both its oxidation states. In thinking about this experiment, I was influenced by Farver and Pecht's work on $Cr^{3+}$ derivatives of blue copper proteins,[16–18] which I felt was a step in the right direction. The chromium derivatives themselves, however, were not suitable for the type of experiment I had in mind, because the $Cr^{2+}$ state is substitutionally labile.

After a number of false starts, my co-workers and I found that ruthenium reagents were the right choice. To begin with, the reagent $a_5Ru(OH_2)^{2+}$ ($a = NH_3$) will bind to histidine on protein surfaces at rates that are reasonable for protein modification experiments.[19–22] Each of the resulting 'ruthenated' derivatives, $a_5Ru(His)^{2+}$-protein, can be oxidized readily to $a_5Ru(His)^{3+}$-protein, and in this form the derivative can be purified by ion-exchange chromatography. The $a_5Ru(His)^{3+/2+}$ label is substitution-inert and its reduction potential of $\sim 0.1$ V *vs.* NHE is convenient for electron-transfer studies involving blue proteins.[22] Perhaps the most important property of $a_5Ru^{3+/2+}$, however, is its hydrophilicity; it is this property that vastly reduces the affinity of the label for the protein interior, thereby making it structurally non-perturbing to the protein. Indeed, based on the spectroscopic and electrochemical studies done to date on $a_5Ru^{3+}$-modified proteins, the evidence is overwhelming that the protein's native conformation is not affected significantly.[19–28]

[13] P. M. Handford, H. A. O. Hill, R. W.-K. Lee, R. A. Henderson, and A. G. Sykes, *J. Inorg. Biochem.*, 1980, **13**, 83.
[14] D. J. Cookson, M. T. Hayes, and P. E. Wright, *Nature (London)*, 1980, **283**, 682.
[15] D. J. Cookson, M. T. Hayes, and P. E. Wright, *Biochim. Biophys. Acta*, 1980, **591**, 162.
[16] O. Farver and I. Pecht, *Isr. J. Chem.*, 1981, **21**, 13.
[17] O. Farver and I. Pecht, *Proc. Natl. Acad. Sci. U.S.A.*, 1981, **78**, 4190.
[18] O. Farver, Y. Blatt, and I. Pecht, *Biochemistry*, 1982, **21**, 3556.
[19] K. M. Yocom, J. B. Shelton, J. R. Shelton, W. A. Schroeder, G. Worosila, S. S. Isied, E. Bordignon, and H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 7052.
[20] K. M. Yocom, J. R. Winkler, D. G. Nocera, E. Bordignon, and H. B. Gray, *Chem. Scr.*, 1983, **21**, 29.
[21] R. Margalit, I. Pecht, and H. B. Gray, *J. Am. Chem. Soc.*, 1983, **105**, 301.
[22] R. Margalit, N. M. Kostić, C.-M. Che, D. F. Blair, H.-J. Chiang, I. Pecht, J. B. Shelton, J. R. Shelton, W. A. Schroeder, and H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, 1984, **81**, 6554.
[23] J. R. Winkler, D. G. Nocera, K. M. Yocom, E. Bordignon, and H. B. Gray, *J. Am. Chem. Soc.*, 1982, **104**, 5798.
[24] S. S. Isied, G. Worosila, and S. J. Atherton, *J. Am. Chem. Soc.*, 1982, **104**, 7659.
[25] S. S. Isied, C. Kuehn, and G. Worosila, *J. Am. Chem. Soc.*, 1984, **106**, 1722.
[26] D. G. Nocera, J. R. Winkler, K. M. Yocom, E. Bordignon, and H. B. Gray, *J. Am. Chem. Soc.*, 1984, **206**, 5145.
[27] N. M. Kostić, R. Margalit, C.-M. Che, and H. B. Gray, *J. Am. Chem. Soc.*, 1983, **105**, 7765.
[28] R. J. Crutchley, W. R. Ellis, Jr., and H. B. Gray, *J. Am. Chem. Soc.*, 1985, **107**, 5002.

### 3 Ruthenated Azurin

A bacterial protein, *Pseudomonas aeruginosa* azurin (Az), has been the main focus of our long-range electron-transfer studies involving blue copper. The crystal structure of Az[29] shows that the blue copper site ($CuN_2SS^*$; N = His, S = Cys, S* = Met) is buried in the hydrophobic interior of the protein (Figure 1). The intense absorption band at 625 nm in the oxidized protein, $Az(Cu^{2+})$, is attributable to an allowed $S(Cys) \rightarrow Cu^{2+}$ charge-transfer transition.[30,31] The reduced protein, $Az(Cu^{+})$, does not absorb appreciably in this region.

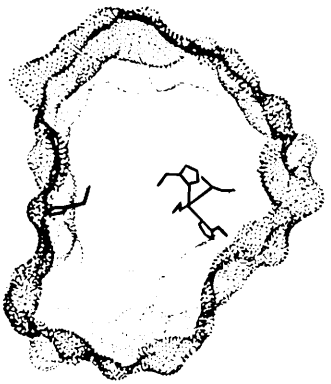
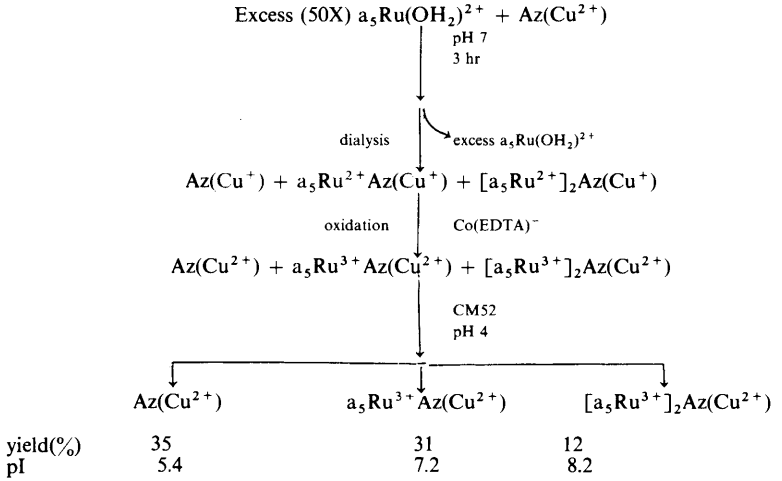


**Figure 1** *Surface of azurin, with the front section removed to show the imidazole ring of* His-83 *(left surface) and the blue copper site* ($CuN_2SS^*$) *in the interior. The copper ligands are* His-46, His-117, Cys-112, *and* Met-121. *The surface consists of points of contact between a sphere of* 3 Å *radius (to simulate* $a_5Ru(OH_2)^{2+}$*) and the atomic van der Waals spheres of the protein* (Reproduced by permission from *ref.* 22)

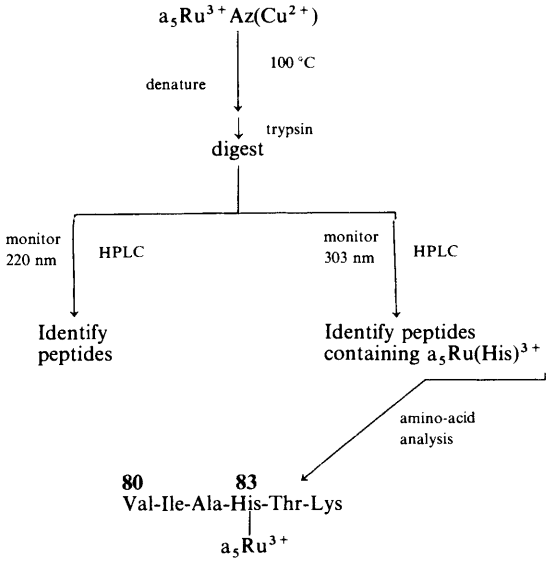
In addition to the copper ligands, Az possesses two histidines, His-35 and His-83, that are potentially available for $a_5Ru^{3+}$-modification. However, His-35 is buried in the protein near the blue copper site, and it appears to be inaccessible to $a_5Ru(OH_2)^{2+}$. On the other hand, His-83 is exposed at the surface, providing an attractive site for $a_5Ru^{3+/2+}$ binding (Figure 1).

The reaction of $a_5Ru(OH_2)^{2+}$ with azurin produces two ruthenated products, RuAz and $Ru_2Az$, as outlined in Scheme 1.[22] Only the singly modified derivative, RuAz, will be discussed here. As expected, the $a_5Ru^{3+}$ binding site was found to be His-83 in peptide-mapping experiments (Scheme 2). The absorption spectrum of the isolated $a_5Ru(His\text{-}83{:}peptide)^{3+}$ is virtually the same as the spectrum of an independently characterized $a_5Ru(His)^{3+}$ complex (Figure 2).[22]

[29] E. T. Adman and L. H. Jensen, *Isr. J. Chem.*, 1981, **21**, 8.
[30] E. I. Solomon, J. W. Hare, D. M. Dooley, J. H. Dawson, P. J. Stephens, and H. B. Gray, *J. Am. Chem. Soc.*, 1980, **102**, 1168.
[31] D. R. McMillin, R. C. Rosenberg, and H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, 1974, **71**, 4760.




**Scheme 1** *Preparation and purification of ruthenated azurins*




**Scheme 2** *Peptide mapping of* $a_5Ru^{3+}Az(Cu^{2+})$

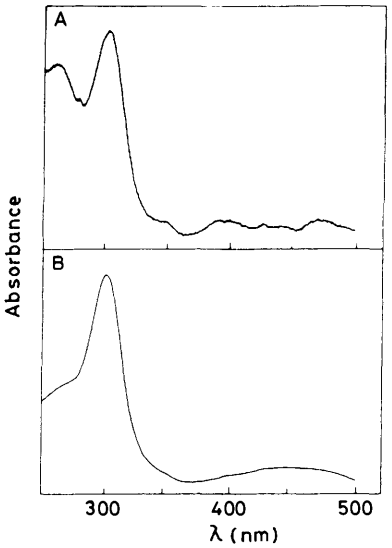



**Figure 2** *A: Absorption spectrum of* $a_5Ru(Val\text{-}Ile\text{-}Ala\text{-}(His\text{-}83)\text{-}Thr\text{-}Lys)^{3+}$ *(Scheme 2); B: Absorption spectrum of an aqueous solution of* $[a_5Ru(His)]Cl_3$
(Reproduced by permission from *ref.* 22)

## 4 Electron-transfer in $a_5Ru(His\text{-}83)^{2+}Az(Cu^{2+})$

The two redox-active units in $a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+})$ are separated by 11.8 Å (Figure 3). Electrochemical (Table 1) and spectroscopic (Table 2) data show that the blue copper site is not perturbed by the presence of the $a_5Ru(His\text{-}83)^{3+}$ group. The ruthenated protein is ideally structured for a long-range electron-transfer experiment. Selective reduction of the surface-accessible $a_5Ru(His\text{-}83)^{3+}$ group will produce $a_5Ru(His\text{-}83)^{2+}Az(Cu^{2+})$. If the experiments are done at very low protein concentrations, then the electron-transfer should be intramolecular.

**Table 1** *Thermodynamic parameters for the reduction of native and ruthenated azurins* (0.1 M *phosphate*, pH 7.0)

| *Parameter* | $Az(Cu^{2+/+})^a$ | $a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+/+})^b$ | $a_5Ru(His)^{3+/2+c}$ |
|---|---|---|---|
| $E^0$ (mV *vs.* NHE, 25 °C) | 308(±2) | 320(±2) | 80(±5) |
| $\Delta S^0$ (cal deg-mol$^{-1}$) | −31.7(±1.2) | −26.8(±0.8) | −3.4(±0.2) |
| $S^0_{red} - S^0_{ox}$ (cal deg-mol$^{-1}$) | −16.1(±1.2) | −11.2(±0.8) | 12.2(±2) |
| $\Delta G^0$ (kcal mol$^{-1}$) | −7.10(±0.05) | −7.39(±0.05) | −1.96(±0.12) |
| $\Delta H^0$ (kcal mol$^{-1}$) | −16.6(±0.4) | −15.4(±0.3) | −3.0(±0.8) |

[a] From V. T. Taniguchi, N. Sailasuta-Scott, F. C. Anson, and H. B. Gray, *Pure Appl. Chem.*, 1980, **52**, 2275. [b] *Ref.* 22. [c] *Ref.* 26

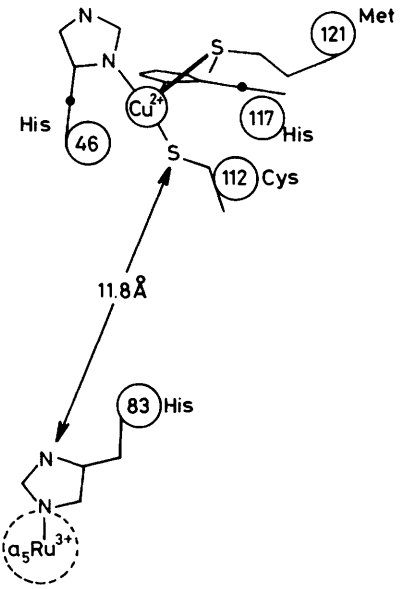



**Figure 3** *View of selected parts of* $a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+})$. *The edge-to-edge distance between the two electron-transfer units is* 11.8 Å
(Reproduced by permission from *ref.* 27)

**Table 2** *Spectroscopic parameters for the blue copper sites in native and ruthenated azurins*[a]

| *Spectroscopy* | *Parameter* ($Cu^{2+}$) | $Az(Cu^{2+})$[b] | $a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+})$ |
|---|---|---|---|
| E.p.r. | $g_{\|\|}$ | 2.271 | 2.271 |
| | $a_{\|\|}$ (G) | 57 | 57 |
| | $g_x$ | 2.065 | 2.065 |
| | $g_y$ | 2.043 | 2.044 |
| Resonance Raman | $\nu(cm^{-1})$ | 369 | 370 |
| | $[CuN_2SS^*]$ | 404 | 404 |
| | | 424 | 425 |

[a] *Ref.* 22. [b] Resonance Raman data for the native protein are from T. J. Thamann, P. Frank, L. J. Willis, and T. M. Loehr, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 6390

Rapid reduction of the surface $a_5Ru(His\text{-}83)^{3+}$ group is achieved by flash photolysis of solutions containing $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) and EDTA:

$$Ru(bpy)_3^{2+} \xrightarrow{h\nu} {}^*Ru(bpy)_3^{2+} \quad (1)$$

$${}^*Ru(bpy)_3^{2+} + a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+}) \xrightarrow{\text{fast}} a_5Ru(His\text{-}83)^{2+}Az(Cu^{2+}) + Ru(bpy)_3^{3+} \quad (2)$$

$$*Ru(bpy)_3^{2+} + a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+}) \xrightarrow{fast} a_5Ru(His\text{-}83)^{3+}Az(Cu^{+}) + Ru(bpy)_3^{3+} \quad (3)$$

$$Ru(bpy)_3^{3+} \xrightarrow{EDTA} Ru(bpy)_3^{2+} \quad (4)$$

Following the flash, all the $a_5Ru(His\text{-}83)^{2+}Az(Cu^{2+})$ formed in equation 2 is converted into $a_5Ru(His\text{-}83)^{3+}Az(Cu^{+})$ by long-range electron-transfer (Figure 4).

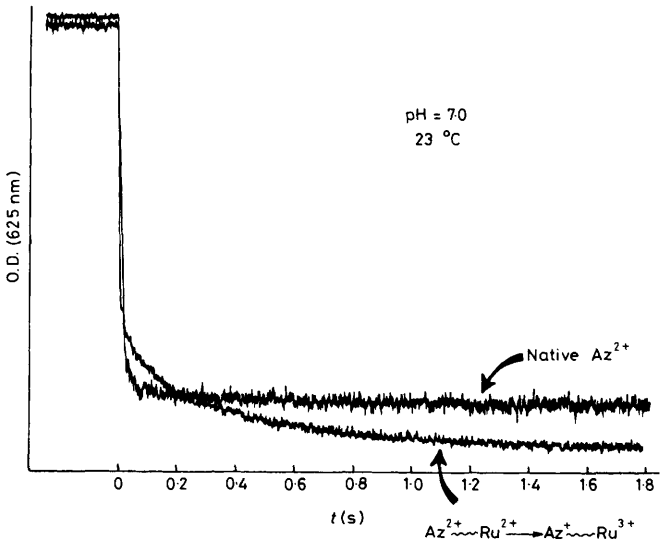



**Figure 4** *Changes in* 625 nm *absorbance following flash photolysis of solutions containing* $Az(Cu^{2+})$ *or* $a_5Ru(His\text{-}83)^{3+}Az(Cu^{2+})$ *and* $Ru(bpy)^{2+}$/EDTA. *The initial O.D. decrease is due to reduction of* $Cu^{2+}$ *by* $*Ru(bpy)_3^{2+}$
(Reproduced by permission from *ref.* 27)

$$a_5Ru(His\text{-}83)^{2+}Az(Cu^{2+}) \xrightarrow{k_{et}} a_5Ru(His\text{-}83)^{3+}Az(Cu^{+}) \quad (5)$$

Strict first-order kinetics are observed, yielding a rate constant of 1.9 $s^{-1}$ that, surprisingly, is temperature-independent from −8 to 53 °C (Figure 5).

## 5 Reorganization Energy

The temperature-independent rate of long-range electron-transfer ($Ru^{2+} \longrightarrow Cu^{2+}$) in ruthenated azurin is strong evidence that the reorganization enthalpy of the blue copper is relatively small. Taking the electron-transfer exothermicity into account[32] (Table 1), the blue copper reorganization enthalpy, $\Delta H^*(Cu)$, is estimated to be ~0.3 eV,[22] a value comparable to that obtained for the low-spin haem in $a_5Ru(His\text{-}33)$ cytochrome *c*.[26] The small reorganization enthalpy confirms that blue copper is optimally structured for its electron-transfer

[32] R. A. Marcus and N. Sutin, *Inorg. Chem.*, 1975, **14**, 213.

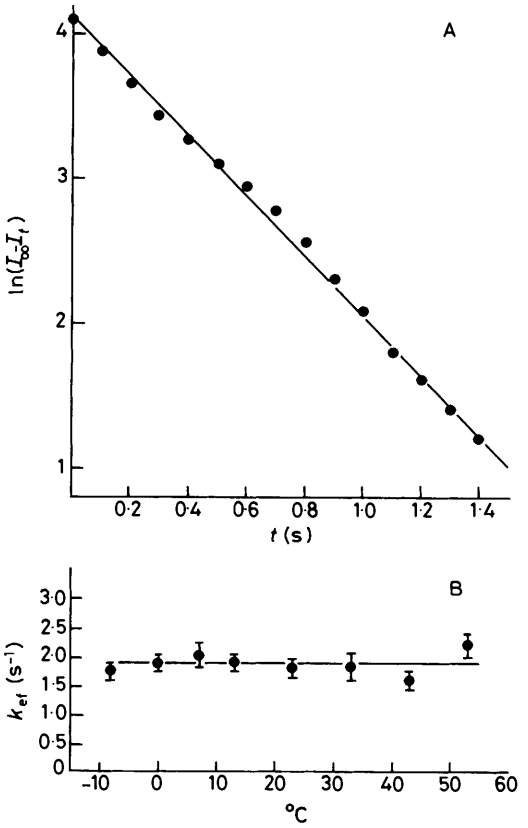



**Figure 5** *A: First-order kinetic plot for the reduction of* $Cu^{2+}$ *in flash-generated* $a_5Ru(His\text{-}83)^{2+}Az(Cu^{2+})$ *at* 23 °C; *B: Temperature dependence of the rate constant for long-range* $Ru^{2} \longrightarrow Cu^{2+}$ *electron-transfer*
(Reproduced by permission from *ref.* 27)

function;[2,33–35] relatively small changes in the copper–ligand bonds occur when blue $Cu^{2+}$ is reduced to $Cu^{+}$,[9] and, since the blue copper is embedded in the hydrophobic interior of azurin, the outer sphere (solvation) contribution to the reorganization enthalpy also is very small. The situation is parallel to that for cytochrome *c*, which has been treated in detail by Warshel and co-workers.[36]

A summary of the results of long-range electron-transfer experiments on ruthenated derivatives of azurin, horse heart cytochrome *c* (cyt *c*), and sperm whale

[33] H. B. Gray and B. G. Malmström, *Comments Inorg. Chem.*, 1983, **2**, 203.
[34] D. R. McMillin, *J. Chem. Educ.*, 1985, **62**, 997.
[35] O. Farver and I. Pecht, in *ref.* p. 151.
[36] A. K. Churg, R. M. Weiss, A. Warshel, and T. Takano, *J. Phys. Chem.*, 1983, **87**, 1683.

myoblogin (Mb) is given in Table 3. The long-range ($Ru^{2+} \longrightarrow Fe^{3+}$) electron-transfer is much slower in $a_5$Ru(His-48)Mb than the corresponding transfers in the two other proteins, which is attributable in part to the relatively large reorganization enthalpy of the Mb high-spin haem ($\sim 0.9$ eV).[28] The high-spin ferrihaem must lose a water ligand upon reduction, and this required rearrangement in the co-ordination environment is a substantial barrier to electron-transfer.

**Table 3** *Long-range electron-transfer data for ruthenated proteins at* 25 °C[a]

| *Protein* | *d*(Å) | $\Delta E^0$ (V) | $k(Ru^{2+} \longrightarrow Fe^{3+}$ or $Cu^{2+})$ ($s^{-1}$) | $\Delta H^*$(eV) (Fe or Cu) |
|---|---|---|---|---|
| $a_5$Ru(His-33)$^{2+}$cyt*c*($Fe^{3+}$) | 11.8 | 0.18 | 30 | 0.3 |
| $a_5$Ru(His-83)$^{2+}$Az($Cu^{2+}$) | 11.8 | 0.24 | 2 | 0.3 |
| $a_5$Ru(His-48)$^{2+}$Mb($Fe^{3+}$) | 13.3 | −0.02 | 0.02 | 0.9 |

[a] *d* is the closest edge-to-edge donor–acceptor distance; data for cyt *c* from *ref.* 26; data for Az from *ref.* 22; data for Mb from *ref.* 28

## 6 Donor–Acceptor Electronic Coupling

It is interesting that the temperature-independent rate of long-range (11.8 Å) electron-transfer in $a_5$Ru(His-33)cyt *c* is an order of magnitude faster than the 11.8 Å $Ru^{2+} \longrightarrow Cu^{2+}$ transfer in ruthenated azurin. Since the driving force is slightly larger in the azurin case, one possibility is that the enhanced rate in cytochrome *c* is due to better electronic coupling between the redox partners, that is, between $a_5$Ru-(His-33)$^{2+}$ and the low-spin haem *c* ($Fe^{3+}$). The origin of the apparent enhancement in donor–acceptor coupling in the low-spin haem system, however, is not known.

The rates of long-range (12—14 Å) electron-transfer we have found in three proteins (Table 3) are strikingly slower than those observed by Calcaterra, Closs, and Miller (CCM)[37,38] in their studies of relatively rigid donor–(hydrocarbon spacer)–acceptor organic molecules. In CCM's work on the 16-biphenyl-5-α-androstane-acceptor system, for example, pulse radiolysis triggers the reduction of biphenyl to its radical anion, and electron-transfer from biphenyl$^{\cdot-}$ across $\sim 10$ Å of steroid frame to an acceptor (*e.g.*, naphthyl) is monitored. Transfer rates of $10^6$ to $10^9$ $s^{-1}$ have been measured by CCM, upwards of $10^5$ times the protein rates at comparable donor–acceptor edge–edge distances.

The factor that could be responsible for this impressive difference in rates is the energy (reduction potential) of the donor. In the hydrocarbon (steroid) experiments, the donor (*e.g.*, biphenyl$^{\cdot-}$) has a very negative reduction potential ($< -2$V *vs.* NHE), and this could greatly enhance its coupling to the acceptor through the steroid (put simply, a 'through-bonds' mechanism[39–42]). In contrast, the donor

[37] L. T. Calcaterra, G. L. Closs, and J. R. Miller, *J. Am. Chem. Soc.*, 1983, **105**, 671.
[38] J. R. Miller, L. T. Calcaterra, and G. L. Closs, *J. Am. Chem. Soc.*, 1984, **106**, 3047.
[39] D. N. Beratan and J. J. Hopfield, *J. Am. Chem. Soc.*, 1984, **106**, 1584.
[40] N. S. Hush, M. N. Padon-Row, E. Cotsaris, H. Oevering, J. W. Verhoeven, and M. Heppener, *Chem. Phys. Lett.*, 1985, **117**, 8.
[41] S. Larsson, *J. Chem. Soc., Faraday Trans. 2*, 1983, **79**, 1375.
[42] K. Ohta, G. L. Closs, K. Morokuma, and N. J. Green, *J. Am. Chem. Soc.*, in press.

reduction potential is much more positive in the protein experiments ($\sim$0.1V *vs.* NHE), which could explain the extremely weak donor–acceptor electronic coupling that is implicated by the slow temperature-independent rates.

## 7 Excited-state Electron-transfer

A very convenient way to study protein long-range electron-transfer is to generate a powerful oxidant or reductant by photoexcitation. Excited zinc porphyrins, for example, have been used as reductants both in fixed-distance experiments[43–45] and in studies of long-range electron-transfer in protein–protein complexes.[46–48] Experiments of this sort on blue copper proteins have involved electronically excited $Ru(bpy)_3^{2+}$ {$^*Ru(bpy)_3^{2+}/Ru(bpy)_3^{+}$ = 0.84 V *vs.* NHE} and $Cr(phen)_3^{3+}$

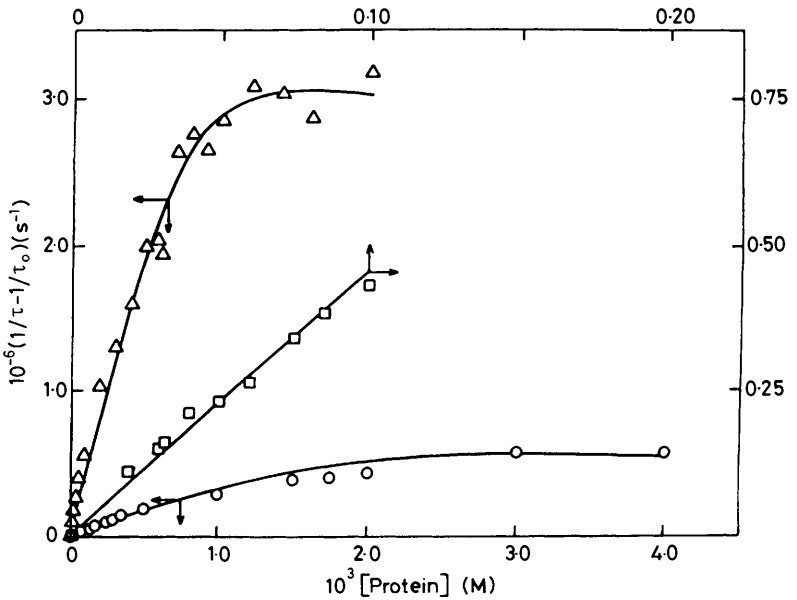
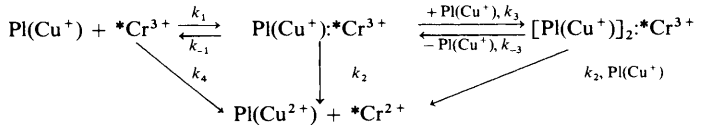



**Figure 6** *Protein concentration dependence of the observed rate of oxidation of* $Pl(Cu^+)$ (△), $Az(Cu^+)$ (□), *and* $St(Cu^+)$ (○) *by* $^*Cr(phen)_3^{3+}$ *at* 25 °C (pH 7.0, 0.1 M *ionic strength*) (Reproduced by permission from *ref.* 50)

[43] J. L. McGourty, N. V. Blough, and B. M. Hoffman, *J. Am. Chem. Soc.*, 1983, **105**, 4470.
[44] S. E. Peterson-Kennedy, J. L. McGourty, and B. M. Hoffman, *J. Am. Chem. Soc.*, 1984, **106**, 5010.
[45] H. B. Gray, S. L. Mayo, A. W. Axup, W. R. Ellis, Jr., and R. J. Crutchley, unpublished results.
[46] K. P. Simolo, G. L. McLendon, M. R. Mauk, and A. G. Mauk, *J. Am. Chem. Soc.*, 1984, **106**, 5012.
[47] G. L. McLendon, J. R. Winkler, D. G. Nocera, M. R. Mauk, A. G. Mauk, and H. B. Gray, *J. Am. Chem. Soc.*, 1985, **107**, 739.
[48] P. S. Ho, C. Sutoris, N. Liang, E. Margoliash, and B. M. Hoffman, *J. Am. Chem. Soc.*, 1985, **107**, 1070.
[49] A. M. English, V. R. Lum, P. J. DeLaive, and H. B. Gray, *J. Am. Chem. Soc.*, 1982, **104**, 870.
[50] B. S. Brunschwig, P. J. DeLaive, A. M. English, M. Goldberg, H. B. Gray, S. L. Mayo, and N. Sutin, *Inorg. Chem.*, 1985, **24**, 3743.

$\{*Cr(phen)_3^{3+}/Cr(phen)_3^{2+} = 1.42$ V *vs.* NHE$\}$ as oxidants.[49,50] The kinetics of these reactions exhibit saturation (Figure 6), suggesting that at high protein concentrations the excited reagent is bound to the reduced protein in an electron-transfer precursor complex.[50] Extensive data have been collected for the reaction of reduced bean plastocyanin with $*Cr(phen)_3^{3+}$, and these data have been analysed in terms of a mechanistic model that includes both 1:1 and 2:1 $Pl(Cu^+):*Cr^{3+}$ complexes:[50]




In this mechanistic scheme $Pl(Cu^+):*Cr^{3+}$ is a complex in which the excited chromium reagent is bound at a site that is relatively distant from the copper. The formation constant of the 1:1 complex is $K_1(= k_1/k_{-1})$, the formation constant of the 2:1 complex is $K_3(= k_3/k_{-3})$, $k_2$ is the intracomplex (remote, or long-range) electron-transfer rate constant, and $k_4$ is the bimolecular rate constant for adjacent attack, that is, for the pathway that involves close approach of $*Cr^{3+}$ to the $Cu^+$ site. It is assumed that the ground and excited states of $Cr(phen)_3^{3+}$ bind with the same equilibrium constant to the protein, and that the bound chromium reagent has the same intrinsic lifetime as the free reagent.

The same mechanistic model has been used to fit the data obtained for the reactions of $Az(Cu^+)$ and $St(Cu^+)$ (St = *Rhus vernicifera* stellacyanin) with $*Cr(phen)_3^{3+}$, as well as the results for the $*Ru(bpy)_3^{2+}$ oxidations. Values of the rate and binding constants that have been extracted from the analyses are given in Table 4.

**Table 4** *Reactions of* $*Cr(phen)_3^{3+}$ *and* $*Ru(bpy)_3^{2+}$ *with reduced blue copper proteins at* 22 °C[a]

| *Protein* | $*M^{n+}$ | $-\Delta G^0$ (V) | $10^{-9}$ ($k_1$ or $k_4$) ($M^{-1}$ $s^{-1}$) | $K_1$ ($M^{-1}$) | $10^{-6}$ $k_2$ ($s^{-1}$) | $10^{-3}$ $K_3$ ($M^{-1}$) | $\frac{k_{adj}}{k_{rem}}$[b] |
|---|---|---|---|---|---|---|---|
| $Pl(Cu^+)$ | $*Cr^{3+}$ | 1.06 | 3.5 | 250 | 2.5 | 4.2 | 7 |
| $Pl(Cu^+)$ | $*Ru^{2+}$ | 0.48 | 1.9 | 100 | 3.0 | 0.2 | 7 |
| $Az(Cu^+)$ | $*Cr^{3+}$ | 1.11 | 0.40 | 60 | 1.2 | — | 7 |
| $Az(Cu^+)$ | $*Ru^{2+}$ | 0.53 | 0.65 | 40 | 1.2 | 2.3 | 14 |
| $St(Cu^+)$ | $*Cr^{3+}$ | 1.23 | 0.35 | 60 | 0.20 | 1.3 | 30 |
| $St(Cu^+)$ | $*Ru^{2+}$ | 0.65 | 0.40 | 40 | 0.20 | 1.4 | 51 |

[a] *Ref.* 50. [b] The ratio of the adjacent to remote rates at low protein concentration calculated from $k_4(k_{-1} + k_2)/k_1k_2$

At low protein concentrations the reactions go mainly by the adjacent-attack pathway; these reactions all exhibit very large rate constants, as expected because

the driving forces are high. The 1:1 protein:reagent binding constant, $K_1$, is somewhat larger for Pl($Cu^+$):*$Cr(phen)_3^{3+}$ than for Pl($Cu^+$):*$Ru(bpy)_3^{2+}$, indicating that electrostatic interactions between the negatively charged protein and the inorganic reagent make a contribution to the stability of the complex. The $K_1$ value for Pl($Cu^+$):*$Cr(phen)_3^{3+}$ is in reasonable agreement with the $\sim 170\ M^{-1}$ reported by Segal and Sykes for Pl($Cu^+$):$Co(phen)_3^{3+}$,[12] which is encouraging because there is every reason to believe that the protein:reagent interactions are very similar for these two reagents. A small difference in these two equilibrium constants would be expected in any case, since the Segal–Sykes experiments were performed with the parsley protein.

N.m.r. experiments have indicated that $Cr(phen)_3^{3+}$ binds to Pl($Cu^+$) near tyrosine-83 and the negative patch formed by the carboxylates of residues 42—45 (plus 51 and 59).[13–15] The region is particularly attractive, because of the possibility of associating the hydrophobic phenyl ring of Tyr-83 with a phenanthroline ligand of $Cr(phen)_3^{3+}$. A computer-generated model of this docking of Pl($Cu^+$) and $Cr(phen)_3^{3+}$ shows that good overlap between the phenyl group and a phenanthroline can be achieved (Figure 7). In the docked complex the closest donor–acceptor contact is 10.3 Å, which is the distance from the co-ordinated sulphur atom of cysteine-84 to the nearest phenanthroline carbon of the bound $Cr(phen)_3^{3+}$. This is the edge-to-edge distance for the long-range (remote) electron-transfer pathway. (Note that the Cu to Cr distance in the docked complex is 18.4 Å.[50]).

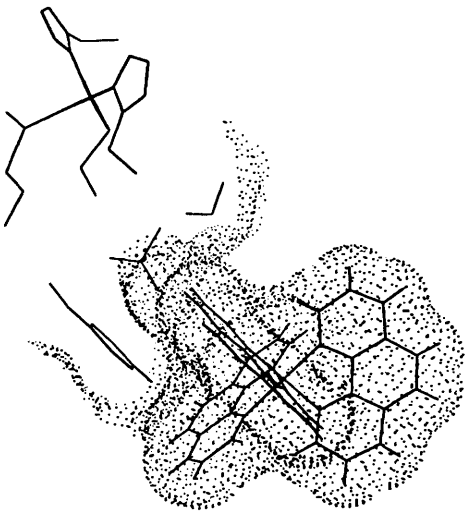


**Figure 7** *View of a computer-generated model of the complex between* Pl($Cu^+$) *and* $Cr(phen)_3^{3+}$. *Shown are the copper atom with its four ligands, three nearby residues* (Val-40, Ser-85, Tyr-83), *and the docking between the solvent-accessible protein surface and the* $Cr(phen)_3^{3+}$ *surface*
(Reproduced by permission from *ref.* 50)

The long-range electron-transfer rate constants ($k_2$) for the Pl($Cu^+$):*$Cr(phen)_3^{3+}$ and Pl($Cu^+$):*$Ru(bpy)_3^{2+}$ complexes are virtually the same ($\sim 3 \times 10^6$ $s^{-1}$), which is reasonable because the driving forces are large enough to overcome the reorganization energies for the reactions. In this situation the only barrier to the reaction is the electron-transfer distance itself:[50]

$$k_2 = 10^{13}\exp(-\beta d)\ s^{-1} \quad (6)$$

Since the closest edge-to-edge distance in the docked complex is 10.3 Å, the value of $\beta$ that reproduces the long-range electron-transfer rate is $\sim 1.4$ $Å^{-1}$. Similar values of $\beta$ have been suggested previously for long-range electron-transfer in proteins.[50–54]

## 8 Concluding Remarks

The relatively high rate of electron-transfer over 10 Å in Pl($Cu^+$):*$Cr(phen)_3^{3+}$ contrasts with the $\sim 2$ $s^{-1}$ that we found for the long-range $Ru^{2+} \longrightarrow Cu^{2+}$ transfer in $a_5$Ru(His-83)$^{2+}$Az($Cu^{2+}$). In addition to the weak donor–acceptor coupling, it appears that in the latter case there is an entropic reorganization barrier associated with the ordering of water about $a_5$Ru(His-83)$^{3+}$ in the final product. The effects of variations in the entropic barrier for long-range electron-transfer are now being investigated by appropriate ligand modifications in ruthenated proteins.

Returning to the Segal–Sykes Pl($Cu^+$):$Co(phen)_3^{3+}$ experiment,[11–12] it is likely that the limiting rate of 20 $s^{-1}$ is a long-range $Cu^+ \longrightarrow Co^{3+}$ electron-transfer within a complex analogous to the one modelled for Pl($Cu^+$):$Cr(phen)_3^{3+}$ (Figure 7). The rate is five orders of magnitude slower than the $Cu^+ \longrightarrow$ *$Cr^{3+}$ transfer, because there is a large free-energy barrier (the driving force for the $Co(phen)_3^{3+}$ reaction is only 0.02 V).[53] In this respect the Pl($Cu^+$):$Co(phen)_3^{3+}$ reaction is similar to the long-range $Ru^{2+} \longrightarrow Cu^{2+}$ electron-transfer in $a_5$Ru(His-83)$^{2+}$ Az($Cu^{2+}$).

It appears from the results obtained thus far that blue copper is not as strongly coupled to a distant donor or acceptor as a low-spin haem or an electronically excited porphyrin.[45] Although the reason for this difference in donor–acceptor electronic coupling is not known, it may be related to the relatively localized nature of the relevant blue copper wavefunction.[55] It will be interesting to see as more fixed-distance electron-transfer experiments are done whether blue copper also is less strongly coupled to a donor or acceptor at short-range (near van der Waals contact) than a low-spin haem. Questions of this sort will need to be answered before we can claim to have any understanding of protein-mediated donor–acceptor interactions.

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