Articles

Contribution from the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel, and Department of Physical Chemistry, Royal Danish School of Pharmacy, DK-2100, Copenhagen, Denmark

Energetics of Intramolecular Electron Transfer in Ruthenium-Modified Stellacyanin‡

Ole Farver* and Israel Pecht†

Received April 4, 1990

Stellacyanin (St) from *Rhus vernicifera* contains two neighboring and free imidazole residues on the surface that provide the possibility of their modification using $Ru(NH_3)_5H_2O^{2+}$. This has indeed been achieved, and the preparation of a St–Ru derivative and preliminary results describing the intramolecular electron transfer between the surface-exposed Ru(II) and the intrinsic Cu(II) at room temperature have been reported (Farver, O.; Pecht, I. *FEBS Lett.* **1989**, *244*, 376–378, 379–382). The energetics of the intramolecular electron transfer from Ru(II) to Cu(II) in $((NH_3)_5Ru)_2$(His-32/His-100)–stellacyanin have now been studied. CO_2^- radical anions produced by pulse radiolysis, reduce Ru^{III}–$StCu^{II}$ to Ru^{II}–$StCu^{II}$, which by an intramolecular electron transfer yields Ru^{III}–$StCu^{I}$ with a rate constant of $k = 0.07 \pm 0.01$ s^{-1} ($T = 298$ K, $I = 0.10$ M, pH 7.0). This reaction was studied over a temperature range from 2.5 to 39.7 °C, and from the variation of the rate constant with temperature, an activation enthalpy, $\Delta H^* = 19.1 \pm 3.1$ kJ mol^{-1}, was calculated. We have further determined the standard enthalpy and entropy for the protein-coordinated $Ru(NH_3)_5His^{3+/2+}$ redox couple. Using the Marcus theory and the above thermodynamic parameters yields a reorganization energy of 42 kJ mol^{-1} for the Cu(II)/Cu(I) center in stellacyanin. The entropy of activation derived for the intramolecular electron-transfer process is $\Delta S^* = -201 \pm 40$ J K^{-1} mol^{-1}. Using the Marcus theory to calculate the activation entropy and introducing a value of 1.6 nm estimated from the tentative three-dimensional St model for the electron-transfer distance yield a value of $\Delta S^* = -193$ J K^{-1} mol^{-1}. This calculated value is in good agreement with the one obtained experimentally.

Introduction

Electron-transfer reactions within and between metalloproteins in solution have been the subject of extensive studies during the past decade.[1] Not only are these reactions of fundamental intrinsic interest but also they are involved in practically all energy conversion processes in living cells. It is now established that electrons can transit distances or more than 2.0 nm in protein molecules at rather fast rates. Theoretical quantitative treatments have been extended to electron transfer in biological systems, along with emerging experimental results.[2] Here we report a study of an intramolecular electron-transfer process and its examination with Marcus theory.

Rhus stellacyanin is an interesting blue single-copper protein. It has a considerably lower redox potential than all other blue proteins. Nevertheless, its electron-transfer reactivity with small redox agents is among the highest observed.[3] It is a glycoprotein with 40% carbohydrate content, and so far no crystals of this protein have been produced. Therefore we have constructed a three-dimensional model by energy minimization,[4] which, although hypothetical and based on amino acid sequence homology with plastocyanin, has proven to be in very good agreement with all the recent spectroscopic studies.[5] The NMR study of Dahlin et al.[5b] of the metal-binding region in Co(II)–stellacyanin is particularly noteworthy, since the proton–metal distance determinations support our notion of a disulfide serving as a Cu ligand.[4] The proposed three-dimensional model suggests that two histidines (32 and 100) are located near each other on the surface of stellacyanin at a distance of about 1.6 nm from the copper center. Cucumber blue protein (CBP) shows a high sequence homology with stellacyanin,[6] and the three-dimensional structure of the former protein has recently been determined.[7] However, CBP contains no histidines homologous with His-32/His-100 in St. Thus no comparison can be made on the basis of the CBP structure.

Solvent-exposed imidazole residues of proteins have been shown to produce a highly stable coordination bond with $Ru(NH_3)_5^{3+/2+}$.[8] Intramolecular electron-transfer processes between such extrinsic metal ions and the native intrinsic metal centers have been investigated extensively.[8-11]

We have previously described a stellacyanin derivative[12] where two a_5Ru complexes are coordinated to the two exposed imidazoles of His-32 and His-100. Furthermore, preliminary studies have demonstrated that an intramolecular electron transfer takes place from Ru(II) to Cu(II) in this modified protein.[13] The temperature dependence of this electron-transfer rate in the Ru-modified stellacyanin has now been investigated. Moreover, the standard thermodynamic properties of the protein-bound Ru ions were determined. This enabled an analysis of the activation parameters

* To whom correspondence should be addressed at the Royal Danish School of Pharmacy.
† The Weizmann Institute of Science.

‡ Abbreviations: a, NH_3; St, stellacyanin; His, histidine; py, pyridine. The notations introduced by Marcus and Sutin[2a] for activation parameters (* and ‡) are being used throughout.

(1) Gray, H. B. *Chem. Soc. Rev.* **1986**, *15*, 17–30.
(2) (a) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265–322. (b) Larsson, S.; Broo, A.; Kallebring, B.; Volosov, A. *Int. J. Quantum Chem.* **1988**, *15*, 1–22.
(3) Farver, O.; Pecht, I. *Copper Proteins and Copper Enzymes*; Lontie, R., Ed.; CRC Press, Inc.: Boca Raton, FL, 1984; pp 183–209.
(4) Wherland, S.; Farver, O.; Pecht, I. *J. Mol. Biol.* **1988**, *204*, 407–415.
(5) (a) Feiters, M. C.; Dahlin, S.; Reinhammar, B. *Biochim. Biophys. Acta* **1988**, *955*, 250–260. (b) Dahlin, S.; Reinhammar, B.; Ångström, J. *Biochemistry* **1989**, *28*, 7224–7233.
(6) Murata, M.; Begg, G. S.; Lambrou, F.; Leslie, B.; Simpson, R. J.; Freeman, H. C.; Morgan F. J. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6434–6437.
(7) Guss, J. M.; Merritt, E. A.; Phizackerley, R. P.; Hedman, B.; Murata, M.; Hodgson, K. O.; Freeman, H. C. *Science* **1988**, *241*, 806–811.
(8) (a) Yocom, K. M.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Worosila, G.; Isied, S. S.; Bordignon, E.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7052–7055. (b) Margalit, R.; Pecht, I.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 301–302. (c) Margalit, R.; Kostic, N. M.; Che, C.-M.; Blair, D. F.; Chiang, H.-J.; Pecht, I.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6554–6558.
(9) (a) Kostic, N. M.; Margalit, R.; Che, C.-M.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 7765–7767. (b) Mayo, S. L.; Ellis, W. R.; Crutchley, R. J.; Gray, H. B. *Science (Washington, D.C.)* **1986**, *233*, 948–952. (c) Axup, A. W.; Albin, M.; Mayo, S. L.; Crutchley, R. J.; Gray, H. B. *J. Am. Chem. Soc.* **1988**, *110*, 435–439.
(10) (a) Isied, S. S.; Worosila, G.; Atherton, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 7659–7661. (b) Isied, S. S.; Kuehn, C.; Worosila, G. *J. Am. Chem. Soc.* **1984**, *106*, 1722–1726. (c) Bechtold, R.; Kuehn, C.; Lepre, C.; Isied, S. S. *Nature* **1985**, *322*, 286–288.
(11) (a) Jackman, M. P.; Sykes, A. G.; Salmon, G. A. *J. Chem. Soc., Chem. Commun.* **1987**, 65–67. (b) Jackman, M. P.; McGinnis, J.; Powls, R.; Salmon, G. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 5880–5887. (c) Osvath, P.; Salmon, G. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 7114–7118.
(12) Farver, O.; Pecht, I. *FEBS Lett.* **1989**, *244*, 376–378.
(13) Farver, O.; Pecht, I. *FEBS Lett.* **1989**, *244*, 379–382.

0020-1669/90/1329-4855$02.50/0

for this long-range electron-transfer reaction based upon the Marcus theory and available structural data.

Experimental Section

Preparations. Stellacyanin was isolated and purified from an acetone extract of the Japanese lacquer tree *Rhus vernicifera* according to the method of Reinhammar.[14] $a_5Ru(H_2O)^{2+}$ was generated by reduction of a_5RuCl^{2+} over zinc amalgam. Preparation and characterization of Ru-modified stellacyanin (Ru_2-St) have been described earlier.[12] Employing this protocol, we have consistently found that, when the product is separated and purified chromatographically, only doubly labeled and unmodified proteins are obtained.

Instrumentation and Methods. Pulse radiolysis experiments were carried out by using the Varian V-7715 linear accelerator of the Hebrew University in Jerusalem. Pulse lengths employed were in the range from 0.1 to 1.5 μs of 5-MeV electrons. All optical measurements were carried out in a 4 × 2 × 1 cm Spectrosil cuvette, using three light passes which result in an overall path length of 12.3 cm. A 150-W xenon lamp produced the analyzing light beam, and appropriate optical filters with a cutoff at either 385 or 590 nm were used to avoid photochemistry and light scattering. The data acquisition system consisted of a Tektronix 390 A/D programmable digitizer and a Micro PDP 1123 computer. Temperature of the reactant solutions was continuously monitored by an attached thermistor and controlled by a thermostating system. Most reactions were performed under pseudo-first-order conditions, with typically a 10-fold excess of oxidized protein over reductant. Each kinetic run was repeated at least three times. The copper redox state was monitored at 605 nm (ϵ_{605} = 4030 M^{-1} cm^{-1}), while that of the a_5Ru^{III} ion coordinated to imidazole side chains was followed at its maximal absorption at 310 nm (ϵ_{310} = 1910 M^{-1} cm^{-1}).[12] Neither Cu(I) nor Ru(II) contributes significantly to the absorption in the 300–700-nm region.

When N_2O-saturated, 0.1 M sodium formate aqueous solutions are irradiated, all the primary radicals are converted into CO_2^- radical anions, as described earlier.[13] Yields of CO_2^- radicals produced were determined by monitoring the reduction of 0.1 mM $Fe(CN)_6^{3-}$ (ϵ_{410} = 1020 M^{-1} cm^{-1}). Buffer-free aqueous solutions were adjusted to pH 7.0 by titration of 0.10 M formic acid with NaOH. Aliquots of concentrated (1–2 mM) modified stellacyanin were added after N_2O saturation to yield the desired protein concentration for the experiment. pH was routinely checked both before and after irradiation. The deviation from pH 7.0 was never more than 0.05.

Nonisothermal cyclic voltammetry was performed with a PAR 174A potentiostat in a 0–25 °C temperature range. The electrode system consisted of a gold working electrode, a platinum-wire auxiliary electrode, and a saturated sodium chloride reference electrode. The measurements were performed at pH 7.0 in 0.05 M Hepes/0.100 M NaCl. The protein concentration was 0.2 mM, and the solution was degassed and blanketed by a nitrogen flow before and during the electrochemical measurements.

Results

Two distinct reduction steps could be monitored at 605 nm upon reacting Ru-modified stellacyanin with CO_2^- radical ions (cf. Figure 1). The fast one is due to the bimolecular reduction of the Cu(II) site by CO_2^-. The rate of this reaction was found to increase linearly with the protein concentration over a 2–20-μM range, as well as with the pulse width (0.2–1.0 μs, corresponding to a CO_2^- radical concentration range from 1.2 to 6.0 μM). The second-order rate constant determined from this concentration dependence at 298 K is (9.9 ± 2.0) × 10^8 M^{-1} s^{-1}.

A second, slower process is observed at 605 nm (Figure 1). This is also expressed in an absorption decrease, which represents further reduction of Cu(II), and depends on neither protein nor radical-ion concentration (2–20 μM protein, 0.2–1.0-μs pulse width).

Two reaction phases were also observed when the absorption changes at 310 nm were monitored following the pulse: a fast phase, in the time domain of 0.1 ms, and a slow one, in the 10-s time range. The rate of the fast phase was also found to depend on both protein and radical-ion concentrations and is attributed to the direct reduction by CO_2^- of Ru(III) ions coordinated to His-32 or His-100. Since a large excess (10-fold or more) of protein over radical-ion concentration was employed, at most only one of the two St-coordinated Ru(III) ions is expected to be reduced. The rate constant of the direct reduction of the Ru(III) site, k = (1.2 ± 0.5) × 10^9 M^{-1} s^{-1} (298 K, calculated based on protein concentration), is slightly higher than that for direct reduction of the Cu(II) site.

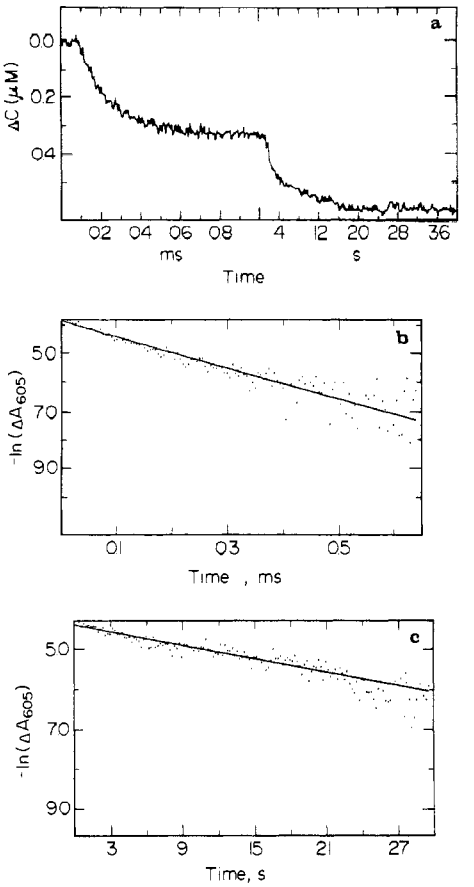
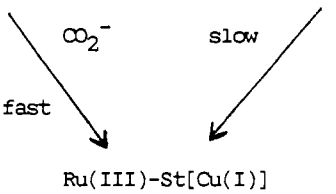


Figure 1. (a) Absorption changes of Ru^{III}–$StCu^{II}$ solution monitored at 605 nm following the radiation pulse: fast phase, bimolecular reduction of the Cu(II) center by CO_2^-; slow phase, intramolecular Ru(II) to Cu(II) electron transfer. Conditions: protein concentration, 6.6 μM; pulse width, 0.1 μs; T = 6.5 °C. (b) Logarithmic plot of the fast decay in absorbance at 605 nm. (c) Logarithmic plot of the slow decay in absorbance at 605 nm.

Scheme I

$$\text{Ru(III)-St[Cu(II)]} \xrightarrow[\text{fast}]{CO_2^-} \text{Ru(II)-St[Cu(II)]}$$



The slow absorption increase observed at 310 nm has a rate similar to that of the slow reduction of the Cu(II) site and most probably reflects the reoxidation of Ru(II). The rate of this process is indeed found to be independent of protein and CO_2^- radical concentrations with a rate constant k = 0.07 ± 0.01 s^{-1} (298 K). Moreover, the amplitudes of the slow phases monitored at 605 and 310 nm correspond, within experimental error, to the same concentration change in Cu(II) and Ru(II), respectively. This supports the assignments of both absorption changes to the same intramolecular electron-transfer step from Ru(II) to Cu(II) in the modified protein. The overall reaction is depicted in Scheme I.

The yields of Ru(II) and Cu(I) are relatively high: with a 10-fold excess of modified protein, more than 90% of the CO_2^-

(14) (a) Reinhammar, B. *Biochim. Biophys. Acta* **1970**, *205*, 35–47. (b) Reinhammar, B. *Biochim. Biophys. Acta* **1972**, *275*, 245–259.
(15) Wherland, S.; Gray, H. B. In *Biological Aspects of Inorganic Chemistry*; Dolphin, D., Ed.; J. Wiley, Inc.: New York, 1977; pp 289–368.

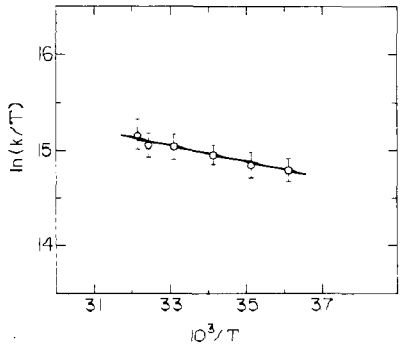


Figure 2. Eyring plot of the fast bimolecular reduction of Cu(II) in Ru-modified stellacyanin by CO_2^-.

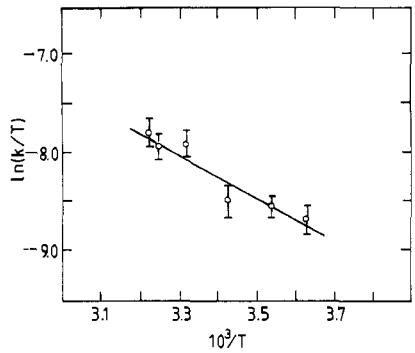


Figure 3. Eyring plot of the slow intramolecular electron-transfer rate from Ru(II) to Cu(II) in Ru-modified stellacyanin.

radical ions transferred an electron to one of the metal ion centers with a relative yield of Ru(II):Cu(I) = 1.2:1.

The temperature dependencies of both the fast bimolecular and the slow intramolecular Cu(II) reduction rates were measured over the temperature range from 2.5 to 39.7 °C. The respective Eyring plots are shown in Figures 2 and 3. From the slopes and intercepts, the activation enthalpies and entropies were determined. These parameters are as follows: for the fast, direct reduction step $\Delta H^* = 6.8 \pm 1.2$ kJ mol^{-1} and $\Delta S^* = -49.8 \pm 8.5$ J K^{-1} mol^{-1}, while for the slow intramolecular process $\Delta H^* = 19.1 \pm 3.1$ kJ mol^{-1} and $\Delta S^* = -201 \pm 40$ J K^{-1} mol^{-1}. The entropies of activation, however, include a contribution from the transmission coefficient κ, which is expected to decrease exponentially with the distance between electron donor and acceptor (cf. Discussion).

The temperature dependence of the $Ru(NH_3)_5$(His-32/His-100)$^{3+/2+}$ redox couple was determined by nonisothermal cyclic voltammetry. The redox potential was found to be 61 ± 8 mV at 25 °C, which is quite similar to that for $Ru(NH_3)_5His^{3+/2+}$, 80 ± 5 mV.[8c] The reduction potential of Cu(II)-St is 184 mV.[14] This yields a 12 kJ mol^{-1} driving force for the Ru(II) to Cu(II) electron transfer. From the temperature dependence of the redox potential at pH 7.0 we determined $\Delta H^\circ = -10.5 \pm 1.6$ kJ mol^{-1} and $\Delta S^\circ = -15.6 \pm 2.0$ J K^{-1} mol^{-1}.

Discussion

Fast Bimolecular CO_2^- Reduction of the Cu(II) Site. The bimolecular, direct reduction of the Cu(II) center by CO_2^- radicals is most probably a diffusion-controlled process. This is supported by both the high bimolecular rate constant ($k = 9.9 \times 10^8$ M^{-1} s^{-1}) and the low activation enthalpy ($\Delta H^* = 6.8$ kJ mol^{-1}). Since the charge of the redox center in this protein is essentially zero,[14] the Smoluchowsky equation can be applied to calculate the expected diffusion-controlled rate:

$$k_{diff} = 4\pi(D_P + D_R)r_{PR}N/1000 \quad (1)$$

where D_P and D_R represent the diffusion coefficients of the protein and the reductant, respectively, and N is Avogadro's number. The intermolecular distance between the reactants' centers, r_{PR}, is approximately the same as that of the protein radius, which according to our computer-calculated model is 2.3 nm[4] (cf. Figure 4). Relative to CO_2^-, the protein is essentially stationary, and thus only the diffusion coefficient for the radical anion, $D_R = 1.5 \times 10^{-5}$ cm^2 s^{-1}, is used. This yields a calculated $k_{diff} = 2.6 \times 10^{10}$ M^{-1} s^{-1}. Considering the limited spherical angle of approach to the Cu center, the agreement between the observed ($(9.9 \pm 2.0) \times 10^8$ M^{-1} s^{-1}) and the calculated rate constant is satisfactory, supporting the notion that the fast bimolecular direct reduction of Cu(II) in stellacyanin by CO_2^- is essentially diffusion controlled.

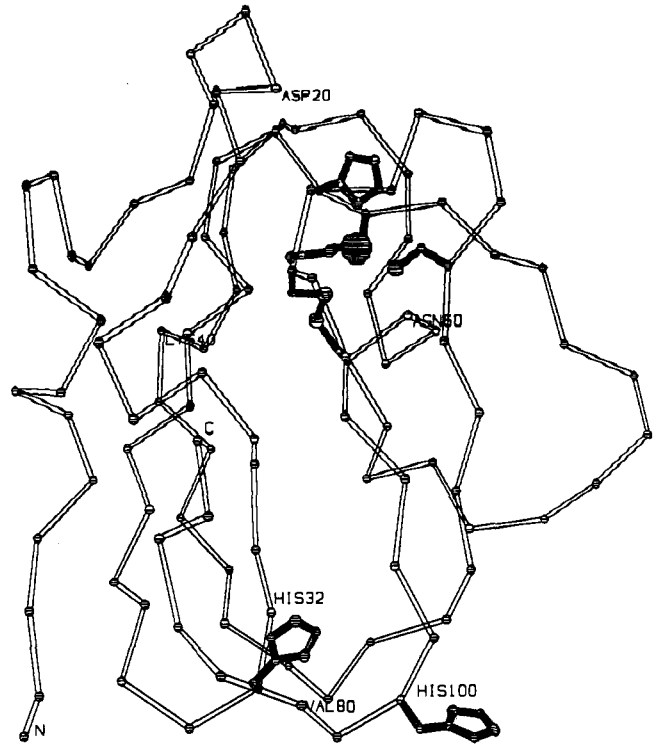


Figure 4. Three-dimensional structure of *Rhus* stellacyanin based on the calculated model.[4] Drawn in bold lines are the four copper coordinating residues and the two ruthenium-binding histidines.

Slow Intramolecular Ru(II) to Cu(II) Electron Transfer. The change in enthalpy due to the Ru(II) to Cu(II) electron-transfer reaction can be derived from the known thermodynamics of the redox reactions of stellacyanin-[Cu(II)/Cu(I)] (ΔH°_{St}),[16] and of protein-bound $a_5RuHis^{3+/2+}$ (ΔH°_{Ru}; determined here): $\Delta H^\circ = \Delta H^\circ_{St} - \Delta H^\circ_{Ru} = -32.6$ kJ mol^{-1}. When the driving force of the reaction ($-\Delta G^\circ$) is much smaller than the reorganization energy, λ, of the reaction, then[2a]

$$\Delta H^* = \lambda/4 + \Delta H^\circ/2 \quad (2)$$

which means that $\lambda = 141$ kJ mol^{-1}. Now, assuming that the reorganization energy for the self-exchange of $a_5RuHis^{3+/2+}$ is the same as that for $a_5PyRu^{3+/2+}$ namely $\lambda_{22}/4 = 29$ kJ mol^{-1},[1,14] we may calculate the reorganization energy for the Cu(II)/Cu(I) center of stellacyanin, λ_{11}. For the intramolecular electron transfer from Ru(II) to Cu(II) in the Ru-derivatized St, λ is taken as approximately the mean value of those of the self-exchange reactions λ_{11} and λ_{22}:

$$\lambda = \lambda_{11}/2 + \lambda_{22}/2 \quad (3)$$

The reorganization energy for electron self-exchange of Cu(II)/Cu(I) stellacyanin thus becomes $\lambda_{11}/4 = 42$ kJ mol^{-1}. For the copper site in azurin an upper limit for the reorganization energy has been estimated to be 30 kJ mol^{-1}.[8c] The higher energy requirement in stellacyanin is hardly surprising, considering the better accessibility of the copper site here as compared with that of azurin. Thus, the contribution to reorganization of the solvent water molecules is expected to be larger in stellacyanin. Since the electrostatic work involved in bringing together two stellacyanin molecules may be assumed to be negligibly small,[15] ΔG^* for

(16) Tanigushi, V. T.; Sailasuta-Scott, N.; Anson, F. C.; Gray, H. B. *Pure Appl. Chem.* **1980**, *52*, 2275–2281.
(17) Brown, G. M.; Sutin, N. *J. Am. Chem. Soc.* **1979**, *101*, 883–892.

St(Cu^{II}/Cu^{I}) self-exchange can be calculated from[2a]

$$\Delta G^* = \lambda/4(1 + \Delta G^\circ/\lambda)^2 \quad (4)$$

This means the standard free energy of activation is 42 kJ mol^{-1}. Thus, assuming the reaction to be essentially adiabatic ($\kappa \approx 1$), we find $k_{11} = 2 \times 10^5$ M^{-1} s^{-1} at 20 °C. The self-exchange rate constant has been determined experimentally to be 1.2×10^5 M^{-1} s^{-1} at 20 °C.[18] Thus, the agreement between the two values is satisfactory indeed. The electron self-exchange most likely takes place via the partially exposed imidazole rings of the copper-ligating His-92.[4] A κ value of about unity is expected for a system with delocalization of the metal ion electron density onto the π^* orbitals of the imidazole ligand.

The entropy of activation ΔS^* for the intramolecular electron transfer from Ru(II) to Cu(II) in modified stellacyanin can be calculated by using the theory as formulated by Marcus and Sutin:[2a]

$$\Delta S^* = \Delta S^\circ/2 - R\beta d \quad (5)$$

ΔS° is the difference between the standard entropies for St(Cu^{II}/Cu^{I}) (−82.8 J K^{-1} mol^{-1})[16] and for protein-bound Ru(NH_3)$_5$His$^{3+/2+}$ (−15.6 J K^{-1} mol^{-1}; this study). For the distance d separating the electron donor and acceptor, we use 1.6 nm as derived from our model,[4] while for β, the electron-tunneling barrier, the widely employed value is 12 nm^{-1}.[2] We thus obtain $\Delta S^* = -193$ J K^{-1} mol^{-1}, as compared with the experimentally determined value of -201 ± 40 J K^{-1} mol^{-1}. The excellent agreement between the experimentally observed value and that calculated with an electron-transfer distance deduced from our tentative St model lends further support to the usefulness of this computer-calculation-based model.

Finally, the relatively slow rate that is observed for this intramolecular electron transfer deserves attention. It proceeds over a relatively long distance (1.6 nm deduced from the model), yet the intervening medium, as perceived in our model, contains several aromatic residues. The driving force of the reaction is however smaller (12 kJ mol^{-1}) than in other modified redox proteins studied so far. In azurin modified with Ru at His-83 the intramolecular electron-transfer rate from the Ru(II) to Cu(II) over a 1.8-nm distance is 1.9 s^{-1} at 25 °C.[9a] The driving force for this reaction is 27 kJ mol^{-1}. Thus, it is most probably the combination of the large separation distance between the electron donor and acceptor with a low driving force that leads to the unusually low rate of intramolecular electron transfer in Ru-modified stellacyanin.

Acknowledgment. O.F. acknowledges a short-term EMBO fellowship (ASTF 5830). We are grateful to Dr. Gideon Czapski, Hebrew University of Jerusalem, for allowing us generous use of the pulse radiolysis system. We thank Dr. Scot Wherland for his interest in and helpful criticism of this work. The electrochemical equipment was put at our disposal by Prof. Erik Larsen, Department of Chemistry, The Royal Veterinary and Agricultural University, Copenhagen. Carsten Heegaard from the same department helped with the measurements, which we gratefully acknowledge.

Registry No. Ru, 7440-18-8; Cu, 7440-50-8; CO_2^-, 14485-07-5.

(18) Dahlin, S.; Reinhammar, B.; Wilson, M. T. *Biochem. J.* **1984**, *218*, 609-614.

Contribution from the Departments of Chemistry, University of California, Irvine, California 92717, and The University, Newcastle upon Tyne, U.K., and Department of Biochemistry, The University, Liverpool L69 3BX, U.K.

Direct Cyclic Voltammetry of Three Ruthenium-Modified Electron-Transfer Proteins

F. A. Armstrong,*,† J. N. Butt,† K. Govindaraju,‡ J. McGinnis,‡ R. Powls,§ and A. G. Sykes*,‡

Received April 16, 1990

A comparison is made between the voltammetric behavior of three electron-transfer proteins and that of their derivatives obtained by attachment of a single Ru(NH_3)$_5^{3+/2+}$ group to specific histidine (imidazole) residues. The native proteins—plastocyanin (PCu) from the green alga *Scenedesmus obliquus* ("blue" $Cu^{2+/1+}$ center, $M_r \sim 10.5 \times 10^3$), cytochrome c_{551} from *Pseudomonas stutzeri* (porphyrin $Fe^{3+/2+}$ center, $M_r \sim 9.25 \times 10^3$), and (to a lesser extent) high-potential iron–sulfur protein (HiPIP) from *Chromatium vinosum* ([4Fe–4S]$^{3+/2+}$ center, $M_r \sim 9.5 \times 10^3$)—require the presence of a cationic reagent (neomycin is used here) to promote their interaction and electron exchange with the pyrolytic graphite-"edge" electrode. By contrast, each of the derivatives PCu(His59)Ru(NH_3)$_5$, HiPIP(His42)Ru(NH_3)$_5$, and c_{551}(His47)Ru(NH_3)$_5$ displays well-defined peak-type cyclic voltammograms without inclusion of such reagents in the electrolyte. The results indicate the importance of localized (as opposed to overall) protein surface charge as a determining factor underlying protein–electrode interactions that lead to reversible electron exchange. It is shown that reduction potentials of the intrinsic and Ru centers in such derivatives may be significantly different from the respective values for native proteins and the complex [Ru(NH_3)$_5$(imid)]$^{3+/2+}$.

Introduction

An important strategy for understanding long-range electron transfer in biological molecules has been to study intramolecular processes in chemically modified redox proteins, particularly those derivatized by attachment of Ru(NH_3)$_5$ at a specific histidine (imidazole) group.[1,2] A separate yet related development in metalloprotein chemistry has been the application of direct (unmediated) voltammetric techniques.[3,4] In the latter area it has been important to determine the factors that allow proteins to interact with electrode surfaces in such a manner as to afford reversible electron exchange. In order to extend our understanding of these factors and simultaneously derive a more quantitative picture of the comparative redox equilibrium properties of Ru-modified proteins, we have investigated the cyclic voltammetry of three representative classes of proteins and their derivatives. The proteins selected are plastocyanin (PCu) from the green algae *Scenedesmus obliquus* ("blue" $Cu^{2+/1+}$ center, $M_r \sim 10.5 \times 10^3$), high-potential iron–sulfur protein (HiPIP) from *Chromatium vinosum* ([4Fe–4S]$^{3+/2+}$ center, $M_r \sim 9.5 \times 10^3$) and cytochrome c_{551} from *Pseudomonas stutzeri* (porphyrin $Fe^{3+/2+}$ center, $M_r \sim 9.25 \times 10^3$). Crystal structure information is available for each example: for poplar plastocyanin,[5] supplemented by recent 2D

* To whom correspondence should be addressed.
† University of California.
‡ The University, Newcastle upon Tyne.
§ The University, Liverpool.

(1) Gray, H. B. *Chem. Soc. Rev.* **1986**, *15*, 17.
(2) Sykes, A. G. *Struct. Bonding* **1990**, *73*, 1.
(3) Armstrong, F. A.; Hill, H. A. O.; Walton, N. J. *Acc. Chem. Res.* **1988**, *21*, 407.
(4) Armstrong, F. A. *Struct. Bonding* **1990**, *72*, 135.
(5) Guss, J. M.; Freeman, H. C. *J. Mol. Biol.* **1983**, *169*, 521. Guss, J. M.; Harrowell, P. R.; Murata, M.; Norris, V. A.; Freeman, H. C. *J. Mol. Biol.* **1986**, *192*, 361.

0020-1669/90/1329-4858$02.50/0