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

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

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]

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

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.

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Registry No. Ru, 7440-18-8; Cu, 7440-50-8; CO$_2^-$, 14485-07-5.

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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*[‡]

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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

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* To whom correspondence should be addressed.

[†] University of California.

[‡] The University, Newcastle upon Tyne.

[§] The University, Liverpool.

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NMR studies[6] on the *S. obliquus* protein, for *C. vinosum* HiPIP,[7] and for *Pseudomonas aeruginosa* cytochrome c_{551},[8] which has a sequence similar to that of the *P. stutzeri* protein.[9] All three proteins have one free histidine (His) available for attachment of the $Ru(NH_3)_5$ moiety. The preparation and characterization of the products—$PCu(His59)Ru(NH_3)_5$,[10] $HiPIP(His42)Ru(NH_3)_5$,[11] and $c_{551}(His47)Ru(NH_3)_5$[12] —have been described.

Experimental Section

Samples of *S. obliquus* plastocyanin, *C. vinosum* high-potential iron-sulfur protein, and *P. stutzeri* (strain 224) cytochrome c_{551} were isolated and then converted to their Ru derivatives and characterized as described previously,[10-12] except that a modified procedure was used to purify Ru-modified c_{551}. Here the sample was fully oxidized and chromatographed at pH 5.5 (Mes) by using a Pharmacia FPLC Mono S column, rechromatographed at pH 8.0 (Tris) on a Mono Q column, and then subjected to gel filtration on a column of Sephadex G-25-50 (10 × 2.5 cm) using 20 mM phosphate at pH 7.0 as the eluent. The unbound fraction was collected in each case. A sample of spinach plastocyanin was prepared according to adaptations of literature procedures.[13] The complex $[Ru(NH_3)_5(imid)](PF_6)_2$ was prepared as described previously.[10]

Deionized water was used to prepare all electrochemical solutions. Most voltammetric experiments were carried out after ultrafiltration-dialysis of the protein samples (Amicon 8MC, YM5 membrane) into a mixed-buffer electrolyte medium comprising 5 mM acetate (from BDH or Aldrich glacial acetic acid) and 5 mM Mes, 5 mM Hepes, and 5 mM Taps (each purchased from Sigma) together with 0.10 M NaCl (Aldrich or BDH Aristar grade). Protein concentrations were typically of the order of 100 μM. Unless otherwide stated, all samples were adjusted to pH 7.0 by addition of small aliquots of concentrated HCl or NaOH solutions. Some experiments were carried out by using 20 mM Hepes with 0.10 M NaCl or by using 0.10 M sodium phosphate (Aldrich) at pH 7.0 with 0.10 M NaCl added (I = 0.32), as used in earlier pulse-radiolysis experiments.[10-12] Neomycin sulfate (Sigma) was added by using a 0.20 M stock solution adjusted to pH 7.2.

For cyclic voltammetry, a nonisothermal cell featuring a three-electrode configuration was used[13-16] in which the saturated calomel reference (SCE) was held at 25 °C (at which E_{SCE} = 244 mV vs the standard hydrogen electrode SHE) in a jacketted side arm linked to the sample compartment through a Luggin capillary. The sample compartment, holding typically 450 μL of solution, was immersed in a water bath that could be maintained at various temperatures. The pyrolytic graphite-"edge" (PGE) working electrode was constructed as described previously.[14] Prior to each experiment it was polished with an aqueous alumina slurry (Banner or Boehler, 0.3 μm) and sonicated thoroughly. The auxiliary electrode was a piece of platinum gauze positioned opposite the Luggin tip. The sample was made anaerobic by passing humidified Ar across the surface of the stirred solution. Stirring was stopped for measurement of cyclic voltammograms. Where necessary, the electrochemical response was promoted by addition of small aliquots of neomycin from a glass syringe. Cyclic voltammetry was carried out with an Ursar Instruments potentiostat used in conjunction with a Bryans-Gould 60000 or a Houston Instruments 2000 XY recorder. Formal reduction potentials $E°'$ were determined from the average of reduction (E_{pc}) and oxidation (E_{pa}) peak potentials as recorded once a steady state had been achieved, i.e. typically after four cycles at a scan rate of 20 mV s^{-1}.

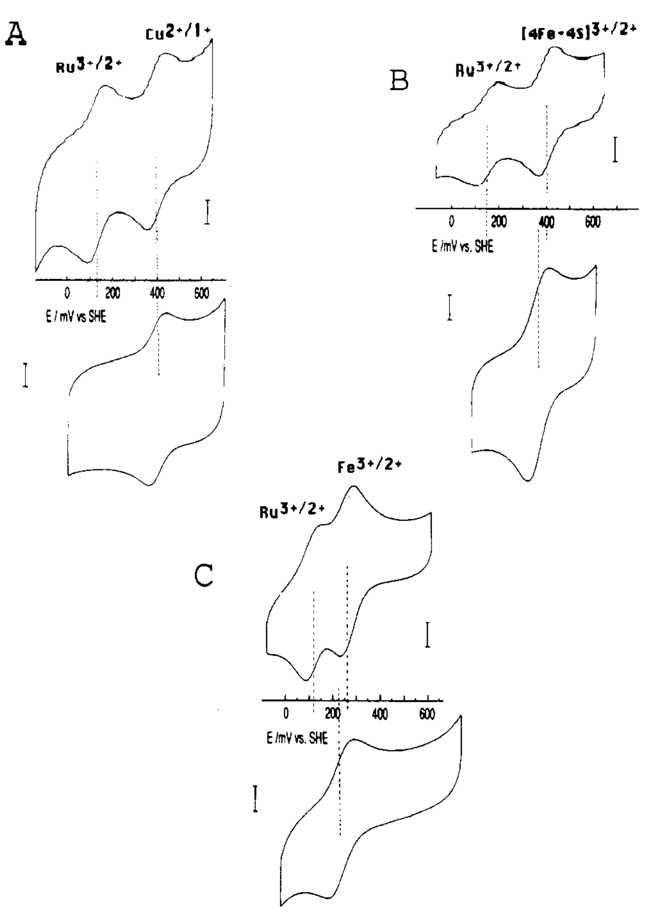

Figure 1. Cyclic voltammograms of *S. obliquus* plastocyanin, *C. vinosum* HiPIP, and *P. stutzeri* cytochrome c_{551} and their Ru derivatives: (A) (bottom) native PCu, 100 μM in 0.10 M NaCl, 20 mM mixed buffer, 0.4 mM neomycin, pH 7.0 (temperature 2 °C, scan rate 100 mV s^{-1}, current scale 1.0 μA), (top) $PCu(His59)Ru(NH_3)_5$, 100 μM in 0.10 M NaCl, 20 mM mixed buffer, pH 7.0 (temperature 2 °C, scan rate 20 mV s^{-1}, current scale 0.2 μA); (B) (bottom) native HiPIP, 100 μM in 0.10 M NaCl, 20 mM Hepes, 0.4 mM neomycin, pH 7.0 (temperature 4 °C, scan rate 10 mV s^{-1}, current scale 0.1 μA), (top) $HiPIP(His42)Ru(NH_3)_5$, 130 μM in 0.10 M NaCl, 20 mM mixed buffer, pH 6.9 (temperature 1 °C, scan rate 10 mV s^{-1}, current scale 0.2 μA); (C) (bottom) native cytochrome c_{551}, 150 μM in 0.10 M NaCl, 20 mM mixed buffer, 0.6 mM neomycin, pH 7.0 (temperature 25 °C, scan rate 10 mV s^{-1}, current scale 0.2 μA), (top) $c_{551}(His47)Ru(NH_3)_5$, 100 μM in 0.10 M NaCl, 20 mM mixed buffer, pH 7.0 (temperature 25 °C, scan rate 20 mV s^{-1}, current scale 0.4 μA). Broken vertical lines indicate positions of $E°'$.

Results

Representative cyclic voltammograms are shown in Figure 1. Importantly, when examined using mixed-buffer electrolyte, each of the Ru-modified proteins gave stable cyclic voltammograms exhibiting the expected two sets of well-defined peak-type waves, *without* any requirement for a cationic promoter such as the aminocyclitol neomycin. By contrast, for native *S. obliquus* (and spinach) plastocyanins and cytochrome c_{551}, achievement of a satisfactory peak-type response from the single intrinsic metal center required the addition of small amounts of neomycin. As this was titrated in, weak and impersistent sigmoidal-type waves developed into stable peaks with typical separations (E_{pa} - E_{pc}) less than 70 mV at a scan rate of 20 mV s^{-1}. A peak-type response for native HiPIP was evident without neomycin, although its addition to a concentration of 0.8 mM resulted in further sharpening of the waves. Experiments with native HiPIP carried out with 20 mM Hepes yielded results that were essentially identical with tests made by using mixed buffer. In the case of either of the native plastocyanins, the voltammetric response obtained with a 0.1 mM protein solution at 25 °C was not stable even in the presence of neomycin. For plastocyanin and HiPIP

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Table I. Summary of Electrochemical Data for Native and Ru-Modified Proteins and $[Ru(NH_3)_5(imid)]^{3+/2+}$ [a]

couple	pH	temp/°C	$E°'$/mV vs SHE native	$E°'$/mV vs SHE modified	$\Delta S°_{rc}$/J K^{-1} mol^{-1} native	$\Delta S°_{rc}$/J K^{-1} mol^{-1} modified
			PCu (*S. obliquus*)			
$Cu^{2+/+}$	7.0	25	376 (±5)[b,c]			
$Cu^{2+/+}$	7.0	25	389 (±5)[d]		−26 (±5)	
$Cu^{2+/+}$	7.0	25		392 (±5)		−36 (±16)
$Ru^{3+/2+}$	7.0	25		142 (±5)		+58 (±14)
$Cu^{2+/+}$	7.0	20		397 (±8)[e]		
$Ru^{3+/2+}$	7.0	20		120 (±8)[e]		
			HiPIP (*C. vinosum*)			
$[4Fe\text{-}4S]^{3+/2+}$	6.9	25	357 (±5)[f]		−40 (±5)	
$[4Fe\text{-}4S]^{3+/2+}$	6.9	25		387 (±5)		−41 (±10)
$Ru^{3+/2+}$	6.9	25		150 (±8)		+12 (±3)
$[4Fe\text{-}4S]^{3+/2+}$	7.0	20		389 (±8)[e]		
$Ru^{3+/2+}$	7.0	20		122 (±8)[e]		
			cyt c_{551} (*P. stutzeri*)			
$Fe^{3+/2+}$	7.0	25	228 (±5)[g]			
$Fe^{3+/2+}$	7.0, 7.7	25		264, 268 (±5)		
$Ru^{3+/2+}$	7.0, 7.7	25		116, 119 (±8)		
$Fe^{3+/2+}$	6.5, 7.0, 7.7	25		(269, 266, 266) (±5)[e]		
$Ru^{3+/2+}$	6.5, 7.0, 7.7	25		(109, 106, 102) (±8)[e]		
			$[Ru(NH_3)_5(imid)]^{3+/2+}$			
$Ru^{3+/2+}$	7.0	25	109 (±5)			+37

[a] All data obtained with use of mixed-buffer electrolyte containing 0.10 M NaCl except where specified. [b] From kinetic studies with $[Co(phen)_3]^{3+/2+}$.[22] [c] Value 363 mV obtained by titration with $[Fe(CN)_6]^{3-/4-}$.[10] [d] $E°'$ value at 25 °C obtained by extrapolation of $E°'$ vs T plot, where values are determined in presence of 0.4 mM neomycin. [e] 0.10 M phosphate buffer with 0.10 M NaCl ($I \sim 0.32$ at pH 7). [f] In presence of 0.8 mM neomycin. [g] In presence of 0.6 mM neomycin.

and their derivatives, we made voltammetric measurements at various temperatures over the range 1–25 °C and determined values for the reaction center entropy $\Delta S°_{rc}$ ($S°_{red} - S°_{ox}$) from the temperature coefficient of plots of $E°'$ versus T,[17,18] as shown in Figure 2. Voltammograms obtained for Ru-modified plastocyanin and Ru-modified HiPIP in phosphate buffer were less satisfactory, since they showed broader peaks with larger separations ($E_{pa} - E_{pc}$). On the other hand, those obtained for Ru-modified cytochrome c_{551} were sharp and well-defined at 25 °C irrespective of whether mixed or phosphate buffers were used. In all cases, voltammetric peak currents were proportional to (scan rate)$^{1/2}$ over at least part of the range of scan rates used (5–500 mV s^{-1}), showing that reaction was controlled by diffusion of free species to the electrode surface. All potential data were obtained from voltammograms that met this criterion.

For the Ru-modified proteins we assigned the high- and low-potential couples in each case to the intrinsic metal center and $[Ru(NH_3)_5(His)]^{3+/2+}$, respectively. This is reasonable if we assume that reduction potentials are unlikely to be shifted greatly from their values for the native proteins (and the complex $[Ru(NH_3)_5(imid)]^{3+/2+}$). Measured formal reduction potentials, relevant conditions, and reaction entropies (where determined) are shown in Table I.

Discussion

By the attachment to each protein of a *single* Ru complex having a formal 3+ charge, the electrochemical requirement of plastocyanin, cytochrome c_{551}, and to some extent HiPIP for the presence of a cationic promoter (neomycin) is removed. Such promoters probably act by creating a noncovalent bridge between the negatively charged surface of the protein and that of polished PGE, which is rich in oxide functionalities and is believed to bear an effective negative charge at pH 7 over the potential range of these experiments.[14] This ternary interaction allows the protein to bind at sites on the electrode surface in such a manner as to enable fast electron transfer to occur[3,4,13-16]. For HiPIP, which is a weakly acidic protein, the gross effect of Ru modification on the overall protein charge is to transform it from slightly negative to neutral (or slightly positive). This property is suggested from the amino acid composition[19] and from the ion-exchange behavior of each form: both oxidized and reduced HiPIP bind (respectively more tightly) to an anion-exchange column (DE23) at pH 7.3,[20] whereas the oxidized Ru-modified form binds weakly to a cation-exchange column at pH 5.2.[11] Ru modification also transforms the overall charge on cytochrome c_{551} to approximately neutral (pH 7.0), as judged by amino acid composition[9] and ion-exchange behavior. On the other hand, by the same criteria, it is certain

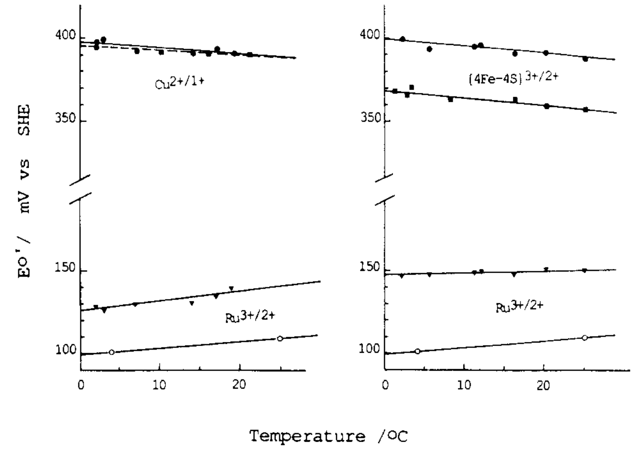

Figure 2. Variation of $E°'$ with temperature for native and Ru-modified proteins, showing least-squares fits: (left) (●) $Cu^{2+/1+}$ couple in *S. obliquus* PCu(His59)Ru$(NH_3)_5$, (■) $Cu^{2+/1+}$ couple in native *S. obliquus* PCu, solution containing 0.4 mM neomycin (least-squares fit indicated by broken line), (▼) $Ru^{3+/2+}$ couple in *S. obliquus* PCu(His59)Ru$(NH_3)_5$, (○) $[Ru(NH_3)_5(imid)]^{3+/2+}$; (right) (●) $[4Fe\text{-}4S]^{3+/2+}$ couple in *C. vinosum* HiPIP(His42)Ru$(NH_3)_5$, (■) $[[4Fe\text{-}4S]^{3+/2+}$ couple in native *C. vinosum* HiPIP, solution containing 0.4 mM neomycin, (▼) $Ru^{3+/2+}$ couple in *C. vinosum* HiPIP(His42)Ru$(NH_3)_5$, (○) $[Ru(NH_3)_5(imid)]^{3+/2+}$. All data were obtained with 0.10 M NaCl, 20 mM mixed buffer.

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that plastocyanin *retains an overall negative charge* at pH 7.0 after single-site modification.[6,10] The latter result, in particular, suggests the importance of *localized* charge (as opposed to the *sign* of the *overall* protein charge) in underlying the protein-electrode interaction that is necessary for reversible electron exchange.

For native and derivatized plastocyanin and HiPIP, and for $[Ru(NH_3)_5(imid)]^{3+/2+}$, graphs of $E^{\circ\prime}$ vs T were linear (Figure 2). In addition to yielding values for ΔS°_{rc}, this enabled us to determine the reduction potential of plastocyanin at 25 °C (for which voltammetry at this temperature was not stable) by simple extrapolation. Referring to Table I, we are able to make the following comments.

We first compare the voltammetrically measured formal reduction potentials for the native proteins with earlier determinations.

For *S. obliquus* plastocyanin ($Cu^{2+/1+}$), the value of 389 mV at 25 °C obtained in the presence of neomycin is appreciably higher than in previous studies.[10] Thus, titration of the *S. obliquus* protein with $[Fe(CN)_6]^{3-/4-}$ (for which an $E^{\circ\prime}$ value of 410 mV was used) at 25 °C in 0.10 M phosphate (pH 7.0, I = 0.21 M) gave reduction potentials for native and Ru-modified proteins of 363 and 385 mV, respectively. If ϵ(597 nm) is 5000 M^{-1} cm^{-1} instead of 4500 M^{-1} cm^{-1} as normally used (a value of 5160 M^{-1} cm^{-1} has recently been reported by Gewirth and Solomon[21]), then the $E^{\circ\prime}$ value must be raised by 5 mV. Furthermore, in this investigation we have measured the reduction potential for $Fe(CN)_6{}^{3-/4-}$ under identical conditions used for the earlier titration and found it to be 418 mV. This correction also increases $E^{\circ\prime}$ for plastocyanin. From kinetic studies on *S. obliquus* plastocyanin with $[Co(phen)_3]^{3+/2+}$, $E^{\circ\prime}$ is calculated to be 376 mV at pH 7.0.[22] We have also checked the reduction potential of spinach plastocyanin for which direct electrochemistry is also promoted by neomycin. This was found to be 384 mV when an electrode predipped in neomycin is used (this gives a transient peaklike voltammetric response when it is used with a solution of plastocyanin containing no neomycin) and 387 mV in the presence of 0.6 mM neomycin. We do not fully understand these differences and are uncertain whether the discrepancy arises entirely from the presence of neomycin. It is possible therefore that the $E^{\circ\prime}$ value for native plastocyanin is slightly higher than the value 370 mV that is widely used and may be closer to 380 mV. Some other reported determinations of $E^{\circ\prime}$ for plastocyanins cover a surprisingly large range: a value of 340 mV has been obtained for bean plastocyanin by thin-layer potentiometry using $[Co(phen)_3]^{3+}$ as mediator,[18] a value of 390 mV has been measured for the protein from *Chlorella ellipsoidea* by equilibration against $[Fe(CN)_6]^{3-/4-}$,[23] and a value of 360 mV has been determined for spinach plastocyanin by direct cyclic voltammetry in the presence of $[Pt(NH_3)_6]^{4+}$.[14]

The reduction potential of 228 mV for native cytochrome c_{551} ($Fe^{3+/2+}$) is somewhat higher than the value of 200 mV (pH 7) measured at ambient temperature by equilibration against $[Fe(CN)_6]^{3-/4-}$.[24] We found that the reduction potential of cytochrome c_{551} did not change on increasing the neomycin concentration from 0.4 to 0.8 mM.

The reduction potential of HiPIP ($[4Fe-4S]^{3+/2+}$) as measured at 4 °C in 20 mM Hepes, 0.10 M NaCl, increased from 367 to 371 mV as the neomycin concentration was raised from 0 to 0.8 mM. Our value of 357 mV at 25 °C, obtained by using mixed buffer in the presence of 0.8 mM neomycin, is in good agreement with the value originally reported by Bartsch and co-workers using the $Fe(CN)_6{}^{3-/4-}$ equilibration method.[19]

For all three proteins, attachment of the $Ru(NH_3)_5{}^{3+}$ group causes an increase in the reduction potential of the metal center of the protein. For plastocyanin, the effect is quite mild (taking the above-mentioned variations into consideration, the increase is within 21 mV when compared at 25 °C), but for HiPIP and cytochrome c_{551}, $E^{\circ\prime}$ is changed more significantly, the increases being around 30 and 36 mV, respectively. Placing a positive charge in the vicinity of a protein redox center is expected to raise its reduction potential (relative stabilization of the reduced state), and the magnitude of this effect is expected to depend upon the separation distance and effective dielectric constant of the intervening medium.[25] From the respective crystal structures we do indeed note that for plastocyanin[5] the through-space separation distance (Cys84{S} to His59{ring}) is of the order of about 12 Å, whereas for HiPIP (Cys43{S} to His42{ring})[7] and for cytochrome c_{551} (axial Met61 to His47 {ring}),[6] much shorter separations of about 8 Å are estimated. In each case, $E^{\circ\prime}$ values at pH 7.0 were similar regardless of whether studies were made in mixed or phosphate buffer. For cytochrome c_{551}, the absence of any variation of $E^{\circ\prime}$ with pH over the range 6.5–7.7 contrasts with the pH dependence in this range that has been reported for the native protein and attributed to ionization of His47.[22] Disappearance of this acid–base equilibrium (pK = 7.8 for c_{551} from *P. stutzeri* strain 224) is expected if His47 is coordinated by $Ru(NH_3)_5{}^{3+}$.

For the intrinsic metal centers in plastocyanin and HiPIP, the temperature coefficient $d(E^{\circ\prime})/dT$ is slightly negative, yielding small negative values of ΔS°_{rc}. The results suggest that for the reduced forms there is a small increase in solvent ordering or a more compact conformation. Entropy changes for native and derivatized proteins are very similar in view of the error (at least ±5 J K^{-1} mol^{-1}) that we estimate to be likely in the determinations. The respective values of −26 and −36 J K^{-1} mol^{-1} for native and Ru-modified plastocyanin may be compared with the value −10 J K^{-1} mol^{-1} obtained[18] by optically transparent thin-layer potentiometry studies using $[Co(phen)_3]^{3+}$ as mediator.

For each modified protein, as studied in mixed-buffer electrolyte, reduction potentials of the Ru centers are higher than that measured under the same conditions for $[Ru(NH_3)_5(imid)]^{3+/2+}$. Although the increase in $E^{\circ\prime}$ is consistent with the decrease in solvation and dielectric shielding that is expected upon attachment to the protein surface, such a simple view is not altogether clear from the values for $\Delta S^{\circ\prime}_{rc}$. Thus, while the positive ΔS°_{rc} determined for the free complex is certainly consistent with relaxation of solvent ordering accompanying the decrease in charge,[17] the differing relative directions of change in ΔS°_{rc} observed for modified plastocyanin (larger) and HiPIP (smaller) show that other, less tangible influences are operative. Furthermore, reduction potentials for the Ru site are significantly lower when measured in phosphate buffer as compared with mixed buffer. This suggests that decreased solvation and dielectric shielding at the protein-bound positively charged Ru center may be compensated for by binding of $HPO_4{}^{2-}$ (or $H_2PO_4{}^-$) close by. Such an interaction could stabilize the oxidized state of Ru while having a negligible influence on the redox properties of the intrinsic center.

Determinations of intramolecular rate constants for electron transfer from $[Ru(NH_3)_5(his)]^{2+}$ to the intrinsic metal center have each been carried out by pulse radiolysis[10–12] on solutions containing 0.10 M phosphate at pH 7. It is thus appropriate to use our measurements of reduction potentials obtained in the presence of phosphate to estimate driving forces (ΔE) for those reaction systems. Our values of ΔE are as follows: plastocyanin, 277 mV; HiPIP, 267 mV; cytochrome c_{551}, 260 mV. However, it should be emphasized that the measured ΔE corresponds to a state of equilibrium and need not necessarily describe, accurately, the energetics that are appropriate for the intramolecular electron-transfer system. In our experiments, the measured $E^{\circ\prime}$ value for the Ru site is obtained under the condition of the intrinsic site being reduced, and the $E^{\circ\prime}$ value for the intrinsic site is that measured while the Ru site is oxidized. By contrast, for the kinetic experiment, the initial state (that is, with the Ru site reduced while the intrinsic site is oxidized) is by necessity an unstable one for which we are unable to determine reduction potentials. The driving

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force for intramolecular electron transfer may thus differ from the voltammetrically determined value of ΔE depending upon the degree to which the reduction potential of one site is sensitive to the oxidation state of the other site close by.

In conclusion, the experiments demonstrate that interaction of proteins with electrode surfaces leading to reversible electron exchange is critically influenced by specific attachment of a single Ru complex. The loss of requirement for a cation promoter even though the sign of the protein's overall charge remains unchanged following modification (as with plastocyanin) provides support for the importance of localized protein surface charge, rather than overall charge alone, in determining its interaction with an electrode surface. The voltammetric measurements show further that the reduction potentials of redox sites in modified proteins may be significantly different from values assumed on the basis of the isolated components. In particular, the reduction potential of the histidine-attached Ru center (which is expected to be largely exposed at the protein surface) is sensitive to the composition of the electrolyte.

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Contribution from the Department of Chemistry,
University of Washington, Seattle, Washington 98195

Oxygen Atom Transfer Reactions of Cationic Rhenium(III), Rhenium(V), and Rhenium(VII) Triazacyclononane Complexes

Rebecca R. Conry and James M. Mayer*[1]

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$Re(O)Cl_3(Me_2S)(OPPh_3)$ reacts readily with 1,4,7-trimethyltriazacyclononane (Me_3tacn) to form the rhenium(V) oxo cation $[Re(O)Cl_2(Me_3tacn)]^+$ (**1**) in good yield. With the unsubstituted triazacyclononane (tacn), however, both $[Re(O)Cl_2(tacn)]^+$ (**2**) and $[Re(O)_3(tacn)]^+$ (**3**) are formed, even under anaerobic conditions. Oxidation of **2** to **3** [Re(V) → Re(VII)] can be easily accomplished with a variety of mild oxidizing agents such as Me_2SO and I_2, but the oxidation of **1** requires over a month at 80 °C in aqueous nitric acid. Complex **1** is reduced [Re(V) → Re(III)] by oxygen atom transfer to phosphines, forming $[Re(OPR_3)Cl_2(Me_3tacn)]^+$ (R = Ph, **4**; Me, **5**). The $OPPh_3$ ligand in **4** is easily displaced by other neutral ligands such as acetonitrile or acetone. The acetone complex $[Re(O{=}CMe_2)Cl_2(Me_3tacn)]^+$ (**7**) is readily oxidized back to **1** [Re(III) → Re(V)] by the oxygen atom donors ^nBuNCO, $OAsPh_3$, Me_2SO, ethylene oxide, pyridine *N*-oxide, and N_2O. These reactions require an open coordination site at the rhenium(III) center. Surprisingly, it is not substantially easier to oxidize the rhenium(III) complex **7** than the rhenium(V) species **2**. On the basis of these reactions, simple thermochemical cycles are used to estimate the rhenium-oxo bond strength in **1** to be 141 ± 9 kcal/mol.

Transfer of an oxygen atom between a metal center and a substrate is one of the most fundamental reactions of metal oxo complexes.[2,3] Oxygen atom transfer has also received attention because of its importance in biological systems, in organic synthesis, and in industrial processes. For example, it has been suggested as the critical step in catalysis by cytochrome P-450[4] and molybdenum hydroxylase enzymes.[5] Despite the interest in this reaction, oxygen atom transfer is less well understood than transfer of a univalent atom by classical inner-sphere electron transfer.[6] Only oxygen atom transfer processes involving molybdenum have received systematic study; a recent comprehensive review states that there are no reports of oxygen atom transfer to rhenium.[2]

A primary goal of this study was to examine the effect of metal oxidation state on oxygen atom transfer reactivity. We describe here[7] a series of oxygen atom transfer reactions that interconvert rhenium(III), rhenium(V), and rhenium(VII) complexes, which enable, for the first time, a comparison of the oxygen atom transfer reactivity of two different redox couples, $d^4 \rightleftharpoons d^2$ and $d^2 \rightleftharpoons d^0$. Studies of the related d^3 oxo complex, which would likely have one electron in a metal-oxygen antibonding orbital,[8] were however thwarted by the instability of this compound. Triazacyclononane (tacn) and its methylated analogue (1,4,7-trimethyltriazacyclononane, Me_3tacn) have been used as supporting ligands because they bind well to both high- and low- oxidation state complexes.[9] The observed reactions are used to derive an estimate of the $Re(V){\equiv}O$ bond strength and to discuss the mechanism of oxygen atom transfer.

Experimental Section

Syntheses were performed with standard Schlenk or vacuum-line techniques and a continuous nitrogen flow glovebox except as indicated. Solvents were dried and deoxygenated by standard methods.[10] All reactions were executed at ambient temperatures unless otherwise stated. NMR spectra were obtained on Varian VXR-300 or Bruker WM-500 spectrometers. Chemical shifts are reported in ppm downfield from TMS: δ (multiplicity, number of hydrogens). NMR spectra in D_2O were referenced to DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid, assigning the most upfield resonance to 0.015 ppm) for ^1H NMR and to MeOH (49.3 ppm) for ^{13}C. IR spectra were obtained as Nujol mulls on NaCl plates with Perkin-Elmer 283, FT 1604, or FT 1800 spectrometers and are reported in cm^{-1}. Elemental analyses were performed by Canadian

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