Metalloprotein Electron-Transfer Mechanisms. Quenching of Electronically Excited Tris(2,2'-bipyridine)ruthenium(II) by Reduced Blue Copper Proteins

Ann M. English, Vanessa R. Lum, Patricia J. DeLaive, and Harry B. Gray*

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from the Arthur Amos Noyes Laboratory
California Institute of Technology
Pasadena, California 91125

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The kinetics of electron transfer between blue copper proteins and inorganic complexes in aqueous solution have been studied extensively in our laboratory.[1] The results have been analyzed in terms of a bimolecular mechanistic model featuring close approach of the inorganic complex to the protein redox site at the instant of electron transfer. The inorganic redox reagents we have examined fall into two main groups: reagents that possess hydrophobic, π-conducting ligands [e.g., $Ru(NH_3)_5py^{3+}$, $Co(phen)_3^{3+}$] and those with relatively nonconducting, hydrophilic ligands [$Fe(EDTA)^{2-}$, $Ru(NH_3)_6^{2+}$]. The reactivities of the protein/π-conducting complex pairs are relatively high, and within the framework of a bimolecular mechanism this suggests that the redox site-to-site electron-transfer distances are not greater than $\sim$5 Å.[2]

In our previous work we have assumed that copper protein/inorganic complex electron-transfer reactions can be treated as uniformly nonadiabatic Marcus-type processes.[3] One important aspect of this treatment is the predicted dependence of the electron-transfer rate constants on the thermodynamic driving force ($\Delta E°$) for the reaction. Our earlier studies were not designed to test this aspect of the theory, as we chose protein/complex pairs with $\Delta E°$ values in the range of nearest neighbors in biological electron-transport chains ($\leq$0.25 V). An attractive means of manipulating $\Delta E°$ involves the use of an electronically excited inorganic complex as the oxidant or reductant; a specific system that we have found amenable to detailed study involves quenching of the luminescent excited state of $Ru(bpy)_3^{2+}$ (bpy = 2,2'-bipyridine; $E°$ (Ru^{2+*}/Ru^+) = 0.830 V)[4] by electron transfer from reduced blue copper proteins. Here we communicate our initial results involving three single-site copper proteins, *Pseudomonas aeruginosa* azurin, *Phaseolus vulgaris* plastocyanin, and *Rhus vernicifera* stellacyanin.

The lifetime of the Ru^{2+*} emission (610 nm) was monitored in aqueous solutions as a function of the PCu^I concentration.[5] For all three reduced copper proteins, the Stern-Volmer plots (τ_0/τ vs. PCu^I) are linear for protein concentrations up to 2 mM (Figure 1).[6] The observed second-order quenching rate constants are as follows: stellacyanin, 4.2 (1) $\times 10^8$; plastocyanin, 1.6 (1) $\times 10^9$; azurin, 6.9 (1) $\times 10^8$ M^{-1} s^{-1} (pH 7.0; μ = 0.1 M (phosphate); 25 °C).

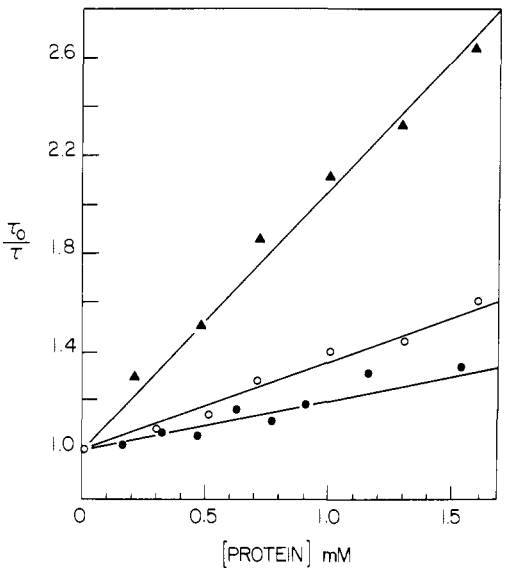

Figure 1. Plot of τ_0/τ vs. [protein] for plastocyanin (▲), azurin (○), and stellacyanin (●). Conditions: pH 7.0 (sodium phosphate), μ = 0.1 M, 25 °C, λ(excitation) = 532 nm, λ(emission) = 610 nm.

Several lines of evidence show conclusively that the quenching mechanism involves electron transfer from PCu^I to Ru^{2+*}:

$$PCu^I + Ru^{2+*} \xrightarrow{k_q\ (\equiv k_{12})} Ru^+ + PCu^{II}$$

Electronic energy transfer cannot contribute to k_q, because the lowest electronic excited states of PCu^I lie far above the emissive state of $Ru(bpy)_3^{2+}$. Relevant to this point is our observation that the emission from Ru^{2+*} is not quenched in solutions containing apoazurin. Furthermore, transient absorptions attributable to Ru^+ ($\sim$520 nm)[7] and PCu^{II} ($\sim$590 nm)[8] have been observed[9] in laser flash photolysis experiments on the PCu^I/Ru^{2+*} system, thereby confirming the electron-transfer pathway.

The PCu^I/Ru^{2+*} rate constants are near the diffusion limit for electron-transfer reactions in aqueous solutions. Such high rates of electron transfer are entirely consistent with a bimolecular close-contact mechanistic model involving an oxidant with π-conducting ligands. Indeed, calculations based on the same mechanistic model developed previously[2] for $PCu^I/Co(phen)_3^{3+}$ systems yield rate constants for electron transfer from PCu^I to Ru^{2+*} that are in good agreement with the experimentally derived values for plastocyanin (k_{12}^{∞} = 6.3 $\times 10^8$; k_{12}^{∞}(calcd) = 1.0 $\times 10^9$ M^{-1} s^{-1}) and azurin (k_{12}^{∞} = 5.9 $\times 10^8$; k_{12}^{∞}(calcd) = 1.6 $\times 10^9$ M^{-1} s^{-1}).[10,11] The probable contact areas for Ru^{2+*} are in the hydrophobic surface regions near the copper-histidine redox units in these proteins (His-87 in plastocyanin;[12] His-117 in azurin[13]). Outer-sphere electron transfer from Cu^I through the imidazole groups of these ligated histidines to Ru^{2+*} should be

(1) (a) Cummins, D.; Gray, H. B. *J. Am. Chem. Soc.* **1977**, *99*, 5158. (b) McArdle, J. V.; Coyle, C. L.; Gray, H. B.; Yoneda, G. S.; Holwerda, R. A. *J. Am. Chem. Soc.* **1977**, *99*, 2483. (c) Wherland, S.; Gray, H. B. In "Biological Aspects of Inorganic Chemistry"; Addison, A. W., Cullen, W. R., Dolphin, D., James, B. R., Eds.; Wiley: New York, 1977; p 289 and references therein. (d) Mauk, A. G.; Bordignon, E.; Gray, H. B., to be submitted for publication.

(2) Mauk, A. G.; Scott, R. A.; Gray, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 4360.

(3) (a) Marcus, R. A.; Sutin, N. *Inorg. Chem.* **1975**, *14*, 312. (b) Marcus, R. A. *J. Phys. Chem.* **1963**, *67*, 853.

(4) Sutin, N.; Creutz, C. *Adv. Chem. Ser.* **1978**, *No. 168*, 1.

(5) The proteins were isolated by standard methods (see ref 1a). Experiments on the reduced proteins (PCu^I, prepared by ascorbate reduction) were performed under anaerobic conditions. Dehydroascorbate and excess ascorbate were removed from the PCu^I solutions by dialysis against phosphate buffer. Excited-state lifetimes were determined by using a Nd:YAG (fwhm, 8 ns) laser system (excitation: second harmonic, 532 nm).

(6) At relatively high protein concentrations (>2 mM) the τ_0/τ value for each system reaches a limiting value (plastocyanin, $\sim$4.2; azurin $\sim$1.9; stellacyanin, $\sim$1.4). Among the several possible explanations of the observed lifetime saturation, an attractive one involves electron transfer from Cu^I to Ru^{2+*} within a precursor complex. A detailed discussion of the several mechanisms that could account for Ru^{2+*} lifetime saturation will be given in a subsequent paper.

(7) Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1976**, *98*, 6384.

(8) Solomon, E. I.; Hare, J. W.; Dooley, D. M.; Dawson, J. H.; Stephens, P. J.; Gray, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 168.

(9) English, A. M.; Lum, V. R.; Horwitz, J.; Kliger, D. S.; Gray, H. B.; DeLaive, P. J.; Humphry-Baker, R., unpublished results.

(10) The experimental second-order quenching rate constants were corrected for nonspecific protein-reagent electrostatic interactions as described in ref 2; they are given for $\mu = \infty$ (k_{12}^{∞}). Protein self-exchange rate constants (k_{11}^{∞}) and reduction potentials for the Marcus-type calculations were taken from analyses of $PCu^I/Co(phen)_3^{3+}$ reactions.[2] Final input parameters were k_{22}^{∞}(Ru^{2+*}/Ru^+) = 10^8 M^{-1} s^{-1} (uncorrected k_{22}^{∞}, see ref 4); ΔE^{∞}(stellacyanin) = 0.649 V; ΔE^{∞}(plastocyanin) = 0.467 V; ΔE^{∞}(azurin) = 0.529 V.

(11) Reduced stellacyanin, however, is not as efficient in electron-transfer quenching as our model predicts (k_{12}^{∞} (calcd) = 8 $\times 10^9$; k_{12}^{∞} = 4 $\times 10^8$ M^{-1} s^{-1}).

(12) Freeman, H. C. In "Coordination Chemistry-21"; Laurent, J. P., Ed.; Pergamon: Oxford, 1981; p 29.

(13) Adman, E. T.; Jensen, L. H. *Isr. J. Chem.* **1981**, *21*, 8.

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very facile. Estimated electron-transfer distances (R_p's)[2] are about the same for the two proteins (3.3 Å for plastocyanin; 3.8 Å for azurin), suggesting that the hydrophobic bpy ligands of Ru^{2+*} can penetrate the three residues (Met-13, Met-44, Phe-114)[13] that isolate the Cu-(His-117) unit from water molecules as well as hydrophilic redox agents.[1d]

The PCu^I/Ru^{2+*} system represents an unambiguous case of electron-transfer quenching of an electronically excited metal complex by a metalloprotein.[14] This type of system offers great advantages in studying the kinetics of very rapid electron-transfer reactions, owing to the relative ease of in situ preparation of the required oxidants and reductants. In future work we will attempt to attach certain photoredox agents directly to selected binding sites on PCu^I surfaces, with the goal of investigating the kinetics of intramolecular electron transfer between copper and relatively remote (>5-Å site-to-site distances) acceptors and donors.

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Registry No. $Ru(bpy)_3^{2+}$, 15158-62-0; Cu, 7440-50-8.

(14) Quenching of Ru^{2+*} by ferricytochrome *c* has been reported previously (Sutin, N. *Adv. Chem. Ser.* **1977**, *162*, 156). However, in this case a significant contribution to the quenching rate may come from electronic energy transfer (McLendon, G.; Lum, V. R.; English, A. M.; Gray, H. B., unpublished results).

Studies of Enzyme Stereochemistry. Elucidation of the Stereochemistry of *S*-Adenosylmethionine Formation by Yeast Methionine Adenosyltransferase

Ronald J. Parry* and Akwasi Minta

Department of Chemistry, Rice University
Houston, Texas 77001

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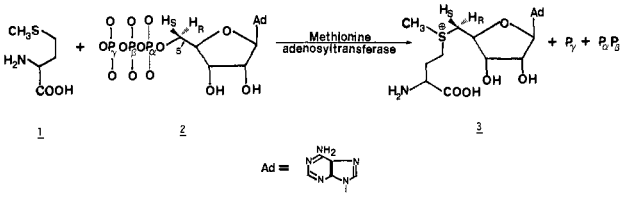
S-Adenosylmethionine (SAM, **3**), the principal agent for biological transmethylations, is biosynthesized from methionine (**1**) and adenosine triphosphate (ATP, **2**) via a reaction catalyzed by the enzyme methionine adenosyltransferase (Scheme I). This enzyme has been isolated in purified form from a number of sources, and it has been subjected to considerable mechanistic scrutiny.[1] These studies allow one to formulate two plausible reaction paths for the generation of SAM from ATP and methionine (Scheme II). The first pathway (Scheme IIA), which is consistent with all the available evidence,[1] assumes that ATP and methionine bind to the enzyme in such a way that a single displacement takes places at C-5′ of ATP to give SAM and enzyme-bound tripolyphosphate. This reaction path would presumably lead to overall inversion of configuration at C-5′ of the resulting SAM. The second pathway (Scheme IIB) would proceed by displacement of tripolyphosphate from C-5′ of ATP by a nucleophilic group present at the active site of the enzyme. A second displacement at C-5′ would then result in the transfer of the adenosyl group to the sulfur atom of methionine. Since two displacements occur in the second pathway, overall retention of configuration could be expected at C-5′ of SAM. The double displacement mechanism for SAM formation appears to be a less likely possibility than the single displacement mechanism due to the failure of attempts to detect an adenosyl-enzyme intermediate.[1] We would now like to report the results of stereochemical studies that also support the single displacement mechanism.

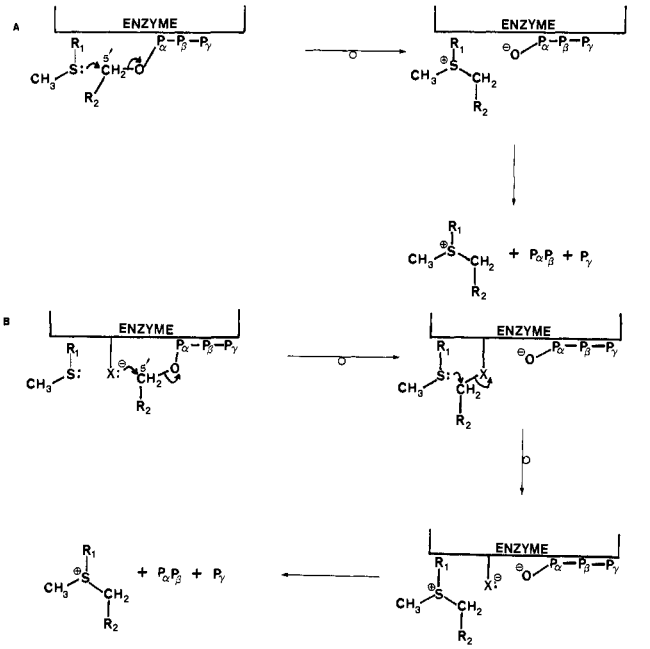
The stereochemistry of *S*-adenosylmethionine formation has been elucidated with the aid of adenosine derivatives that are

(1) Mudd, S. H. *Enzymes, 3rd Ed.* **1973**, *8*, 121-154.

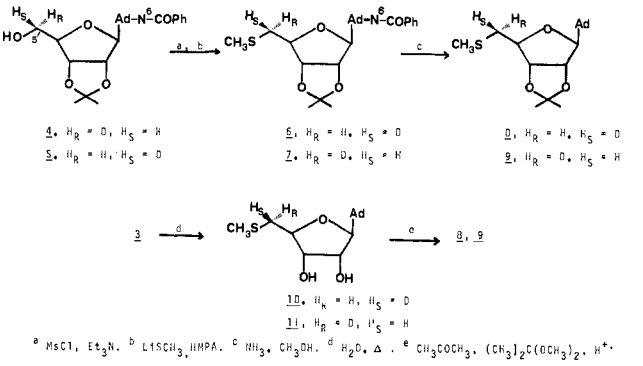
Scheme I



Scheme II



Scheme III



chirally deuterated at C-5′. The initial stages of the investigation were concerned with the synthesis of [5′(*S*)- and [5′(*R*)-$^2H_1$]-2′,3′-*O*-isopropylidene-5′-deoxy-5′-(methylthio)adenosine (**8**, **9**, Scheme III) as NMR reference samples. These reference compounds were synthesized from [5′(*R*)- and [5′(*S*)-$^2H_1$]-N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine (**4**, **5**).[2] The N^6-benzoyl derivatives were converted to the corresponding mesylates and then treated with lithium methylthiolate[3] in HMPA to give [5′(*S*)- and [5′(*R*)-$^2H_1$]-N^6-benzoyl-2′,3′-*O*-isopropylidene-5′-deoxy-5′-(methylthio)adenosine (**6**, **7**) by a displacement reaction that is presumed to occur with inversion of configuration.[4] Removal

(2) (a) Parry, R. J. *J. Chem. Soc., Chem. Commun.* **1978**, 294. (b) Midland, M. M.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1977**, *99*, 5211. (c) Dupre, M.; Gaudemer, A. *Tetrahedron Lett.* **1978**, 2783.

(3) Kelly, T. R.; Dali, B. B.; Tsang, W. G. *Tetrahedron Lett.* **1977**, 3859.

(4) The use of the N^6-benzoyl derivatives avoids the possibility[5] that the sequence might proceed via the formation of a N^3,5′-cyclonucleoside followed by attack of the methylthiolate anion. If the latter reaction course were to obtain, then the stereochemical result would be retention of configuration at C-5′.

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