Evaluation of the Role of Polyisoprenyl Functional Groups in Biological Electron Transfer. Transition Metal Models†

James K. Hurst

ABSTRACT: Copper(I) coordination to olefin bonds in pyridine compounds containing di- and triisoprenyl substituent groups has been investigated. Results from Raman and optical spectroscopic studies in aqueous ethanolic solutions indicate formation of π complexes of 1:1 stoichiometry, with $K \simeq 10^4$ M^{-1}. Despite there being several potential Cu(I) ligation sites on the alkyl side chain, only a single olefin bond is coordinated. The data are consistent with a model comprising extensive folding of the isoprenyl groups in the polar medium, with Cu(I) binding occurring at the exposed olefin group on the terminal unit. Ligand-bridged binuclear ions were formed by simultaneous coordination of an oxidant metal ion, $(NH_3)_5Ru^{III}$, to the pyridine ring nitrogen atoms and Cu(I) to side-chain olefin bonds. Electron-transfer pathways were determined by kinetic analysis; both rate laws and comparative redox rates for complexes containing a variety of 4-alkylpyridine ligands indicate reaction predominantly by intermolecular processes. No evidence for intramolecular electron transfer, i.e., from Cu(I) through the bridging ligand to the bound Ru(III) center, could be found. This result is discussed both in terms of its implications toward the existence of very similar pathways proposed for electron transfer between heme and copper redox sites in cytochrome oxidase and within the wider context of apparent differences in the fundamental mechanisms of electron transfer in biological particles and transition metal ions.

Considerable effort is presently being given to determining the fundamental nature of electron transfer in biological systems. Evidence is accumulating which suggests that discrete redox sites in multiredox particles and complexes between metalloproteins can be separated by relatively large distances, yet still allow for rapid electron transfer between them. Thus, estimates based upon various nuclear magnetic resonance (Gupta & Yonetani, 1973; Dobson et al., 1974; Burns et al., 1975), optical spectroscopic (Leonard & Yonetani, 1974; Vanderkooi et al., 1977; Potasek & Hopfield, 1977; Potasek, 1978; Dockter et al., 1978), and crystallographic methods (Salemme, 1977) have placed heme–heme and heme–substrate distances in several complexes at 15–20 Å, sufficiently large that gaps of 5–10 Å might exist between the edge of the porphyrin rings and second redox sites. Structural and electron paramagnetic resonance studies have indicated comparable or even greater distances between other biological redox sites, namely, between iron–sulfur centers in bacterial ferredoxins (Phillips et al., 1974; Sieker et al., 1972) and the molybdenum–iron sulfur centers in xanthine oxidase (Lowe & Bray, 1978). Because the iron–sulfur group of HIPIP is buried within its protein matrix (Carter et al., 1974 a,b), electron transfer to external redox agents is also likely to be long range for this particle (Mizrahi et al., 1976).

The mechanisms by which electrons are transferred in these instances are not understood. Long-range electron transfer over distances as great as 15 Å has been demonstrated in coordination compounds (Nordmeyer & Taube, 1968; Gaswick & Haim, 1974; Jwo & Haim, 1976; Taube, 1977; Norton & Hurst, 1978) but, with few exceptions,[1] requires formation of binuclear ions containing bridging ligands capable of providing π-delocalized interaction between the metal centers. Although redox proteins do not contain π-conjugated structures similar to the bridging ligands used in the inorganic reactions, the possibility exists that comparable interaction between donor and acceptor sites could be attained through π-delocalized "channels" formed by parallel overlap of aromatic and/or olefinic functional groups that are found in the biological materials. Theoretical models consistent with this suggestion have been developed (Halpern & Orgel, 1960; Dogonadze et al., 1973), the π-stacked channel serving to facilitate electron tunneling between redox centers.

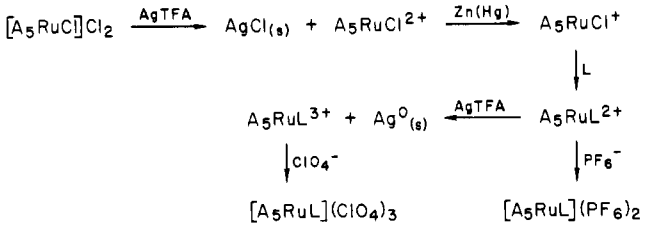
Specific pathways of this nature have been proposed for electron transfer in several redox metalloproteins, including cytochrome *c* (Dickerson & Timkovich, 1975), bacterial ferredoxins (Packer et al., 1972), and cytochrome *c* oxidase (Caughey et al., 1975). There is now fairly conclusive evidence against electron transfer through properly oriented aromatic groups in the former two cases (Dickerson & Timkovich, 1975; Lode et al., 1974), but the proposed pathway in cytochrome oxidase remains largely untested. The suggestion for this pathway is based upon recent structural characterizations of heme *a*, which has been shown to contain an unusual C_{15}-polyisoprenoid (farnesyl) substituent group on the porphine ring. This carbon chain is capable of folding to allow approximately parallel overlap of individual olefin bonds and the entire stack can assume an orientation over the plane and parallel to the ring. Cuprous ion binds strongly to olefins (Hurst & Lane, 1973) and, under appropriate circumstances, can apparently transfer electrons through olefin bonds to suitable oxidant metal centers (Farr et al., 1975; Norton & Hurst, 1978). Considering as well the current evidence favoring cytochrome oxidase functioning in redox reactions as strongly interacting heme–copper pairs (Palmer et al., 1976; Tweedle et al., 1978), the proposed involvement of the farnesyl substituent group seems plausible. Polyisoprenyl side chains of quinones acting as redox carriers in other parts of respiratory chains could, in principle, serve a similar function in providing electron-transfer pathways (Caughey et al., 1975), although studies in quinone specificity suggest side-chain unsaturation

† From the Department of Chemistry and Biochemical Sciences, Oregon Graduate Center, Beaverton, Oregon 97005. *Received September 20, 1978; revised manuscript received January 3, 1979.* This work was supported by a research grant from the U.S. Public Health Service (GM 20943).

[1] One example of electron transfer through a bridge for which π conjugation is disrupted by introduction of a single methylene group has been reported (Rieder & Taube, 1977). Optically induced electron transfer has been measured in binuclear ions containing nonconjugated bridging ligands with sulfur donor atoms (Taube, 1977; Stein & Taube, 1978); sulfur–sulfur distances are probably only 2–3 Å in these compounds.

0006-2960/79/0418-1504$01.00/0

Scheme I



is not obligatory for reaction (Brodie & Ballantine, 1960; Lenaz et al., 1968).

In the present paper we examine redox reactions between metal ions which have been designed to promote electron transfer by π-stacked polyisoprenoid pathways. The Ru(III)–Cu(I) binuclear ions, as discussed in the text, satisfy several criteria as acceptable models for heme–copper pairs that might exist in cytochrome oxidase and, this feature notwithstanding, permit evaluation of the capacity of isoprenyl groups to function in electron transfer. A preliminary account of this work has been published (Norton et al., 1979).

Experimental Procedures

Reagents. Alkylpyridine compounds were synthesized by reacting the corresponding C_{n-1} alkyl bromides with 4-picoline (Brown & Murphey, 1951) and purified by fractional distillation followed by elution with chloroform–ethyl acetate (9:1 v/v) on silica gel. Structures were confirmed by proton NMR analysis in carbon tetrachloride. When commercially unavailable, alkyl bromides were synthesized from the corresponding alcohols (Wiley et al., 1964). Purification after product isolation was unnecessary; in fact, fractional distillation generally gave low yields and evidence of considerable decomposition. Synthesis of pentaammineruthenium (A_5Ru)[2] coordination complexes containing the 4-alkylpyridine ligands (L) followed Scheme I. Stepwise procedures have been described in detail (Norton & Hurst, 1978) excepting the following modification: in the present case, chloride counterion was removed prior to reduction by filtration following precipitation with stoichiometric amounts of Ag(I). The coordination complexes of one ligand, 4-(4′,8′,12′-trimethyl-3′(*E*),7′(*E*),11′-tridecatrienyl)pyridine, hereafter, farnesylpicoline, did not form well-characterized salts. Purification of this complex ion was accomplished by extracting the crude product from 2 M NaTFA, pH 3, into ether and back-extracting into H_2O prior to oxidation with Ag(I). The extractions were done in an oxygen-free argon environment using solvents flushed with argon to minimize losses due to premature oxidation of the Ru(II) ion. Following precipitation and recrystallization with $NaClO_4$, the complex was eluted from a Sephadex G-15 column with 0.01 M NaTFA, pH 5, to remove excess perchlorate ion. The complex was stored as a frozen solution. Concentrations were established by analysis for ruthenium as the perruthenate ion (Larsen & Ross, 1959). The molar extinction coefficient for RuO_4^-, $\epsilon_{383} = 1.95 \times 10^3$ M^{-1} cm^{-1}, was determined from reference solutions containing A_5RuCl_3; this number agrees favorably with literature values. Proton NMR spectra in Me_2SO-d_6 and electronic absorption spectra of each of the coordination complexes were made to establish purities. These measurements were generally made on the complexes in their divalent oxidation state since Ru(II) has a diamagnetic d^6 electronic configuration and its pyridine complexes possess diagnostic Ru(II) → L(π^*) charge-transfer absorption bands in the visible region (Ford et al., 1968). The Ru(III) ions were reduced over amalgamated zinc in anaerobic solutions and loaded into NMR tubes in a glovebox in an argon atmosphere; these procedures effectively prevented paramagnetic line broadening resulting from Ru(II) autoxidation. For each complex, proton NMR spectra were identical with those for the free ligand with two additional bands at δ 2.24 and 2.78, relative to Me_4Si, ascribable to *cis*- and *trans*-NH_3 ligands, respectively; no extraneous peaks were detected. Formation of ruthenium–nitrogen coordinate bonds was evident from the downfield shifts of approximately 0.04 and 0.08 ppm of the doublets arising from protons on the pyridine ring which are centered at δ 8.37 and 6.96, respectively, in the uncoordinated ligands and from the absorption spectra, which gave single, symmetrical bands in the visible region (Ford et al., 1968). Spectral parameters (λ_{max} (log ϵ_λ)) for A_5Ru–4-pyR ions in 0.1 M TFA, 23 °C, which have not previously been reported (Norton & Hurst, 1978), are as follows: R = $(CH_2)_4CH_3$, Ru(II), 398 (3.91), 242 (3.62); R = $(CH_2)_3CH{=}CH_2$, Ru(II), 398 (3.90), 242 (3.67); R = $(CH_2)_5CH{=}CH_2$, Ru(II), 398 (3.88), 242 (3.66); R = $CH_2(CH_2CH{=}C(CH_3)CH_2)_3H$, Ru(II), 402 (3.88), 245 (3.63), Ru(III), 252 (3.87). Electronic energies are nearly identical with values which have been previously reported for other pyridine-containing A_5Ru^{II} ions (Ford et al., 1968). Ultraviolet spectra showed the product complexes to be free of ruthenium-containing impurities, e.g., A_5RuCl^{2+} and A_5RuOH^{2+} ions. Preparation of other reagents used in these studies has been described (Norton & Hurst, 1978).

Caution: Perchlorate salts of these coordination complexes are potentially explosive and should be synthesized only in small quantities.

Copper(I) Binding and Redox Kinetic Studies. Copper(I)–olefin binding stoichiometries and association constants were determined by measuring intensities of Cu(I) → olefin (π^*) near-ultraviolet absorption bands which appear upon π complexation (Hurst & Lane, 1973). Anaerobic reactant solutions were prepared by outgassing with argon and were mixed directly in optical cells using syringe-transfer methods. For farnesylpicoline and its coordination complexes, attempts at bubbling argon through the solutions led to extensive foaming. Deoxygenation was accomplished in these cases by passing a stream of argon over the rapidly stirred solutions. Equilibrium binding calculations were made as previously described (Hurst & Lane, 1973).

Solutions for Raman spectroscopic analysis were prepared by reducing Cu(II) with Cr(II) in the presence of the Cu(I)-coordinating ligands. After reaction, product solutions were transferred to an argon-filled glovebox, filtered through glass wool plugs in Pasteur pipettes, loaded into capillary tubes, and sealed; introduction of adventitious oxygen was thereby minimized. Data collection generally involved repetitive scanning of selected spectral regions. It was occasionally found in solutions with high Cu(I)-to-ligand ratios that Cu^0 precipitated out of solution after exposure for extended periods of time in the laser beam. The procedure of changing the observation area of the tubes every few scans was therefore adopted. It is not known whether the Cu^0 originates from photoinitiated redox processes involving oxidation of ligands or from Cu(I) disproportionation, although the latter seems more likely since the solutions are optically clear in the spectral region of the exciting light.

Electron-transfer rates in solutions containing Cu(I) and Ru(III) ions were measured by following changes in light

[2] Abbreviations used: A_5Ru, pentaammineruthenium moiety; py, pyridine; 4-pyR, 4-alkylpyridines, with R being the alkyl substituent; TFA, trifluoroacetic acid; Me_4Si, tetramethylsilane.

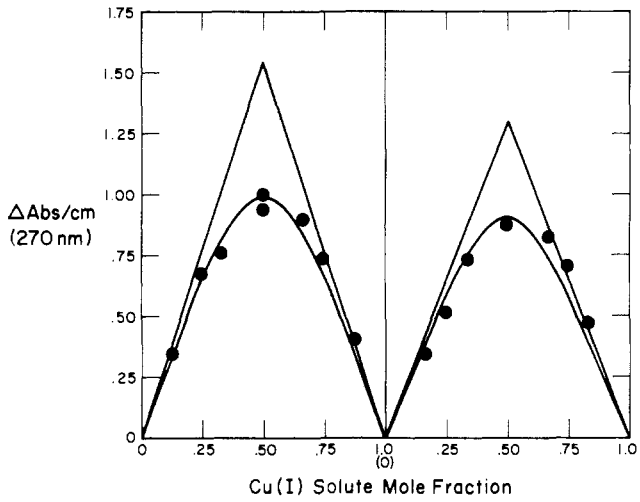


FIGURE 1: Job diagrams of Cu(I) binding to polyisoprenylpyridine (4-pyR) compounds. (Left plot) 4-pyR = $NC_5H_4CH_2(CH_2CH{=}C(CH_3)CH_2)_3H$; (right plot) 4-pyR = $NC_5H_4CH_2(CH_2CH{=}C(CH_3)CH_2)_2H$. Solid circles are averages of duplicate experimental points; (solid lines) theoretical curves assuming 1:1 stoichiometry and the binding parameters given in the text (inner curves), or with $K = \infty$ (outer curves). Total solute mole fraction, $([Cu(I)]_T + [4\text{-pyR}]_T) = 1.0$ mM; other experimental conditions are given in the text. ΔAbs/cm was corrected for the difference in absorptivities of Cu(I) and Cu(II) (Hurst & Lane, 1973).

absorption at the maxima in the visible absorption bands for the A_5Ru^{II}–4-pyR ions (Norton & Hurst, 1978). The Ru(III) complex ions are unstable and undergo slow decomposition, the initial consequences of which are markedly enhanced reactivities toward Cu(I), even when changes in their physical properties are imperceptible. Catalytic impurities, once formed, could not be completely removed either by repeated recrystallization or by chromatography on Sephadex G-15 columns. Kinetic data were therefore taken immediately after preparation of the complexes and considered reliable only after reproducible results from several syntheses were obtained. The problem was more pronounced for the complexes containing larger alkylpyridine ligands.

Instrumentation. Proton NMR spectra were taken on a Varian HA-100 instrument, electronic absorption spectra on a recorder-interfaced Cary 16 spectrophotometer, and Raman spectra on a Jarrell-Ash 25-300 spectrometer equipped with a digital data acquisition and data reduction computer system. A Coherent Radiation Laboratories argon ion laser was used as excitation source.

Results

Cuprous Ion Binding to Polyisoprenylpyridine Compounds. Stoichiometries for Cu(I) π complexation to pyridine compounds containing geranyl and farnesyl substituents, 4-(4′,8′-dimethyl-3′(*E*),7′-nonadienyl)pyridine and 4-(4′,8′,12′-trimethyl-3′(*E*),7′(*E*),11′-tridecatrienyl)pyridine, respectively, were investigated using the method of continuous variations (Rossotti & Rossotti, 1961). Results, given in Figure 1, are indicative of predominantly 1:1 complexation. The slight asymmetry in the plots could be due to binding of additional Cu(I) at the higher Cu(I)/ligand ratios, but this interpretation is not supported by the Raman studies. Alternatively, the asymmetry may represent small losses of the Cu(I) titer arising from introduction of adventitious oxygen during manipulation of reagent solutions; such effects would be more pronounced at lower Cu(I)/ligand ratios.

Equilibrium constants were determined from difference spectral measurements using a method of successive approximations (Hurst & Lane, 1973). Best fit values of K ($= [CuL]/[Cu][L]$) and $\Delta\epsilon$ ($= \epsilon_{CuL} - \epsilon_{Cu} - \epsilon_L$) are, at 23 °C, as follows: for L = 4-pyR, R = $CH_2(CH_2CH{=}C(CH_3)CH_2)_2H$, $K = 1.5\ (\pm 0.5) \times 10^4\ M^{-1}$, $\Delta\epsilon = 2.6 \times 10^3\ M^{-1}\ cm^{-1}$ at 270 nm; for L = 4-pyR, R = $CH_2(CH_2CH{=}C(CH_3)CH_2)_3H$, $K = 9.6\ (\pm 1.9) \times 10^3\ M^{-1}$, $\Delta\epsilon = 3.1 \times 10^3\ M^{-1}\ cm^{-1}$ at 270 nm. Reagent concentrations were varied over the ranges $[Cu(I)]_T = 0.05$–0.87 mM, $[L]_T = 0.05$–0.87 mM, with $[Cu(I)]_T/[L]_T = 0.14$–7.0; subscripts T refer to total concentrations of added reagents. Solutions contained 0.1 M TFA, 0.5 mM Cu(II), and Cr(III) in concentrations equal to Cu(I); for the farnesyl derivative, the medium also contained 20% ethanol to solubilize the ligand. Spectral parameters and association constants are very similar to values recorded for simple Cu(I)–olefin π bonding (Hurst & Lane, 1973; Farr et al., 1975; Hartley, 1973).

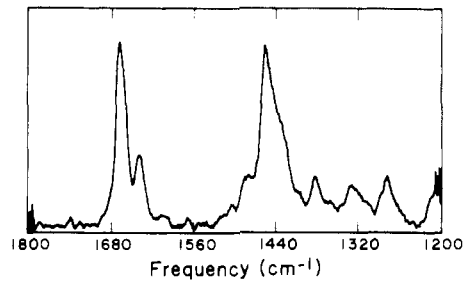


FIGURE 2: Raman spectrum of farnesylpicoline ($NC_5H_4CH_2(CH_2CH{=}C(CH_3)CH_2)_3H$). Conditions: [farnesylpicoline] = 0.5 M, [TFA] = 0.5 M in 60% ethanol; single scan in a sealed capillary, 1.7-mm diameter, obtained at 90° scattering geometry by excitation with the 5145-Å argon ion laser line (400 mW); scan rate, 6 cm^{-1}/s; slit width, 3.6 cm^{-1}. Signal-to-noise ratio was improved using a 25-point smoothing routine (Savitzky & Golay, 1964); background was subtracted using the available computer graphics.

A typical Raman spectrum for the farnesylpicoline compound in 60% ethanol is given in Figure 2. The main feature of interest is the intense olefin carbon–carbon stretching frequency seen at 1665 cm^{-1}; the band at 1638 cm^{-1} is identified with vibrational motions of the pyridine ring and those at 1480, 1451, and 1275 cm^{-1} are due to the alcoholic solvent. There is also a very weak, broad solvent band lying in the region 1600–1740 cm^{-1} which is apparent only in the spectra of considerably more dilute solutions (Figure 3). It has been established that Cu(I) coordination to the olefin leads to a shift in its C=C stretching mode of about 120 cm^{-1} to lower energies (Hurst & Lane, 1973). This property has been exploited to probe the accessibility of various olefin bonds in the polyisoprenyl ligands to Cu(I) coordination. As shown in Figure 3, the intensity of the olefin band at 1660–1670 cm^{-1} is diminished and a new band attributable to the Cu(I)–olefin complex appears at 1540 cm^{-1} on the shoulder of the strong ethanol absorption peak. For these analyses Cu(I)–olefin complexes were prepared and spectra recorded; the reagent solutions were then immediately oxygenated, which removes Cu(I) by oxidation, and rerun to provide reference spectra. The solutions were necessarily dilute to minimize complications associated with Cu(I) instability. As a consequence, the spectra are of insufficient quality to allow accurate integration of peak intensities. Semiquantitative analysis was made, however, by referring changes in peak heights for the 1665-cm^{-1} olefin band under various conditions to that of the pyridine mode at 1638 cm^{-1}. This comparison is of dubious quantitative significance since it is difficult to establish an accurate base line in this spectral region and it is not firmly established that the individual olefin bonds contribute equally to the 1665-cm^{-1} peak. Nonetheless, comparison of peak

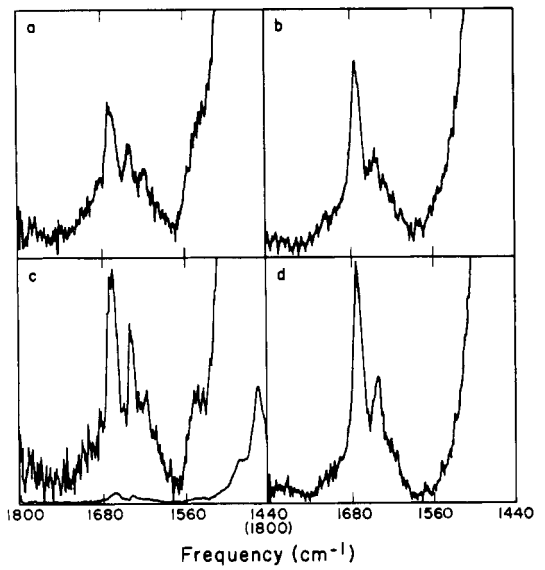


FIGURE 3: Raman spectra of polyisoprenylpyridine compounds (L) and their Cu(I) π complexes in 60% ethanol. (a) Cu(I)–farnesylpicoline, $[Cu(I)]_T$ = 50 mM, $[L]_T$ = 25 mM, [TFA] = 0.1 M, [Cu(II)] = 0.1 M, accumulation of 40 scans; (b) after oxygenation, [Cu(I)] = 0, accumulation of 20 scans, other conditions same; (c) Cu(I)–geranylpicoline, $[Cu(I)]_T$ = 50 mM, $[L]_T$ = 55 mM, [TFA] = 0.1 M, [Cu(II)] = 0.1 M, accumulation of 20 scans; (d) after oxygenation, [Cu(I)] = 0, other conditions same. Relative intensities of lines in spectra a–d are arbitrary; the lower line in panel c displays the averaged scan at reduced intensity. Data acquisition under conditions identical with those of Figure 2, except slit width was 8.5 cm^{-1} and 9-point smoothing routine was used.

Table I: Relative Intensities of Raman Spectral Lines[a]

$[L]_T$ (mM)	$[Cu(I)]_T/[L]_T$	R = 1665/1638	R' = 1665/1638[c] after oxygenation	bonds not π complexed[b]
A. L = $NC_5H_4CH_2(CH_2CH{=}C(CH_3)CH_2)_3H$				
500	0		2.5	
50	0		2.1	
50	0.9	1.9	2.4	2.6
25	1.0	1.6	1.8	2.3
25	2.0	1.4	1.9	1.9
21	3.0	1.6	2.2	2.2
B. L = $NC_5H_4CH_2(CH_2CH{=}C(CH_3)CH_2)_2H$				
25	0		2.2	
50	0.9	1.3	2.0	1.3
25	2.0	1.2	1.7	1.2

[a] At 23 °C, in 60% ethanol, 0.1 M TFA, [Cu(II)] = 0.1 M. Spectra were recorded as described in Figures 2 and 3. [b] Calculated from R/R' × (total no. of olefin bonds) using averaged values for R'. [c] Averages: for A, R' = 2.1; for B, R' = 2.0.

height ratios (Table I) suggests that, for both ligands, a single olefin group is bound, even with metal ion present in considerable excess.

Cuprous Ion Reduction of (Alkylpyridine)pentaammineruthenium(III) Ions. Reduction of $(NH_3)_5Ru^{III}$–4-alkylpyridine ions containing a variety of alkanyl and alkenyl hydrocarbon tails was investigated. For these studies, concentration levels of one of the reactants was maintained in sufficient excess to ensure that pseudo-first-order conditions were attained and, when alkene-containing ligands were used, that the limiting reagent was bound quantitatively as the Ru(III)–Cu(I) binuclear ion. Product solutions gave spectra identical with that for the appropriate Ru(II) ions, and absorbancy changes were within 5% of the values expected for

Table II: Rate Constants for Cu(I) Reduction of $(NH_3)_5Ru^{III}$–4-pyR Ions[a]

R	k (M^{-1} s^{-1})	excess reagent[b]
A. alkanes		
$-CH_2CH_3$	5.4	either (0.5–2.0 mM)[c]
$-(CH_2)_4CH_3$	16	Ru(III) (1.2–4.7 mM)
$-(CH_2)_7CH_3$	17	Ru(III) (0.5–2.5 mM)
B. alkenes		
$-(CH_2)_2CH{=}CH_2$	8.3	Ru(III) (1.7–5.7 mM)
$-(CH_2)_3CH{=}CH_2$	8.9	Cu(I) (0.9–5.5 mM)
$-(CH_2)_5CH{=}CH_2$	6.4	Cu(I) (0.4–9.1 mM)
$-(CH_2)_7CH{=}CH_2$	6.2	Ru(III) (1.4–4.5 mM)
C. polyisoprenes		
$-CH_2(CH_2CH{=}CCH_2)_2H$ (CH₃)	7.8	Cu(I) (0.7–7.6 mM)
	22	Ru(III) (1.3–3.7 mM)
$-CH_2(CH_2CH{=}CCH_2)_3H$ (CH₃)	7.7	Cu(I) (0.4–2.7 mM)
	17	Ru(III) (0.4–2.5 mM)
	10[d]	[Cu(I)] = [Ru(III)] = 0.40 mM
	9.6[d]	[Cu(I)] = [Ru(III)] = 0.15 mM
	22[d]	[Cu(I)] = [Ru(III)] = 0.04 mM

[a] At 23 °C, in 0.1 M TFA. [b] Values in parentheses give concentration ranges for reagents held in excess in pseudo-first-order runs. [c] Norton & Hurst (1978); other data, this work. [d] Calculated from initial rates.

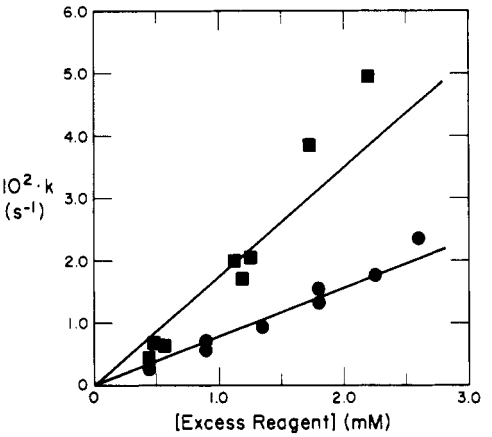


FIGURE 4: Reduction of $(NH_3)_5Ru^{III}NC_5H_4CH_2(CH_2CH{=}C(CH_3)CH_2)_3H$ ion by Cu(I) in 0.1 m TFA, 23 °C. (Ordinate) Pseudo-first-order rate constant; (abscissa) concentration of reagent held in excess. (Solid circles) Average of duplicate determinations with $[Cu(I)]_T > [Ru(III)]_T$; (solid squares) with $[Ru(III)]_T > [Cu(I)]_T$.

stoichiometric one-electron reduction of the Ru(III) complexes. The net reaction, therefore, is

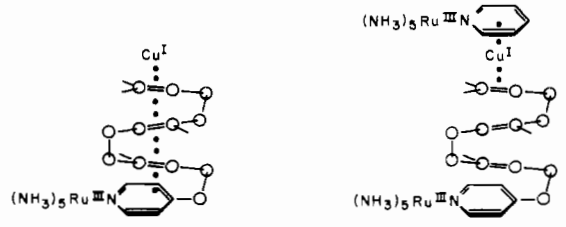
$$(NH_3)_5Ru^{III}\text{–4-pyR} + Cu(I) \rightleftharpoons (NH_3)_5Ru^{II}\text{–4-pyR} + Cu(II)$$

In all cases, the reactions followed the rate law $d[Ru(II)]/dt = k[Ru(III)]_T[Cu(I)]_T$, the subscript T referring to total reagent concentrations; rate constants are summarized in Table II. Kinetic data for the complex containing the farnesyl substituent group, which is typical, are given in Figure 4. This reaction was also studied at considerably lower reagent concentrations under second-order reaction conditions (equimolar reactants). Rate constants, calculated from initial rates of formation of Ru(II) product under these conditions, are also given in Table II.

Discussion

Nature of the Cu(I)–Polyisoprenylpyridine π Complexes. The optical and Raman spectroscopic properties of solutions

Chart I



containing Cu(I) and polyisoprenylpyridines offer clear evidence of π complexation (Hurst & Lane, 1973). Despite there being two or three potential Cu(I) coordination sites on the ligands, predominantly 1:1 metal–ligand complexes are formed. The Raman data further suggest that chelation of olefin bonds does not occur; i.e., each Cu(I) ion is bound to a single olefin group. In the highly acidic media used for these studies, the pyridine ring nitrogen is fully protonated and, therefore, not expected to form a suitable binding site for Cu(I). This assertion receives support from the magnitudes of measured association constants, which are typical for simple Cu(I)–olefin complexation (Hartley, 1973; Hurst & Lane, 1973).

In polar media the alkyl chain is expected to assume a folded conformation.[3] Free rotation of the polyisoprenyl groups is restricted by the olefin bonds in such a manner that folding to minimize exposure to solvent leads to juxtaposition of the double bonds in an approximately parallel π-overlapping arrangement. Overlap of the folded chains with the pyridine ring is suggested from comparison of the Ru(II) $\rightarrow$ pyridine (π^*) charge-transfer energies for the various $(NH_3)_5Ru^{II}$–alkylpyridine complexes. For each of the ions except the farnesyl and geranyl derivatives, this wavelength maximum appears at 398 nm; for the latter ions, the maximum is found at 403 nm. The bathochromic shifts are consistent with the decrease in energy of the pyridine π-antibonding acceptor orbital that would be expected to arise if the side chain were interacting with the ring. Similar arguments have been made for π interactions between the porphine ring and farnesyl substituent in heme *a* (Caughey et al., 1975).

Considering all of this, a self-consistent view of the complex structures is that Cu(I) binds to the exposed terminal olefin bond of ligands that are extensively folded and thereby, more or less, capable of providing electronic interaction via the π-delocalized system with a second metal center coordinated to the pyridine nitrogen (see Chart I).

Electron-Transfer Pathways. It has been established from other studies that Cu(I)–olefin complexation is only weakly influenced by coordination of a second metal ion to nucleophilic sites on the ligand; e.g., association constants for Cu(I) binding to 4-vinylpyridine and the $(NH_3)_5Co^{III}$–4-vinylpyridine ion are 8.1 and 3.3 mM^{-1}, respectively, in 0.1 M TFA at 23 °C (Norton & Hurst, 1978). Differences are expected to be even less in the present studies because electrostatic forces are diminished by the larger size of the ligands and conjugative interaction between the metal coordinate bonds is reduced by the absence of π delocalization along the σ-carbon chain of the ligand. Binding is certainly sufficiently strong that, in solutions containing a 1 mM excess of one reactant, essentially all (80–90%) of the limiting reagent is bound as the Ru(III)–Cu(I) binuclear ion. These conditions are met in the present series of kinetic experiments.

Electron transfer might be either intramolecular, i.e., from Cu(I) through the bridging ligand to its bound Ru(III) partner, or intermolecular, i.e., from Cu(I) to a second oxidant molecule (Chart I). Assuming concurrent reaction by both pathways, the rate law is $d[Ru(II)]/dt = (k_1 + k_2[Ru(III)]_T)[Cu(I)]_T$ when Ru(III) is the reagent in excess and Cu(I) is completely bound as the binuclear ion. A similar expression holds when Cu(I) is in excess, although the values for k_2 need not be identical (see below). The rate constants k_1 and k_2 refer to the intramolecular and intermolecular pathways, respectively. Any contribution by the intramolecular pathways to the overall rate would be manifest in a positive intercept in plots of experimentally determined rate constants against the concentration of excess reagent (Figure 4). Since in no instances have nonzero intercepts been observed, it is apparent that $k_1 \simeq 0$, and electron transfer occurs almost exclusively by intermolecular pathways. This conclusion based upon the rate law is substantiated by comparison of the reactivities of the various ligands (Table II). The rate constants for the polyisoprenyl compounds are nearly identical with those for the alkylpyridines, which necessarily transfer electrons by intermolecular pathways. Furthermore, the constancy of values obtained for the alkenylpyridine and isoprenylpyridine ligands argues against any significant involvement of intramolecular pathways since k_1 should be sensitive to structural variations through the series.

An attempt was made with the farnesyl derivative to minimize the intermolecular reaction by working under conditions less favorable for second-order processes, i.e., equimolar reactants at low concentrations. Even under these limiting conditions, no evidence of intramolecular electron transfer could be found, the experimental rate constant being nearly identical with that determined at higher concentrations. An upper limit for the intramolecular reaction of $k_1 < 10^{-3}$ s^{-1} can be assigned from these experiments. It is clear, then, that polyisoprenoid chains are incapable of providing pathways for facile long-range electron transfer in these reactions. This circumstance contrasts markedly with the reaction of the $(NH_3)_5Ru^{III}$–4-vinylpyridine–Cu(I) binuclear ion, for which electron transfer occurs almost entirely through the π-conjugated bridge (Norton & Hurst, 1978).

Rate constants for reaction of the polyisoprenyl systems with excess Ru(III) are two- to threefold greater than those with excess Cu(I) (Table II). The reaction coordinates are not identical; with excess Ru(III), electron transfer is predominantly from olefin-bound Cu(I) in the binuclear ion to mononuclear Ru(III) complexes and, with excess Cu(I), from free Cu(I) to the binuclear ion. Although this comparison might suggest that the olefin is functioning in promoting electron transfer, the rate constants are not larger than those recorded for the alkylpyridine-containing ions (Table II).

Suitability as Models for Proposed Pathways in Cytochrome Oxidase. Points of similarity between the Cu(I)–Ru(III) reactions and heme–copper reactions in cytochrome oxidase include the following. (a) The energetics are comparable. Standard reduction potentials for the $Cu^{2+/+}$ and $A_5Ru(py)^{3+/2+}$ couples (Latimer, 1952; Lim et al., 1972) are

[3] Evidence for the lipophilic nature of the alkyl substituent groups is found in the solution properties of their compounds. The geranyl- and farnesylpicolines form micelles in aqueous solutions with cmc's $< 10^{-4}$ M. Also, their A_5Ru^{III} and A_5Co^{III} coordination complexes are strongly bound to phospholipid vesicles, whereas analogous complexes containing short-chain alkyl substituents bind only very weakly (L. Lee, unpublished observations).

[4] It is pointless to reduce reactant concentrations below the levels taken in search of an intramolecular pathway since binuclear ion formation becomes inappreciable and both pathways are therefore kinetically second order.

0.15 and 0.30 V, respectively, giving a net driving force for the overall reaction of about 150 mV. Olefin coordination stabilizes Cu(I) by about 0.2 V, so that electron transfer from the π complex is isoenergetic or even slightly unfavorable. By comparison, standard reduction potentials for heme and copper components of the oxidase range from 220 to 350 mV, corresponding to an overall potential difference of 130 mV (Mackey et al., 1973). (b) The electronic configuration of the oxidant molecule is low-spin d^5 and the Ru(II) product is diamagnetic d^6, analogous to iron in the low-spin heme component of cytochrome oxidase (Palmer et al., 1976). The ligand geometries provide C_{4v} site symmetries in both cases. (c) Steric constraints to forming electron-transfer pathways by the ligands are similar, the major differences being the absence of a hydroxy substituent at the C′-1 carbon and a side chain shortened by one methylene unit in the farnesyl model and attachment of the side chain to a pyridine ring, rather than pyrrole as occurs in heme *a*. These structural differences do not present any major barriers to folding and overlap of the isoprenyl groups.

Although the spatial delocalization of electronic charge is considerably less in the pyridine model than occurs across the porphine ring, this deficiency is at least partially compensated by the electronic properties of the metal center. Ruthenium(II) is unique among the divalent transition metal ions in its capacity to delocalize electron density onto ligands which possess vacant orbitals suitable for $d\pi$-$p\pi$ back-bonding (Taube, 1973). Reduction of Ru(III) therefore involves electron transfer into an acceptor orbital which is effectively a π-molecular orbital delocalized over the metal center and pyridine ring. In this sense, the choice of metal center presents the optimal case in models for promoting electron transfer through π-delocalized channels.

Whether or not the protein environment of cytochrome oxidase directs folding of the heme a farnesyl side chain to achieve a level of π overlap unattainable in the model is unknown. Folding appears to be fairly extensive in the Ru(III)–Cu(I) ions; despite the likelihood of substantial π stacking, the absence of any detectable intramolecular electron transfer indicates that a favorable redox pathway has not been created.

Mechanisms of Biological Electron Transfer. These results are one example of an apparent paradox in electron transfer that is becoming increasingly evident; i.e., long-range electron transfer appears to occur between discrete redox sites in physiological reactions of metalloproteins without the strong site–site interaction which our experience with transition metal redox reactions suggests is essential. Reorganization of metal–ligand bonds and surrounding solvent are thought to make dominant contributions to the energetic barriers to electron transfer in coordination complexes, electron-transfer occurring with unitary or near-unitary efficiencies once the nuclear activation barrier has been surmounted (Sutin, 1973; Taube, 1977). Rate-limiting electron tunneling is presumably not frequently encountered because the additional constraint of low tunneling probabilities reduces overall rates to below detectable levels. According to this view, π conjugation provides the necessary degree of interaction between redox sites in long-range transfer to ensure that tunneling probabilities are high. On the other hand, recent theoretical calculations suggest that rates of electron tunneling through barriers comparable to those presented by biological materials (exclusive of special π-stacked arrangements) are sufficiently rapid to account for biological reactivity, even with distances between redox sites of 10 Å or greater (Hopfield, 1974; Jortner, 1976; Van Heuvelen, 1976; Bennett, 1976). If reorganizational barriers in biological environments are small, then the overall reaction rates will be given largely by the tunneling rates. Thus, mechanistic differences may possibly represent a trade-off, the biological milieu serving to reduce reorganizational barriers to permit reaction by pathways associated with inherently low electron tunneling probabilities.

While this viewpoint presents a somewhat comforting rationalization of the problem, it should not be taken as established. Some structural evidence consistent with these suggestions has been presented (Carter et al., 1974a,b; Colman et al., 1978), but other studies suggest that biological reorganizational energies may not be unusually small (Hopfield, 1974; Potasek & Hopfield, 1977; Potasek, 1978). It should be noted, however, that to the extent that these ideas are valid the isoprenyl chain may be capable of promoting electron tunneling in biological particles but not in transition metal ions simply because reorganizational energies in the latter reactions are larger.

Acknowledgments

The author gratefully acknowledges the contributions of Dr. Alan Quenelle, who developed the general protocol for ligand syntheses, Drs. Leslie G. Hulett and David J. Halko, who initiated the sequence of kinetic studies, and Dr. William E. Keyes, who recorded the Raman spectral data. I am also indebted to Dr. Thomas Loehr for making available the instrumentation in his laboratory.

References

Bennett, L. E. (1976) in *The Iron-Sulfur Proteins* (Lovenberg, W., Ed.) Chapter 9, Academic Press, New York.

Brodie, A. F., & Ballantine, J. (1960) *J. Biol. Chem. 235*, 232.

Brown, H. C., & Murphey, W. A. (1951) *J. Am. Chem. Soc. 73*, 3308.

Burns, P. S., Williams, R. J. P., & Wright, P. E. (1975) *J. Chem. Soc., Chem. Commun.*, 795.

Carter, C. W., Jr., Kraut, J., Freer, S. T., & Alden, R. A. (1974a) *J. Biol. Chem. 249*, 6339.

Carter, C. W., Jr., Kraut, J., Freer, S. T., Xuong, Ng. H., Alden, R. A., & Bartsch, R. G. (1974b) *J. Biol. Chem. 249*, 4212.

Caughey, W. S., Smythe, G. A., O'Keeffe, D. H., Maskasky, J. E., & Smith, M. L. (1975) *J. Biol. Chem. 250*, 7602.

Colman, P. M., Freeman, H. C., Guss, J. M., Murata, M., Norris, V. A., Ramshaw, J. A. M., & Venkatappa, M. P. (1978) *Nature (London) 272*, 319.

Dickerson, R. E., & Timkovich, R. (1975) *Enzymes, 3rd Ed. 11*, 397.

Dobson, C. M., Hoyle, N. J., Geraldes, C. F., Bruschi, M., LeGall, J., Wright, P. E., & Williams, R. J. P. (1974) *Nature (London) 249*, 425.

Dockter, M. E., Steinemann, A., & Schatz, G. (1978) *J. Biol. Chem. 253*, 311.

Dogonadze, R. R., Ulstrup, J., & Kharkats, Y. I. (1973) *J. Theor. Biol. 40*, 259.

Farr, J. K., Hulett, L. G., Lane, R. H., & Hurst, J. K. (1975) *J. Am. Chem. Soc. 97*, 2654.

Ford, P., De Rudd, F. P., Gaunder, R., & Taube, H. (1968) *J. Am. Chem. Soc. 90*, 1187.

Gaswick, D., & Haim, A. (1974) *J. Am. Chem. Soc. 96*, 7845.

Gupta, R. K., & Yonetani, T. (1973) *Biochim. Biophys. Acta 292*, 503.

Halpern, J., & Orgel, L. E. (1960) *Discuss. Faraday Soc. 29*, 7.

Hartley, F. R. (1973) *Chem. Rev. 73*, 163.

Hopfield, J. J. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3640.
Hurst, J. K., & Lane, R. H. (1973) *J. Am. Chem. Soc. 95*, 1703.
Jorter, J. (1976) *J. Chem. Phys. 64*, 4860.
Jwo, J. J., & Haim, A. (1976) *J. Am. Chem. Soc. 98*, 1172.
Larsen, R. P., & Ross, L. E. (1959) *Anal. Chem. 31*, 176.
Latimer, W. M. (1952) *Oxidation Potentials*, 2nd ed., Prentice-Hall, Englewood Cliffs, NJ.
Lenaz, G., Daves, G. D., Jr., & Folkers, K. (1968) *Arch. Biochem. Biophys. 123*, 539.
Leonard, J. J., & Yonetani, T. (1974) *Biochemistry 13*, 1466.
Lim, H. S., Barclay, D. J., & Anson, F. C. (1972) *Inorg. Chem. 11*, 1460.
Lode, E. T., Murray, C. L., & Rabinowitz, J. C. (1974) *Biochem. Biophys. Res. Commun. 60*, 163.
Lowe, D. C., & Bray, R. C. (1978) *Biochem. J. 169*, 471.
Mackey, L. N., Kuwana, T., & Hartzell, C. R. (1973) *FEBS Lett. 36*, 326.
Mizrahi, I. A., Wood, F. E., & Cusanovich, M. A. (1976) *Biochemistry 15*, 343.
Nordmeyer, F., & Taube, H. (1968) *J. Am. Chem. Soc. 90*, 1162.
Norton, K. A., & Hurst, J. K. (1978) *J. Am. Chem. Soc. 100*, 7237.
Norton, K. A., Hulett, L. G., Halko, D. J., & Hurst, J. K. (1979) in *Electron Tunneling in Biological Systems*, University of Pennsylvania, Philadelphia, PA (in press).
Packer, E. L., Sternlicht, H., & Rabinowitz, J. C. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3278.
Palmer, G., Babcock, G. T., & Vickery, L. E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2206.
Phillips, W. D., McDonald, C. C., Stombaugh, N. A., & Orme-Johnson, W. H. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 140.
Potasek, M. J. (1978) *Science 201*, 151.
Potasek, M. J., & Hopfield, J. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3817.
Rieder, K., & Taube, H. (1977) *J. Am. Chem. Soc. 99*, 7891.
Rossotti, F. J. C., & Rossotti, H. (1961) *The Determination of Stability Constants and Other Equilibrium Constants in Solution*, McGraw-Hill, New York.
Salemme, F. R. (1977) *Annu. Rev. Biochem. 46*, 299.
Savitzky, A., & Golay, M. (1964) *Anal. Chem. 36*, 1627.
Sieker, L. C., Adman, E., & Jensen, L. H. (1972) *Nature (London) 235*, 40.
Stein, C. A., & Taube, H. (1978) *J. Am. Chem. Soc. 100*, 1635.
Sutin, N. (1973) in *Inorganic Biochemistry* (Eichhorn, G. L., Ed.) Vol. 2, Chapter 19, Elsevier, Amsterdam.
Taube, H. (1973) *Surv. Prog. Chem. 6*, 1.
Taube, H. (1977) *Adv. Chem. Ser. No. 162*, 127.
Tweedle, M. F., Wilson, L. J., Garcia-Iniquez, L., Babcock, G. T., & Palmer, G. (1978) *J. Biol. Chem. 253*, 8065.
Vanderkooi, J. M., Landesburg, R., Hayden, G. W., & Owen, C. S. (1977) *Eur. J. Biochem. 81*, 339.
Van Heuvelen, A. (1976) *Biophys. J. 16*, 939.
Wiley, G. A., Hershkowitz, R. L., Rein, B. M., & Chung, B. C. (1964) *J. Am. Chem. Soc. 86*, 964.

Low-Frequency Vibrations in Resonance Raman Spectra of Horse Heart Myoglobin. Iron–Ligand and Iron–Nitrogen Vibrational Modes†

Alain Desbois,* Marc Lutz, and Ramaprasad Banerjee

ABSTRACT: The low-frequency regions (150–700 cm^{-1}) of resonance Raman (RR) spectra of various complexes of oxidized and reduced horse heart myoglobin were examined by use of 441.6-nm excitation. In this frequency range, RR spectra show 10 bands common to all myoglobin derivatives (numbered here for convenience from I to X). Relative intensities of bands IV, V, and X constitute good indicators of the doming state of the heme and, consequently, of the spin state of the iron atom. An additional band is present for several complexes (fluorometmyoglobin, hydroxymetmyoglobin, azidometmyoglobin, and oxymyoglobin). Isotopic substitutions on the exogenous ligands and of the iron atom ($^{56}Fe \rightarrow {}^{54}Fe$) allow us to assign these additional lines to the stretching vibrations of the Fe–sixth ligand bond. Similarly, bands II are assigned to stretching vibrations of the Fe–N(pyrrole) bonds. An assignment of bands VI to stretching vibrations of the Fe–N_ϵ(proximal histidine) bonds is also proposed. Mechanisms for the resonance enhancement of the main low-frequency bands are discussed on the basis of the excitation profiles and of the dispersion curves for depolarization ratios obtained for fluorometmyoglobin and hydroxymetmyoglobin.

In the past few years, resonance Raman (RR)[1] spectroscopy has been extensively used for the study of hemoproteins [Spiro (1975) and references cited therein]. In particular, the intense lines lying between 1100 and 1650 cm^{-1} have been thoroughly studied. This range of frequencies covers the stretching vibrations of C=C and C=N groups of the porphyrin ring and the bending vibrations of C–H groups of the methine bridges (Ogoshi et al., 1972). Since a proper description of the iron porphyrin complex in hemoproteins also implies a knowledge of the bonds between the iron atom and its five or six ligands, we have given our attention to the low-frequency region; previous work in infrared spectra had indeed suggested that

† From the Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, B.P. 2, 91190 Gif-sur-Yvette, France (A.D. and M.L.), and the Laboratoire de Biophysique, Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris, France (A.D. and R.B.). *Received October 27, 1978.* This work was supported in part by grants from the Centre National de la Recherche Scientifique, the Délégation à la Recherche Scientifique et Technique, and the Institut National de la Santé et de la Recherche Médicale.

* Address correspondence to this author at the Institut de Biologie Physico-Chimique.

[1] Abbreviations used: RR, resonance Raman; Mb, myoglobin; Bistris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol.

0006-2960/79/0418-1510$01.00/0