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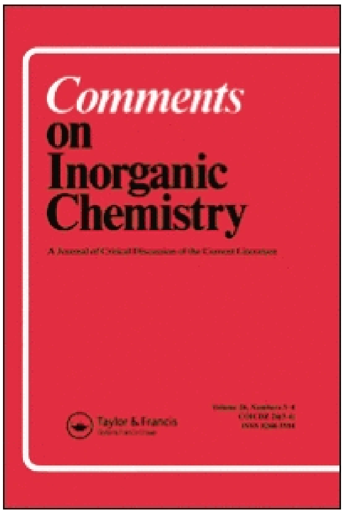

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Electrochemical Effects of Metal Ion Coordination to Noninnocent, Biologically Important Molecules

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Electrochemical Effects of Metal Ion Coordination to Noninnocent, Biologically Important Molecules

INTRODUCTION

As the role of transition metal ions in biological systems becomes better understood, it has become apparent that various biological ligands strongly modulate the oxidation-reduction behavior of metal ions. Less well studied but no less important are the effects exerted by transition metal ions on the redox behavior of biologically important molecules, particularly those which normally function as electron transfer agents. Striking examples of this have been shown for a series of Mg^{II} and Zn^{II} porphyrin complexes in which substitution of the metal ion for two protons sufficiently lowers the oxidation potential of the porphyrin so that it becomes fairly oxidizable in two successive 1*e* steps.[1] Sirohemes in nitrite and sulfite reductases catalyze a remarkable 6*e* reduction in which the reducing ability of both the iron and the isobacteriochlorin are important.[2,3] Metal ions are particularly important in modifying the redox behavior of substrates which coordinate in the active sites of metalloenzymes. Examples of this are nitrogenase and the series of enzymes containing the xanthine-oxidase type of molybdenum center.[4] Some copper enzymes, such as ascorbate oxidase[5] also appear to coordinate the substrate directly in order to effect an efficient electron transfer. Oxygen coordination to copper appears to be especially useful in facilitating critical reductions of O_2.[6]

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In studying the electrochemical effects exerted by transition metal ions on important biochemicals it is convenient to have sufficiently stable complexes of the ligands of interest to apply appropriate electrochemical techniques. This is often not possible with the naturally occurring but relatively substitution-labile metal ions such as Fe^{II}, Fe^{III}, Cu^{I} and Cu^{II}. Also, the tendency of many metal ions such as Fe^{III} and Mo^{IV-VI} to polymerize in aqueous solution severely complicates the synthesis and characterization of the desired model complexes. One way of circumventing these problems is to work with a simple, substitution-inert metal ion, whose other ligands prevent dimerization and are not redox active. Ammineruthenium(II and III) ions are particularly useful in this regard since they tend to bind firmly to nitrogen ligands and Ru^{III} often forms stable species with anionic oxygen ligands.

Most organic reductants normally surrender a second electron at a lower potential immediately following the loss of the first electron and so appear to undergo a simultaneous $2e$ oxidation. On the other hand, most biologically important metal ions, such as Fe^{II} and Cu^{II}, usually proceed to their next stable oxidation state through the loss of a single electron. While in the term "$2e$ transfer" strictly applies in a thermodynamic rather than a kinetic sense, the second electron often transfers so rapidly as to be kinetically indistinguishable on all but the most rapid time scales. It is with this understanding that $2e$ transfers are discussed in this Comment.

When a transition metal ion coordinates to a ligand that is redox-active (noninnocent), (1) the electrochemical properties of both the ligand and metal ion are usually altered, and (2) inner-sphere electron transfer between the two moieties may occur. The metal ion may also provide a catalytic route for electron transfer involving a third agent, which may be facilitated by either (1) or (2) above. Outer-sphere electron transfer to a third agent mediated by an inner-sphere transfer between metal and ligand probably never involves more than $2e$ in single step.

The rudiments of ligand effects on the reduction potentials of metal ions have been summarized by others.[7,8] In addition, it appears that ion–dipole interactions dominate in modulating the electrochemical potential of some metal ions coordinated to anionic ligands. When this case holds, the amount of lowering of the reduction potential of the metal ion depends on the inverse square of the distance between the metal and the ligand atom on which the anionic charge is localized.[9]

If no electron transfer occurs, coordination of a cationic metal ion usually increases the reduction potential of the ligand. The degree of change in the ligand's reduction potential depends upon the extent to which the charge on the metal is transferred to sites that contribute significantly to the orbital receiving the electron. Decreasing the electrochemical potential of the ligand upon cation coordination is less common but may occur when the metal ion increases the electron density residing at sites involving the receptor orbital by (1) inducing deprotonation of the ligand or (2) retrodative bonding. An appropriate charge distribution induced by metal ion coordination, which may be combined with alterations in the acid-base properties of the ligand, can cause the ligand to switch from a 2*e* transfer mode to transferring single electrons (or the reverse).

FLAVIN COMPLEXES

Flavins[10] are nearly ubiquitous in interfacing between 1*e* and 2*e* transfers in biological systems, i.e., between 2*e* transfers involving organic molecules and the single electron transferring metal centers in the proteins donating (or receiving) the electrons. Figure 1 summarizes the electrochemical and acid-base properties of flavins and illustrates the level of complexity that is often encountered in dealing with biologically important redox agents. The long history of metalloflavin chelates and a chronology of speculation as to their biological relevance are summarized elsewhere.[14–16]

Contrary to early expectations[11–13] the N_5-O_4 site on oxidized flavins (Fl_{ox}) is a poor chelator and only Cu^I, Ag^I and Ru^{II} form stable flavin complexes in aqueous solution.[14–16] Since it is relatively easy for the flavin to accept electron density into its π system and the N_5 and O_4 are poor Lewis bases toward metal ions, backbonding appears to be important in stabilizing at least the Cu^I and Ru^{II} complexes. In the case of $[Fl_{ox}(NH_3)_4Ru^{II}]^{2+}$ structural,[14] electrochemical[15,16] and Raman studies[17] are most consistent with the valence-bond resonance forms given in Figure 2.

Comparison of the Pourbaix diagrams for Ru^{II}-Fl_{ox} (Figure 3) and free riboflavin (Figure 4) illustrates the profound effects of metal ion

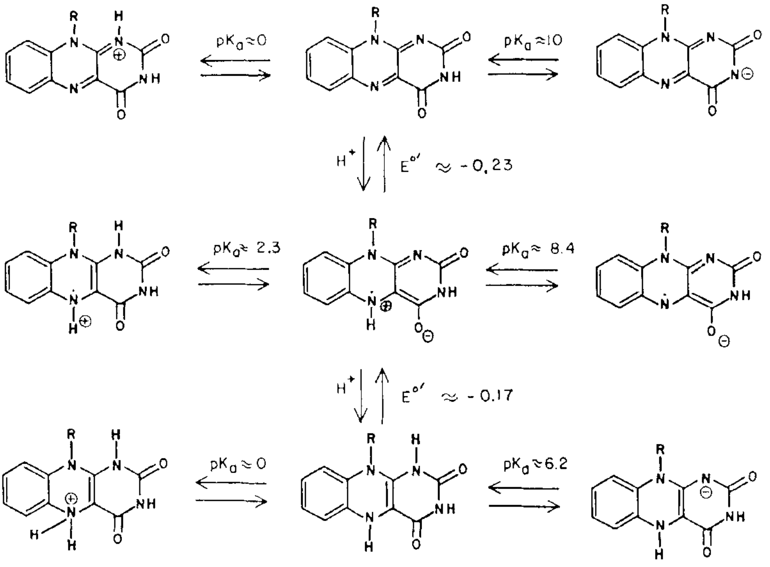


FIGURE 1 Flavin oxidation and protonation states.

coordination. In contrast to free riboflavin, whose reduction potential is pH dependent over the entire range,[18] binding of the metal ion eliminates the overlaps in the proton ionization equilibria of Fl$_{ox}$, HFl$^{\cdot}$ and H$_2$Fl$_{red}$ and so produces regions in which the flavin reduction potentials are essentially independent of pH. Partially due to the electron transfers not always being accompanied by proton transfer, the fully reduced form of the flavin is destabilized so as to cause a reversal in the relative values of the two flavin reduction potentials and the flavin is reduced in two distinct 1*e* steps.

Chelation of the metal by Fl$_{ox}$ at N$_5$ causes the N$_3$ site to become more acidic by 2.6 orders of magnitude in the ionization constant. Owing to a cancellation of resonance (see Figure 2) and electrostatic effects, ionization at N$_1$ is little affected. Metal ion coordination of HFl$^{\cdot}$ prevents protonation at N$_5$ and so forces it to occur at N$_1$. The resulting higher energy tautomer causes an increase in the acidity of HFl$^{\cdot}$ by 4–4.5 units in the pK$_a$. These changes in proton ionization equilibria result in the pH-independent regions in the reduction of Ru^{II}-Fl$_{ox}$.

ESR studies[19] of Fl$^{\cdot}$ have shown that much of the unpaired electron density resides at N$_5$, suggesting that cation coordination at this site favors electron addition and stabilizes the flavosemiquinone radical. Electron density resulting from the addition of a second electron must then accumulate at the next most eletronegative site, which for flavins appears to be N_1-O_2. Alkylation at N_1, where little of the bunpaired electron density in Fl· resides, favors 2*e* transfers.[20] Therefore, the coordination (protonation) position is critical to whether the flavin reacts in a 1*e* or 2*e* mode. Figure 3 shows that at a pH where the N_1 position is partially protonated in Ru^{II}-Fl· the reduction potentials of Ru^{II}-Fl_{ox} and Ru^{II}-Fl· occur within 40 mV, so that these nearly merge into a 2*e* transfer. No doubt nature takes advantage of this as a means of controlling the flavin 1*e*–2*e* interface in proteins. When 1*e* transfer is necessary the protein coordinates a Lewis acid (most likely a proton) at N_5, but when a 2*e* reaction is desired the N_1 site is coordinated.[16,20,21]

These results suggest that amphoteric heterocyclic ligands with nonequivalent coordination sites can be influenced toward 1*e* processes by coordination (or protonation) at the position which localizes the majority of the initial anionic charge. On the other hand, 2*e* transfers should be favored by coordinating a Lewis acid at the site which accepts the charge of the second electron. An implicit as-

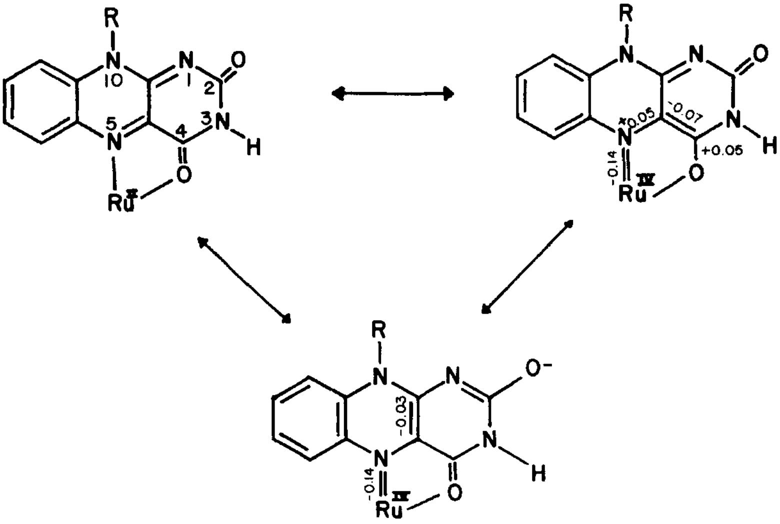


FIGURE 2 Resonance forms of $[Fl_{ox}(NH_3)_4Ru^{II}]^{2+}$. Changes in bond lengths relative to free Fl_{ox} and a Ru^{II}–N single bond length are shown.

sumption here is that the added electronic charge tends to localize on the heteroatoms and is not uniformly distributed over the molecule.

This hypothesis can be tested through the use of MO calculations to select likely heterocyclic systems, followed by the synthesis of the candidate 1*e* and 2*e* transfer agents and verification of the mode of electron transfer by electrochemical techniques. The design of suitable 1*e*–2*e* switching agents could be quite useful in mimicking important

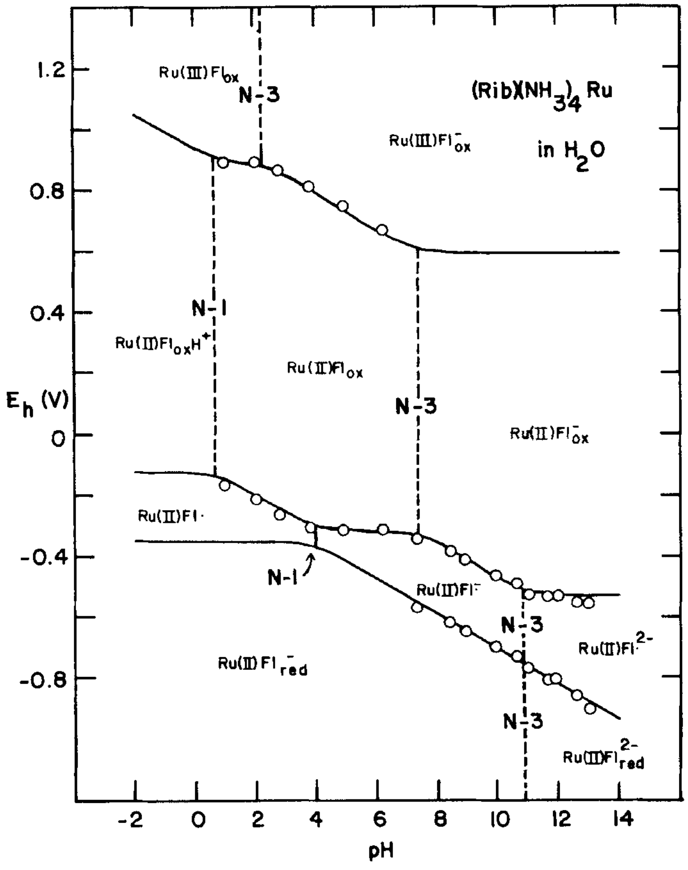


FIGURE 3 Pourbaix diagram of [(Riboflavin)$(NH_3)_4Ru^{II}$]. Crossing a solid horizontal line in this diagram corresponds to a simple 1*e* transfer. Crossing a vertical dashed line indicates a single proton equilibrium at the specified site on the flavin ring. Crossing a diagonal solid line pertains to a 1*e*-1H^+ redox process.

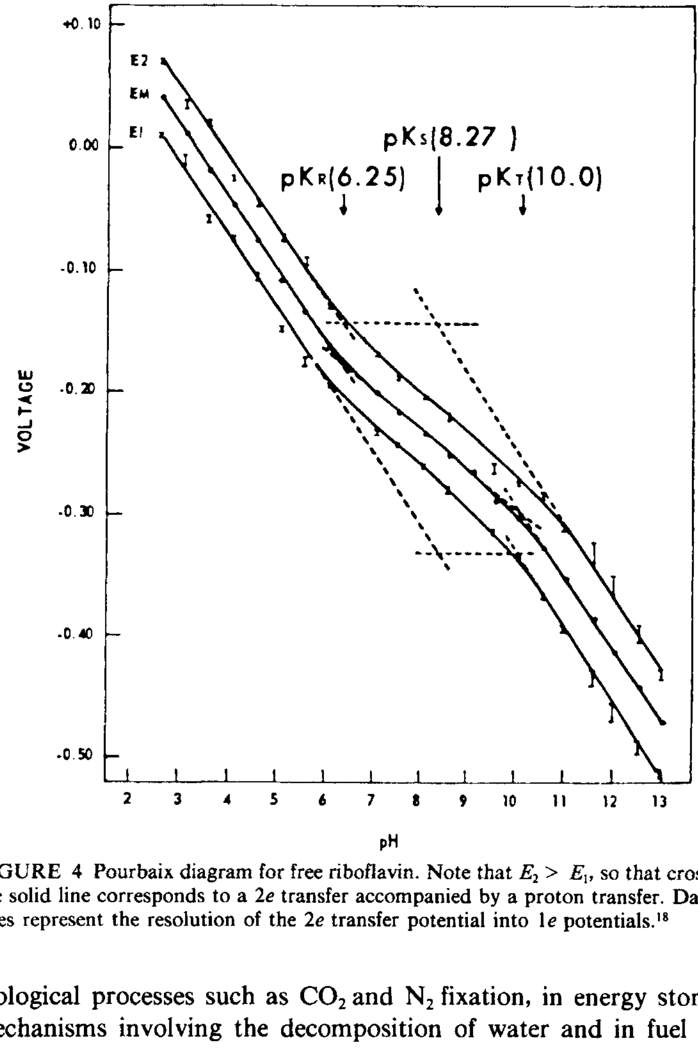


FIGURE 4 Pourbaix diagram for free riboflavin. Note that $E_2 > E_1$, so that crossing the solid line corresponds to a 2*e* transfer accompanied by a proton transfer. Dashed lines represent the resolution of the 2*e* transfer potential into 1*e* potentials.[18]

biological processes such as CO_2 and N_2 fixation, in energy storage mechanisms involving the decomposition of water and in fuel cell applications.

Since much of the charge of a metal cation coordinated to aquo or ammine ligands is largely delocalized onto the ligand protons, its charge to radius ratio is much less than that of a proton and usually even less than that of a methyl group, so that it is less able to stabilize added electronic charge at a given ligand site. In the case of $(NH_3)_4Ru^{II}$ backdonation of electron density into Fl_{ox} appears to cancel the cation stabilization effect on Fl· with the result that the complex reduces at virtually the same potential as the free flavin.[15]

On the other hand, the fully reduced flavin is substantially destabilized by Ru^{II} coordination with the reduction potential for $[Ru^{II}\text{-}Fl\cdot]^- \longrightarrow [Ru^{II}\text{-}Fl_{red}]^=$ being 0.34 V less than that for $[5\text{-}MeFl\cdot] \longrightarrow [5\text{-}MeFl_{red}]^-$. Even when a proton is added to N_1 in both cases, the reduction potential of the Ru^{II} complex is lower by 0.17 V.[15] Since the π-acceptor orbital in Fl· and Fl_{red} is at least partially filled, backbonding should be unimportant in complexes with these ligands and cannot account for the observed decreases in reduction potential. Destabilization of Fl_{red} can be attributed to (1) the relatively low charge to radius ratio of the metal ion, (2) the low affinity for metal ions exhibited by Fl_{red} and (3) the formation of a higher energy N_1 tautomer. It then appears that 1*e* reduction in flavin complexes with $(NH_3)_4Ru^{II}$ is favored as much by destabilization of Fl_{red} as by stabilization of Fl·. Thus, while simple electrostatic effects appear to dominate in proton or alkyl stabilization of reduced states, more subtle effects may come into play with metal ion coordination.[15,16] In general coordination of either "hard" or "soft" cations at N_5 or N_1 will tend to favor 1*e* or 2*e* processes, respectively; however, the harder ions should tend to raise the values of both flavin redution potentials, while softer ions may tend to depress them.

The Pourbaix diagram for $[(Riboflavin)(NH_3)_4Ru^{II}]$ in dimethylformamide is shown in Figure 5 and comparison with Figure 3 gives an approximation of the differences in electrochemistry that might result from transferring the metalloflavin from the exterior to the interior or a protein. Most notable are the decrease in acidity of the N-3 position in both $Ru^{II}\text{-}Fl_{ox}$ and Ru^{II}-Fl·, which is attributed to the stronger electron pair donor characteristics of the nonaqueous solvent and its decreased hydrogen bonding ability.[16] These properties should tend to increase the electron density on the isoalloxazine ring and thereby increases its basicity. For the same reason the redution potentials of the coordinated flavins are subsantially lower than those in aqueous solution. Failure to add a proton on reduction accounts for the extremely negative potential for the reduction of $Ru^{II}\text{-}Fl\cdot^-$, and this in turn is responsible for the enhanced separation between the first and second flavin reduction potentials in DMF relative to water in the mid pH range.[16]

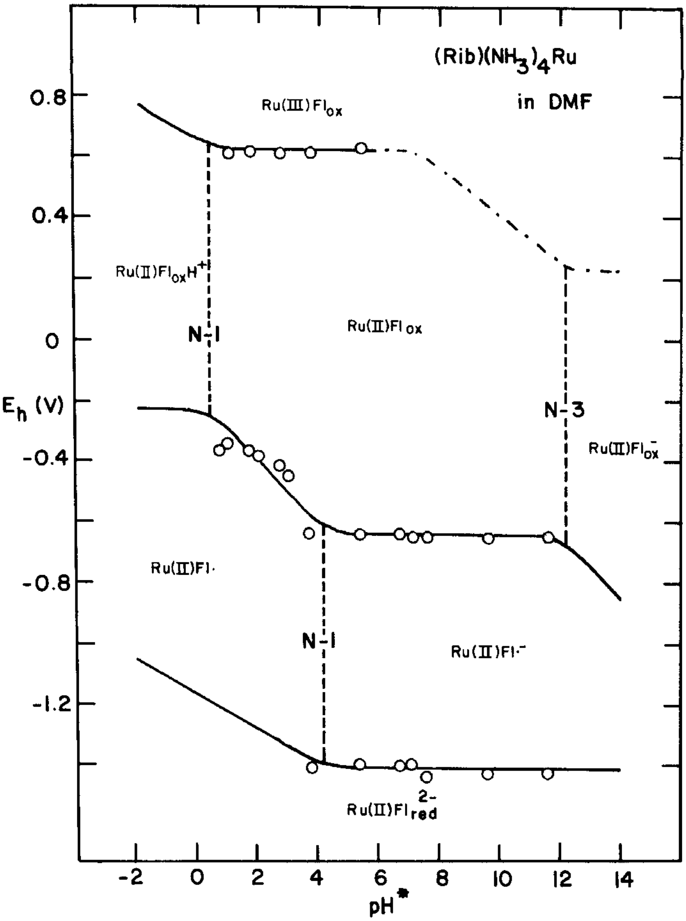


FIGURE 5 Pourbaix diagram of [(Riboflavin)(NH$_3$)$_5$Ru^{III}] in DMF.

A SIMPLE MODEL FOR XANTHINE OXIDASE

Xanthine oxidase provides a good example of the rapid oxidation of a stable heterocycle by a third agent upon coordination of a metal ion. This metalloenzyme contains Fe–S and flavin centers together with a molybdopterin active site to which the purine substrate coordinates before oxidation.[4,23,24] The metabolic function of the enzyme is to catalyze the 2*e* oxidation of xanthine to uric acid; however, it will also oxidize a wide variety of purines to produce oxo addition at C_8 (see Figure 6). Until recently no simple metal ion complex with a purine or purine nucleoside was observed to undergo autooxidation of the purine ligand.[22] Therefore, it was surprising to find that one of the first well characterized metallonculeoside complexes, $[(dG)(NH_3)_5Ru^{III}]^{3+}$, spontaneously undergoes such a reaction.[25]

HPLC studies of the metal ion induced hydrolysis of the sugar–purine bond in Ru^{III}-dG revealed several additional peaks which could be attributed to neither this simple reaction nor metal–purine dissociation.[26] Varying the reaction conditions showed that production of these unknown species was favored in mildly basic media and inhibited by decreasing the oxygen concentration. Acid hydrolysis of the unknown purine complexes showed that they contained 8-hydroxyguanine.[27]

Since the observed rate constants for oxidation of the coordinated nucleoside in air increased with pH ($2.1 \times 10^{-5}\ s^{-1}$ at pH 6 to $1.1 \times 10^{-4} s^{-1}$ at pH 7), it is possible that oxidation of the nucleoside is preceded by deprotonation of C_8 followed by hydroxide or water attack at this site. Application of a modified version of the Kirkwood–Westheimer relationship[9] suggests that the proximity of $(NH_3)_5Ru^{III}$ to the C_8-H should enhance the acidity of this site by more than 10 orders of magnitude in the ionization constant. Since 7-methyldeoxyguanosine undergoes imidazole ring opening rather than oxidation in basic media,[28] it is likely that the presence of the transition metal ion is responsible for the catalytic autooxidation, possibly by facilitating electron transfer to molecular oxygen to yield superoxide.[29]

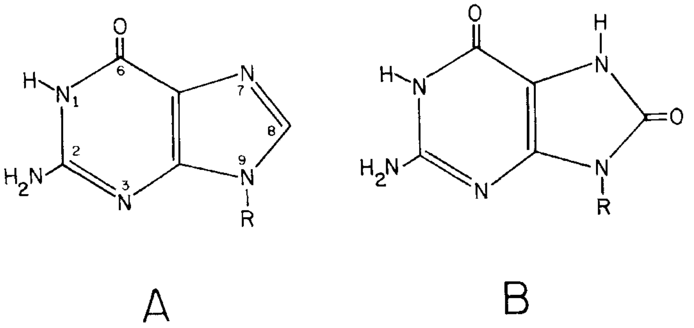


FIGURE 6 Structures of guanine, 8-hydroxyguanine and guanine nucleosides. (A) R = H, guanine; R = deoxyribose, deoxyguanosine. (B) R = H, 8-hydroxyguanosine; R = deoxyribose, 8-hydroxydeoxyguanosine.

While oxygen reduction in xanthine oxidase appears to involve the flavin center, this mechanism is otherwise similar to those which have been proposed for the enzymic action. This model suggests that this activity requires only a relatively "hard" metal cation capable of electron transfer together with hydroxide and molecular oxygen. Moreover, it may be possible to expand on this theme to effect specific oxidations of other heterocyclic ligands.

CATECHOL AND QUINONE COMPLEXES

Catechols represent classic examples of noninnocent ligands which often transfer an electron to a metal ion upon coordination.[30] Indeed, it is not uncommon to form semiquinone complexes following the combination of a catechol and a metal ion.[31] Metal–catechol complexes may also serve as a models for nonheme dioxygenase enzymes[32] and the primary donor-acceptor center in bacterial photosynthesis.[33]

In a series of complexes of the type $[(\mathrm{Cat})(\mathrm{NH_3})_4\mathrm{Ru^{III}}]$, where Cat represents a generic catechol,[30] elemental analysis, PMR, EPR and magnetic susceptibility measurements reveal these to contain $\mathrm{Ru^{III}}$ and the fully reduced catechol coordinated as a diolato chelate.[34] Titration of the blue catecholato complex is complete upon the addition of one equivalent of the oxidant and yields an oxidized, magenta colored species. Cyclic voltammetry on the $\mathrm{Ru^{III}}$-Cat complexes reveals a single, chemically reversible electrochemical couple between 350 mV and 650 mV (versus NHE). When allowed to stand at low pH these complexes generally autooxidize to the magenta species, which usually reverts to the reduced (blue) form at higher pH. Cycling between low and high pH causes some loss of complex in the transition. Therefore, complex dissociation probably releases catechol and $\mathrm{Ru^{II}}$ (see below), which can serve to reduce the complex at higher pH.

PMR spectra of the oxidized (magenta) forms of these complexes are similar to those of the free ligand and show none of the extreme line broadening evident in $\mathrm{Ru^{III}}$ complexes. Magnetic susceptibility studies and the total absence of an ESR signal are also consistent with the oxidized complexes containing the low-spin, diamagnetic $\mathrm{Ru^{II}}$ ion.

An alternative explanation for the diamagnetism for the 1*e*-oxidized complexes is that they contain $\mathrm{Ru^{III}}$ coordinated to the catecholato semiquinone anion. The lack of parmagnetism would then be attributed to antiferromagnetic coupling between ligand and metal portions of the diradical. While this sort of interaction appears to occur with some first-row transitional metal ions[37–40] it is unlikely in the case of ruthenium. Reasons for this are (1) the complete lack of a paramagnetic signal in the oxidized complexes, (2) the relative oxidation and reduction potentials of the *o*-semiquinone anion radical and $[(H_2O(NH_3)_4Ru^{III}]^{3+}$ are approximately 230 mV and 109 mV, respectively,[35,36] so that metal ion oxidation of the semiquinone should be greatly favored, (3) Ru^{II} complexes are known to be greatly stabilized with π-acceptor, quinone ligands[41] and (4) the oxidized species exhibit carbonyl stretching frequencies which are only 20–50 cm^{-1} lower than those exhibited by the free quinone ligands. Therefore, the oxidized forms of these complexes are best formulated as Ru^{II} complexes of *o*-quinones. Lowering of the carbonyl stretching frequencies is consistent with retrodative bonding between a metal ion d_π orbital and the lowest unoccupied π^* orbital on the ligand.

The oxidation process involves the transfer of a single electron to the external oxidizing agent, which induces the intramolecular transfer of a second electron to the metal ion. Similar examples of induced electron transfer have long been known to occur in complexes with $(NH_3)_5Co^{III}$ and heterocyclic ligands.[42] However, in these instances the metal acceptor orbital has σ symmetry and due to a large Franck–Condon barrier in the metal ion and the difference in symmetry between the HOMO of the ligand and the LUMO of the metal ion, a discrete intermediate Co(III) complex of an organic radical can be detected. In the case of $(NH_3)_4Ru^{III}$ as the secondary electron acceptor, the Franck–Condon barrier is probably substantially lower (at least with respect to the ammine ligands) and the electron can be passed directly into a metal d_π orbital by resonance transfer. Therefore, it is likely that the second electron transfer occurs concurrently with the initial electron abstraction. The resulting Ru^{II}-Q species represent the first *o*-benzoquinone complexes to be stable in aqueous solution for extended periods. The unusual stability of these is most likely due to significant retrodative bonding from a metal d_π orbital into the lowest unoccupied π^* orbital on the ligand.[41]

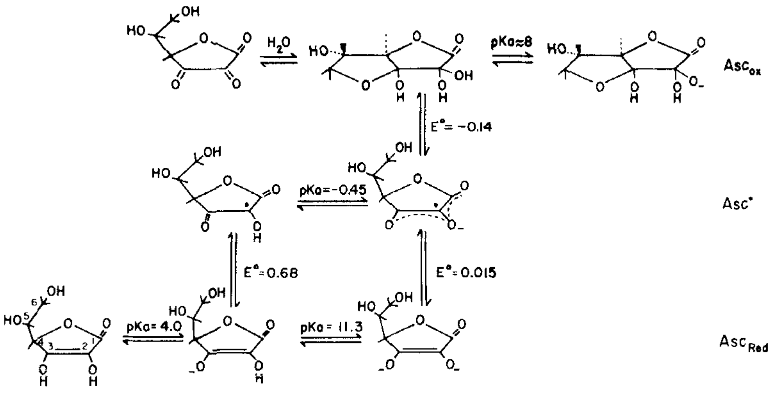
Since chelation eliminates the requirement of proton transfer to the quinone oxygens upon reduction, comparison of the coordinated and free ligand reduction potentials can be misleading. However, the reduction potentials for *o*-quinones when complexed to Ru^{II} are about 0.3 V lower than the 2*e*-2H$^+$ standard reduction potentials for the free quinones.[43–45] While this decrease in reduction potential is consistent with substitution of a metal ion for protons on an organic ligand, ammineruthenium(II) ions are known to stabilize the *quinone* forms of similar ligands through retrodative bonding.[16,41] Such backbonding interactions coupled with the substitution inertness of the low-spin, d^6 metal ion are probably responsible for the unusual stability of these quinone complexes.

Pourbaix plots for the Ru^{III} complexes of DHB, DHA and DHP exhibit pH-dependent regions of 59 mV/pH, which is consistent with a 1*e*-1H$^+$ process. Values of pK_a derived from either Pourbaix plots or spectrophotometric titrations of the reduced, [Ru^{III}-Cat] complexes were in the range 3.8–4.6, while for the corresponding oxidized [Ru^{II}-Q] species these were in the range 3.0–3.5.

ASCORBATE COMPLEXES

Figure 7 summarizes the various oxidation, protonation and hydration equilibria for ascorbic acid.[46–48] Since decomposition reactions can also occur, it is often more convenient to use the more stable TMRA in model studies (see Figure 8).[49] There appear to be no ascorbato complexes which persist in aqueous solution and little reliable structural information on the modes of bonding between transition metal ions and ascorbate. Nevertheless, 2,3-diolato chelate structures have been logically supposed.[50–52] While the kinetics of metal ion catalyzed ascorbate oxidations have been well studied,[50–52] no electrochemical information has been available on any ascorbate complexes, so thermodynamic models have not been presented which address ascorbate binding in enzymic active sites.[5]

Combination of $[Cl(NH_3)_5Ru]Cl_2$ with ascorbate results in two distinct complexes, depending on the pH at which the reaction is run. While these complexes are not fully characterized, they have been isolated in solution and have been determined to be pure by a sensitive HPLC technique.[26] The two ascorbate complexes yield discrete UV-vis, PMR and ESR spectra and can be separated on either ion exchange columns or by HPLC.[53] Entirely analogous results have also been obtained with TMRA as a ligand. Acid-catalyzed disso-



At pH 7

$$\mathrm{Asc_{ox}} + 2e^- + H^+ \longrightarrow \mathrm{Asc^-_{Red}} \qquad E^{0'} = 0.06$$

$$\mathrm{Asc_{ox}} + e^- \longrightarrow \mathrm{Asc^{\cdot}} \qquad E_1^{0'} = -0.14$$

$$\mathrm{Asc^{\cdot -}} + e^- + H^+ \longrightarrow \mathrm{Asc^-_{Red}} \qquad E_2^{0'} = 0.32$$

$$K_d = \frac{[\mathrm{Asc_{Red}}][\mathrm{Asc_{ox}}]}{[\mathrm{Asc^{\cdot}}]^2} = 6.7 \times 10^8$$

FIGURE 7 Oxidation and protonation states of ascorbic acid.

ciation of any of these complexes yields the original ruthenium starting material, which indicates monodentate rather than bidentate coordination of the metal ion. While this is contrary to the general supposition that ascorbate chelates metal ions, the structure of a similar complex with squarate ion has now been determined by x-ray methods.[54]

In the structure of $[\mathrm{Sqr(NH_3)_5Ru}]\mathrm{Cl}$ the metal ion is coordinated to an oxygen atom on the squarate dianion in a monodentate fashion with the ligand bent to one side so that one of its adjacent oxygens enters into symmetric hydrogen bonding with protons on two *cis*-ammines. That strong similarities between the squarate, ascorbate and TMRA complexes exist is reinforced by their all exhibiting broad, intense bands in the visible regions of their spectra, which are attributed to ligand π to metal d_π charge transfer transitions. The structure of the squarate complex indicates that monodentate oxygen coordination to the O_2 of TMRA would not yield a species of C_{2v} symmetry, since the C–O–Ru angle would be in the range of 104–120°. Moreover, hydrogen bonding is likely between one of the adjacent oxygens and *cis*-ammines.

Both the TMRA and Asc complexes prepared at pH 7 show LMCT bands around 680 nm and so will be referred to as the "blue" forms of these complexes. The complexes synthesized at pH 4 exhibit LMCT bands around 550 nm and so are labeled as the "purple" complexes. Spectrophotometric titration and Pourbaix plots of the blue TMRA complex indicate a pK_a of 4.1. Following protonation a subsequent, slower reaction takes place with a half-life of about 5 min. The reduction potentials of both the TMRA and Asc complexes are independent of acid concentration above pH 4 and are 306 mV and 237 mV, respectively.

ESR spectra of the blue complexes are rhombic with *g* values of 2.69, 2.34 and 1.58, while those of the purple species are axial with $g = 2.83$ and $g = 2.42$. These indicate that both forms of the Asc and TMRA complexes contain the metal ion as Ru^{III}. The PMR spectra of the all the complexes reveal broad, highly shifted resonances evident in the spectra of Ru^{III} complexes. Both TMRA complexes exhibit two distinct methyl resonances, consistent with monodentate coordination of $(NH_3)_5Ru^{III}$ at either O_2 or O_3. The blue species exhibits these resonances at 3.67 and 1.16 ppm, while they appear at 13.25 and 6.62 ppm in the purple complex. The most extreme shift and broadening evident in the signal at 13.25 ppm suggests that the paramagnetic center is located closer to one of the methyl groups, i.e., that the metal ion is coordinated to O_3 rather than O_2. The lessened shifting of the methyl proton resonances relative to the free ligand evident in the blue complexes suggests metal ion coordination to the central oxygen. Moreover, the proximity of these

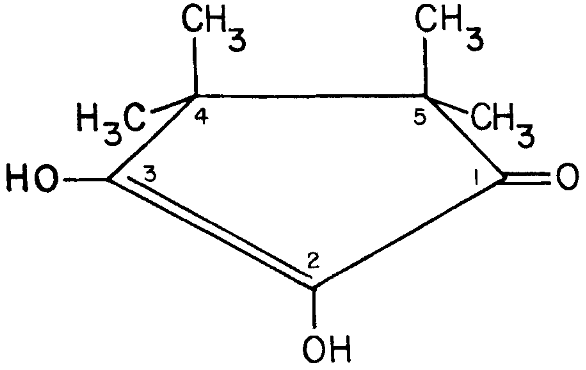


FIGURE 8 Structure of tetramethylreductic acid.

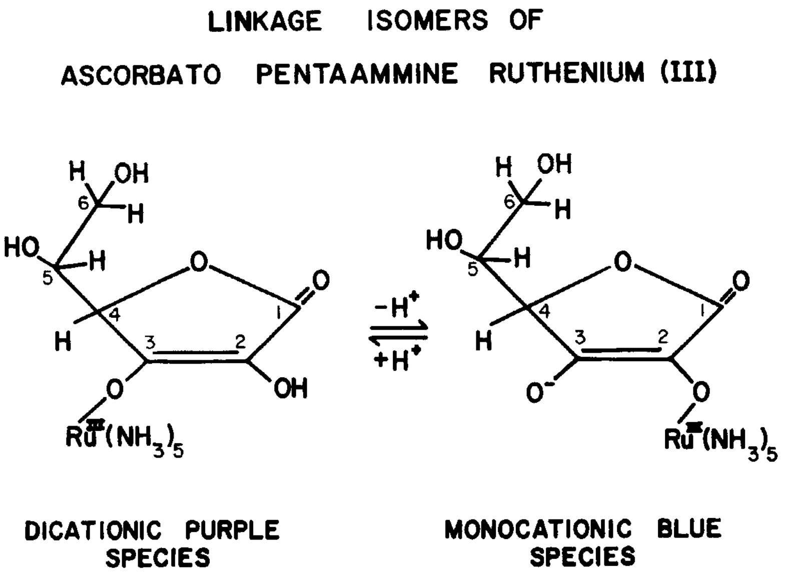


FIGURE 9 Proposed linkage isomers of $[(Asc)(NH_3)_5Ru^{III}]$.

resonances in this complex is consistent with their being more equidistant from the metal ion. The nonequivalence of the signals in the blue TMRA complex is consistent with a bent Ru–O–C_2 bond as was in evidence in the squarato structure.

These results suggest that the blue and purple ascorbato complexes with $(NH_3)_5Ru^{III}$ are the O_2 and O_3 linkage isomers. Moreover, the slower reaction following protonation of the blue Asc or TMRA complexes results in the purple species, so that these species appear to be interconvertable as a function of pH. Such pH-dependent linkage isomerizations are now well known in the chemistry of $(NH_3)_5Ru^{III}$.[9,55,56] By analogy with the electrochemistry of the catecholato complexes discussed previously, it is likely that these complexes are oxidized in 1*e* steps by the external oxidant and that the second electron is passed intramolecularly to reduce the metal ion. The resulting $[Asc_{ox}(NH_3)_5Ru^{II}]$ is stable on a cyclic voltammetry time scale but has not been isolated. The stability of this species is likely due to some degree of backbonding into the π^* system of the oxidized ascorbate and to the usual slowness of Ru^{II} ions to undergo ligand substitution. A precedent for stable monodendate coordination of

$(NH_3)_5Ru^{II}$ to an oxo ligand has already been established in a series of nitropyridine complexes.[57]

These complexes suggest that bidentate chelation to metal ions need not occur in proteins which catalyze the oxidation of ascorbate in biological systems. Moreover, metal ion coordination may facilitate the oxidation of the metalloascorbate moiety in two sequential 1*e* steps. The first involving transfer of 1*e* to an external oxidant in concert with the transfer of the second electron to the metal ion. The second electron can then be extracted from the metal ion by an external oxidant.[9,55,56]

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