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Electron Transfer. 140. Reactions of Riboflavin with Metal Center Reductants[1]

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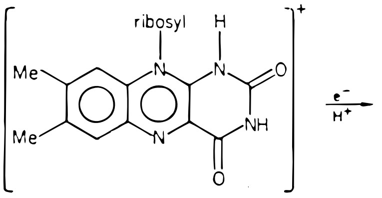
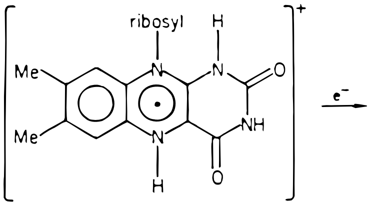
Riboflavin (**I**) is reduced in separable steps by indium(I), vanadium(II), europium(II), and titanium(III) in 0.02−1.0 M H^+, yielding first the radical ion, **II** ($\lambda_{max} = 495$ nm), and then the dihydro compound, **III**. The initial reduction with In^I yields 2 equiv of the radical, but kinetic profiles exhibit no irregularity due to intervention of In^{II}, indicating that participation by the dipositive state is much more rapid than the In^I reaction. Predominant paths involve the protonated form of the flavin, RbH^+, and that of the radical, $RbH_2^{•+}$. Formation of the radical with excess V^{II} and Ti^{III} (but not with In^I) is strongly autocatalytic, reflecting rapid comproportionation involving the flavin and the dihydro compound. The V^{II} and Ti^{III} rates for both steps greatly exceed the substitution-controlled limits for these states and therefore pertain to outer-sphere processes. The very high ratio k_{Eu}/k_V for the first step, however, points to an inner-sphere reduction by the lanthanide cation. A kinetic inversion is observed for In^I ($k_{RbH^{•+}} > k_{RbH_2^{•+}}$), implying a bridged reduction path for the initial step with this center as well.

No other class of oxidation coenzymes rivals flavin derivatives in importance and versatility.[2] The multiformity of their roles stems principally from the readiness of the isoalloxazine system, which characterizes the group, to partake in both one- and two-electron transactions, leading to their effectiveness as mediators between single-electron-transfer processes and those even-electron transformations contributing to metabolic conversions.

The reductions of riboflavin (**I**) to its radical cation (**II**) and its dihydro derivative (**III**), using V^{II}(aq) and Eu^{II}(aq) in aqueous acid media, were briefly considered in earlier studies[3] dealing with the reactions of these reduced forms with metal center oxidants. We here report examination of the analogous conversions using Ti^{III}(aq) and the now available reductant In^I(aq)[4] in addition to a more detailed scrutiny of the V^{II} reaction. Recent adaptable methods for treating complex kinetic sequences have allowed a more precise definition of a previously suggested[3c] mode of catalysis. Note, however, that the present conversions are only indirectly-related to those in biosystems, for our reducing metal centers and the semiquinone intermediate **II** coexist in covenient concentrations only at low pH values.



I



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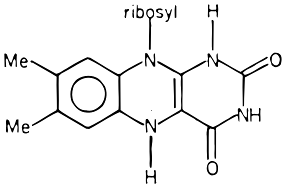

III

Experimental Section

Materials. Solutions were prepared using Millipore water which had been boiled for 2 h and then sparged with N_2 for 2 h more to remove dissolved oxygen. Sodium perchlorate (used as a supporting electrolyte) was prepared in solution from concentrated $HClO_4$ and $NaHCO_3$. Riboflavin, anhydrous CH_3CN, and anhydrous CF_3SO_3Ag (silver triflate)[5] (Aldrich products) and anhydrous lithium chloride (Alfa) were used as received. Indium(I) solutions were prepared as described,[4b] their In^I content was estimated iodimetrically, and the solutions were then used within one week of preparation. Solutions of V^{II}(aq), Eu^{II}(aq), and $Ru(NH_3)_6^{2+}$ were prepared by literature methods.[6] These reductants, and solutions of riboflavin as well, were handled under prepurified argon and generated fresh for each day's experiments. Titanium(III) chloride solution in 2 M HCl (Aldrich) was standardized spectrophotometrically as described.[7]

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(1) Sponsorship of this work by the National Science Foundation (Grant CHE-9714981) is gratefully acknowledged.

(2) Reviews: (a) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256. (b) Niemz, A.; Rotello, V. M. *Acc. Chem. Res.* **1999**, *32*, 44. (c) Signer, T. P.; Edmondson, D. E. *Methods Enzymol.* **1978**, *53*, 397. (d) Muller, F.; Stankovich, M. T. In *Chemistry and Biochemistry of the Flavoenzymes*; Muller, F., Ed.; CRC Press: Boca Raton, FL, 1991; Vol. 1, Chapters 1 and 18.

(3) (a) Singh, A. N.; Gelerinter, E. Gould, E. S. *Inorg. Chem.* **1982**, *21*, 1232. (b) Singh, A. N.; Srinivasan, V. S.; Gould, E. S. *Inorg. Chem.* **1982**, *21*, 1236. (c) Singh, A. H.; Balasubramanian, P. N.; Gould, E. S. *Inorg. Chem.* **1983**, *22*, 655.

(4) (a) Chandra, S. K.; Gould, E. S. *J. Chem. Soc., Chem. Commun.* **1996**, 809. (b) Chandra, S. K.; Gould, E. S. *Inorg. Chem.* **1996**, *35*, 3881.

(5) Preparations of In^I solutions[4] using this very deliquescent silver salt were most successful when freshly opened bottles of the latter were taken.

(6) (a) Chen, J. C.; Gould, E. S. *J. Am. Chem. Soc.* **1973**, *95*, 5593. (b) Dockal, E. R.; Gould, E. S. *J. Am. Chem. Soc.* **1972**, *94*, 6673. (c) Fan, F.-R. F.; Gould, E. S. *Inorg. Chem.* **1974**, *13*, 2647.

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Table 1. Electronic Spectra of Riboflavin at Three Oxidation Levels (1.0 M $HClO_4$)

	λ_{max}, nm (ϵ)[a]
riboflavin, protonated form (**I**)	388 (18.0), 264 (35.0), 218 (27.0)
radical cation (semiquinone) (**II**)	495 (9.0), 374 sh (8.1), 354 sh (12.7), 334 sh (11.5), 318 sh (9.3), 256 (39.3), 210 (32.3)
dihydro product (**III**)	312 (11.9), 292 sh (9.0), 246 sh (13.6), 226 sh (24.2), 208 (39)

[a] ϵ values in M^{-1} cm^{-1} $\times$ 10^{-3}, ref 3a.

Kinetic Studies. Reactions, under argon, were examined at or near 495 nm, the high-wavelength maximum of the riboflavin radical, using a Durrum–Gibson stopped flow spectrophotometer interfaced with an OLIS computer system. Ionic strength was regulated by addition of $NaClO_4/HClO_4$. Most reductions were carried out with the reductant in greater than 20-fold excess. To minimize loss of In^I in acidic media,[4b] reactions with this center were initiated by mixing In^+ solutions in water with riboflavin solutions having the appropriate acidity and ionic strength.

All reactions gave composite kinetic profiles comprising the growth and subsequent disappearance of a strongly absorbing red intermediate. Curves with In^I closely resembled those typifying a pair of consecutive first-order processes (fast rise, much slower decay).[8] Curves generated by V^{II} and Ti^{III} exhibited additional features pointing to autocatalysis—perceptible rate increases during a brief period shortly after mixing and unusually rapid decay of the intermediate after its maximum concentration had been reached.[9]

The reaction of riboflavin with Eu^{2+} was too rapid to measure, even under second-order conditions with [flavin] = 4.8×10^{-5} M and $[Eu^{II}]$ = 9.5×10^{-5} M. With these concentrations, reaction was more than 95% complete within 5 ms after mixing, corresponding to a second-order rate constant $>8 \times 10^7$ M^{-1} s^{-1} (21 °C, $\mu = 0.5$ M). At the other extreme, no reaction between riboflavin and $(NH_3)_6Ru^{2+}$ was observed, even with the latter species at the 10^{-3} M level. In an independent experiment, the dihydro derivative of riboflavin was found to react with the parent flavin at a rate too great to measure.

Results and Discussion

The reductions of riboflavin (**I**), using V^{II}(aq) and Eu^{II}(aq) in acid, have been shown[3] to proceed via consecutive single-electron transactions, yielding first the red radical (semiquinone) cation (**II**), and then the colorless dihydro species (**III**). Single-electron reductions of both the protonated flavin ($E° = +0.220$ V)[3a] and the semiquinone (+0.150 V) are strongly favored potentiometrically for all metal reductants taken. Spectral characteristics of the three oxidation levels are summarized in Table 1.[3a] The growth and disappearance of the semiquinone are likewise observed when the coenzyme is treated with excess Ti^{III}(aq) and In^I(aq). When the flavin is taken in excess, the radical persists, and its yield is doubled when In^I, a 2e reagent, is substituted for an equivalent quantity of V^{II}, Eu^{II}, or Ti^{III}.

$$2RbH^+ \text{ (excess)} + In^I \xrightarrow{2H^+} 2RbH_2^{\bullet +} + In^{III} \qquad (1)$$

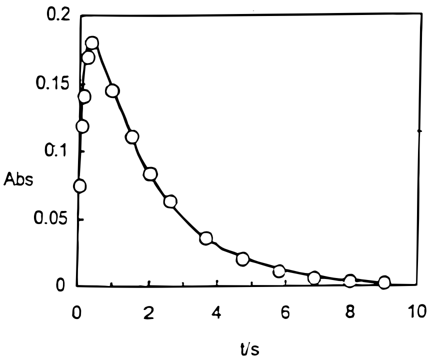

Figure 1. Kinetic profile at 495 nm for the reduction of riboflavin (3.25×10^{-5} M) with In^I (3.3×10^{-4} M) at 21 °C ($\mu = 0.30$ M, $[H^+]$ = 0.10 M). The solid line is the experimental curve whereas the circles are absorbances calculated from numerical integration of differential equations based on sequence 2. The pseudo-first-order rate constants calculated in this manner are $k_1 = 8.8$ s^{-1} and $k_2 = 0.51$ s^{-1}. The final products were considered to be negligibly absorbant. Optical path length 2.0 cm.

Table 2. Kinetic Data for Reductions of Riboflavin (Rb) with Indium (I)[a]

10^3 [In(I)], M	$[H^+]$, M	k_1, s^{-1} [b]	k_2, s^{-1} [b]	$10^{-3}\epsilon$,[c] M^{-1} cm^{-1}
1.60	0.05	29.3 ± 1.5 (29.0)	3.00 ± 0.06 (2.90)	4.8 (4.8)
0.95	0.05	17.8 ± 1.7 (18.1)	1.60 ± 0.02 (1.79)	4.6 (4.8)
0.33	0.30	13.8 ± 0.8 (13.2)	0.64 ± 0.03 (0.61)	7.2 (7.0)
0.33	0.20	10.6 ± 1.0 (11.6)	0.61 ± 0.05 (0.60)	6.7 (6.6)
0.33	0.10	8.8 ± 0.9 (8.4)	0.51 ± 0.04 (0.58)	5.7 (5.7)
0.33	0.05	5.3 ± 0.1 (5.4)	0.49 ± 0.01 (0.54)	4.5 (4.8)
0.33	0.02	2.5 ± 0.1 (2.6)	0.43 ± 0.01 (0.43)	4.1 (3.9)

[a] Reactions were carried out at 21 °C. $\mu = 0.50$ M ($NaClO_4/HClO_4$), $\lambda = 495$ nm, mixing time 4.5 ms, optical path length 2.0 cm, $[Rb]_0$ = 3.25×10^{-5} M. [b] Pseudo-first-order rate constants were determined from treatment of biphasic kinetic profiles in terms of sequence 2; averages from six kinetic runs. Parenthetical values were obtained by refinements of kinetic acidity data using eq 3. [c] Molar absorbances of the riboflavin radical ($Rb^\bullet$) were obtained by treatment of kinetic data as described in ref 10. Parenthetical values were from refinement of ϵ values using an equation analogous to eq 3.

Reduction by In^I. Biphasic kinetic profiles (e.g., Figure 1) obtained with excess In^I may be disentangled using the treatment of Bose, yielding contributing pseudo-first-order rate constants applicable to sequence 2[10] and the extinction coefficient, ϵ, for the radical intermediate. Rate constants resulting from reductions at acidities between 0.01 and 0.30 M H^+ are assembled in Table 2. Both k_1 (pertaining to growth of the intermediate) and k_2 (its disappearance) are very nearly proportional to $[In^I]$.

$$\underset{\textbf{I}}{RbH^+} \xrightarrow[H^+]{k_1} \underset{\textbf{II}}{RbH_2^{\bullet +}} \xrightarrow{k_2} \underset{\textbf{III}}{RbH_2} \qquad (2)$$

The formation of the radical and its further reduction are single-electron transfers, and both therefore utilize conversions $In^I \rightarrow In^{II}$ and $In^{II} \rightarrow In^{III}$.[11] Since no irregularities in profile attributable to intervention of In^{II} were noted, we may infer that, in each case, the $In^I \rightarrow In^{II}$ conversion is rate-determining, whereas the II → III step is much more rapid, i.e., kinetically silent. The situation is thus analogous to that observed for an array of In^I reductions of Co^{III} [4] and Ru^{III} [12a] complexes and is in accord with the much more strongly reducing potential (−0.6 V)[12b] for In^{II}.

Reductions of riboflavin (k_1) and its radical (k_2) are seen to be accelerated by increases in acidity, but the dependencies are

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(7) (a) Birk, J. P.; Logan, T. P. *Inorg. Chem.* **1973**, *12*, 580. (b) Bose, R. N.; Gould, E. S. *Inorg. Chem.* **1985**, *24*, 2645.

(8) See, for example: Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*, 2nd ed.; McGraw-Hill: New York, 1995; Chapter 4.

(9) See, for example, Ghosh, S. K.; Bose, R. N.; Gould, E. S. *Inorg. Chem.* **1987**, *26*, 2684.

(10) Bose, R. N.; Gould, E. S. *Inorg. Chem.* **1985**, *24*, 2832. The ambiguity associated with assignment of rate constants to the two contributors[8] was resolved by choosing values which included an extinction coefficient for the radical in agreement with that obtained by static measurements with the flavin in excess.

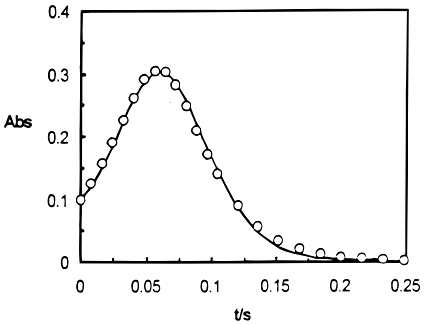

Figure 2. Kinetic profile at 495 nm for the reduction of riboflavin (3.25×10^{-5} M) with V^{II} (3.25×10^{-4} M) at 21 °C ($\mu = 0.3$ M, $[H^+] = 0.20$ M). The solid line is the experimental curve, and the circles are absorbances calculated from numerical integration of differential equations based on the sequence 4−6 and using the kinetic parameters listed for the 0.2 M H^+ entry in Table 3. The final products were considered to be negligibly absorbant. Optical path length 2.0 cm.

less steep than correspond to a $[H^+]$-proportionality. These patterns are consistent with eq 3, which describes the partition

$$k_{obsd} = [In^I]\frac{k_{HA}[H^+] + k_A K_{HA}}{K_{HA} + [H^+]} \tag{3}$$

of the oxidant into a deprotonated and a more reactive protonated form. Here the k_{HA} and k_A are rate constants for the two contributing levels and K_{HA} is the acidity constant for the protonated form. Refinement of values in Table 2 yields bimolecular rate constants in Table 5 for reduction of RbH^+, $RbH_2^{\bullet +}$, and $RbH^\bullet$ by In^I as well as an acidity constant for RbH^+, the protonated flavin. The deprotonated oxidant is negligibly reactive. Observed rates are compared to those calculated from these parameters (in parentheses) in Table 2. An analogous (relatively narrow) variation with $[H^+]$ in the absorptivity of the radical, when treated similarly, allows us to estimate ϵ values for the two protonation levels of this intermediate. The acidity constant obtained for the radical corresponds to the reported value ($pK_A = 2.3$),[13a] but that calculated for the unreduced flavin ($pK_A = 0.9$) agrees poorly (0.3).[13a] For the latter acidity, we favor the older entry, which was determined potentiometrically.

Reduction by V^{II}. The kinetic curves for reduction with excess V^{II} (e.g., Figure 2) also feature the growth and decay of $RbH_2^{\bullet +}$, but are more nearly symmetric than those generated by consecutive first-order reactions, in which the decay of the intermediate is much more gradual than its formation. The profiles are characteristic of an autocatalytic process. As with an earlier V^{II}-flavin reaction system,[3c] the catalyst is taken to be the dihydro compound, RbH_2, which is known to react very rapidly ($k = 10^9$ M^{-1} s^{-1})[2d,13c] with the parent flavin. The reaction sequence then includes a rapid comproportionation step, reaction 6, for which reversibility has been established and which exhibits an equilibrium constant, K_6, known to be strongly dependent on $[H^+]$.[2d,13c,d]

Table 3. Kinetic Data for Reductions of Riboflavin with Vanadium(II)[a]

$[H^+]$, M	k_4, s^{-1} [b]	k_5, s^{-1} [b]	K_6[b]	$10^{-3}\epsilon$,[b,c] M^{-1} cm^{-1}
0.30	11	33 (32)	15	7.8 (7.5)
0.20	9	31 (29)	13	7.0 (7.0)
0.10	6	25 (26)	4	5.8 (6.0)
0.050	4	18 (22)	2	4.8 (4.9)
0.020	2	14 (12)	0.5	4.0 (3.8)

[a] Reactions were carried out at 21 °C. $\mu = 0.30$ M ($NaClO_4/HClO_4$), $\lambda = 495$ nm, mixing time 4.5 ms, opitcal path length 2.0 cm, $[Rb]_0 = 3.25 \times 10^{-5}$ M, $[V^{II}] = 3.2 \times 10^{-4}$ M. [b] Parameters pertain to sequence 4−6 in the text. Values listed give optimum agreement between calculated and observed absorbances for runs in which the acidity was systematically varied. Parenthetical values for k_5 were obtained by refinement of kinetic acidity data using an equation analogous to eq 3. Values of K_6 were estimated from the data of Muller (refs 13d and 2d). [c] Molar absorbances of the riboflavin radical ($Rb^\bullet$). Parenthetical values were from refinement of ϵ values using a partition equation analogous to eq 3.

$$RbH^+ + V^{2+} \xrightarrow[H^+]{k_4} RbH_2^{\bullet +} + V^{3+} \tag{4}$$

$$RbH_2^{\bullet +} + V^{2+} \xrightarrow{k_5} RbH_2 + V^{3+} \tag{5}$$

$$RbH_2 + RbH^+ \overset{K_6}{\rightleftharpoons} 2RbH_2^{\bullet +} \text{ (rapid)} \tag{6}$$

Expression of sequence 4−6 as a series of differential equations and numerical integration using an adaptation of the program KINSIM[14] yield concentrations of the flavin-related species at appropriate time intervals during the course of each reaction. Incorporation of the molar absorbances of riboflavin and the radical cation (the species absorbing appreciably at 495 nm) yields calculated values of the absorbance of the reaction mixtures at each point.

Rate constants and ϵ values for the radical leading to optimal agreement between calculated and observed absorbances are listed in Table 3. Optical densities calculated from a representative set of parameters are compared to an experimental curve in Figure 2. Note that V^{II} reduces the flavin (k_4) more slowly than its radical (k_5), whereas the reverse is the case for In^I. Both steps in the reduction are again seen to be accelerated by increases in $[H^+]$ but exhibit kinetic saturation at high acidity. The estimated values of k_4 are not sufficiently precise to justify further refinement, but the rates for reduction of the intermediate (k_5) may be correlated by eq 3. Refinement gives bimolecular rate constants listed in Table 5.

Reduction by Ti^{III}. Individual profiles for reactions with excess Ti^{III} (e.g., Figure 3) resemble the autocatalytic patterns

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(11) A reviewer points out that the observations in the In^I system are equally in accord with a sequence initiated by a 2e reduction of the flavin to the dihydro derivative:

$$Rb + In^I \rightarrow RbH_2 + In^{III} \ (k_1)$$

$$RbH_2 + Rb \rightarrow 2RbH^\bullet \text{ (rapid)}$$

$$RbH^\bullet + In^I \rightarrow RbH_2 + In^{II} \ (k_2)$$

$$RbH^\bullet + In^{II} \rightarrow RbH_2 + In^{III} \text{ (rapid)}$$

The large Franck−Condon barrier associated with 2e changes (except for atom transfers) is not, in our view, consistent with the high value of k_1 observed (5×10^4 M^{-1} s^{-1}). We therefore strongly favor the proposed sequence of 1e changes.

(12) (a) Chandra, S. K.; Gould, E. S. *Inorg. Chem.* **1997**, *36*, 3485. (b) Al-Ajlouni, A. M.; Gould, E. S. *Res. Chem. Intermed.* **1998**, *24*, 653.

(13) (a) Land, E. J.; Swallow, A. J. *Biochemistry* **1969**, *8*, 2117. (b) Anderson, R. F. *Biochem. Biophys. Acta* **1983**, *723*, 78. (c) Ogura, Y.; Nakamura, S.; Nakamura, T. *Acta Chem. Scand.* **1963**, *17*, S184. (d) Muller, F. *Free Radical Biol. Med.* **1987**, *3*, 217.

(14) (a) Barshop, b. A.; Wrenn, R. F.; Frieden, C. *Anal. Biochem.* **1983**, *130*, 114. (b) Values for the equilibrium constant K_6 were estimated from data obtained[13d] and summarized[2d] by Muller. These were incorporated into the KINSIM sequence by taking k_6 (for the forward reaction) as 10^8 M^{-1} s^{-1} and k_6 (for the reverse) as $10^8/K_6$. The quality of agreement between calculated and observed profiles depended only on the ratio of these k values provided both were large.

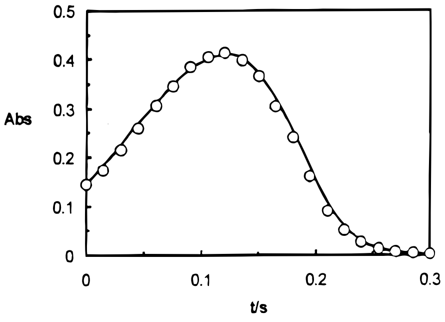

Figure 3. Kinetic profile at 495 nm for the reduction of riboflavin (5.0×10^{-5} M) with titanium(III) (5.0×10^{-3} M) $[H^+] = 0.2$ M, $\mu = 1.0$ M (LiCl/HCl). The solid line is the experimental curve, and the circles are calculated from numerical integration of differential equations based on sequence 6−11 and using the parameters listed for the 0.2 M H^+ entry in Table 4. Optical path length 2.0 cm.

Table 4. Kinetic Data for Reductions of Riboflavin (Rb) with Titanium(III)[a]

$[H^+]$, M	k_{RbH^+}, s^{-1} [b]	$k_{RbH^{\bullet+}}$, s^{-1} [b]	K_6[b]	$10^{-3}\epsilon$,[b,c] M^{-1} cm^{-1}
1.00	0.40(0.35)	15(14)	1.2	14
0.80	0.45(0.43)	16(17)	1.2	14
0.60	0.52(0.54)	18(21)	1.1	13
0.40	0.60(0.70)	27(29)	1.2	13
0.20	1.01(1.1)	50(48)	0.6	13
0.10	1.7(1.6)	75(70)	0.5	10
0.05	2.0(2.0)	80(84)	0.5	9

[a] Reactions were carried out at 21 °C. $\mu = 1.0$ M (LiCl/HCl), $\lambda = 495$ nm, mixing time 4.5 ms, optical path length 2.0 cm, $[Ti^{III}] = 0.0050$ M, $[Rb]_0 = 5.0 \times 10^{-5}$ M. [b] Pseudo-first-order rate constants for the 1e reduction of riboflavin (k_{RbH^+}) and the radical semiquinone) intermediate ($k_{RbH^{\bullet+}}$) with titanium(III). Rate constants, comproportionation equilibrium constants, and extinction coefficients listed give optimum agreement between observed absorbances for individual runs (in which the acidity was varied) and those calculated on the basis of sequence 6−11. Parenthetical values were obtained by refinement of acidity data using eq 12. [c] Molar absorbance for the radical intermediate.

obtained with V^{II}, but reactions were in this case more rapid at low than at high acidities (Table 4).

Acceleration of reaction at high pH is a characteristic of Ti^{III} reductions[15] and is generally attributed to partial deprotonation of the aqua-substituted cation ($pK_A = 2.64$),[16] to the more reactive reductant $Ti(OH)^{2+}$. The Ti^{III} data are then consistent with the expanded sequence

$$\mathrm{Ti^{III}(aq)} \overset{K_A^{Ti}}{\rightleftharpoons} \mathrm{Ti(OH)^{2+}} \qquad (7)$$

$$\mathrm{RbH^+} \overset{K_A^{fl}}{\rightleftharpoons} \mathrm{Rb} + \mathrm{H^+} \qquad (8)$$

$$\mathrm{RbH^+} + \mathrm{Ti(OH)^{2+}} \xrightarrow[H^+]{k_9} \mathrm{RbH_2^{\bullet+}} + \mathrm{Ti(OH)^{3+}} \qquad (9)$$

$$\mathrm{RbH_2^{\bullet+}} \overset{K_A^{rad}}{\rightleftharpoons} \mathrm{RbH^{\bullet}} + \mathrm{H^+} \qquad (10)$$

$$\mathrm{RbH_2^{\bullet+}} + \mathrm{Ti(OH)^{2+}} \xrightarrow[H^+]{k_{11}} \mathrm{RbH_3^+} + \mathrm{Ti(OH)^{3+}} \qquad (11)$$

$$\mathrm{RbH_3^+} + \mathrm{RbH^+} \overset{K_6}{\rightleftharpoons} 2\mathrm{RbH_2^{\bullet+}}\ \text{(rapid)} \qquad (6')$$

Steps 7−9 pertain to reduction to the radical intermediate. If this component proceeds preponderantly via reaction between the deprotonated reductant, $Ti(OH)^{2+}$, and the protonated flavin, RbH^+, rates should conform to eq 12 where $[Ti^{III}]_T$ and $[Rb]_T$

$$(\text{rate})_{\text{rad}} = \frac{K_A^{Ti} k_9 [Ti^{III}]_T [Rb]_T [H^+]}{[H^+]^2 + (K_A^{Ti} + K_A^{fl})[H^+] + K_A^{Ti} K_A^{fl}} \qquad (12)$$

are the total added concentrations of reductant and flavin. Refinement of data in terms of eq 12 yields kinetic parameters k_{RbH^+} ($=k_9$) and $K^A_{RbH^+}$ ($=K_A^{fl}$) in Table 5. Rates obtained from the individual computerized treatments are compared to those calculated from eq 12 at the left of Table 4.[17] Rates for reduction of the radical were treated in an analogous manner, with reaction 8 replaced by equilibrium 10 involving deprotonation of the radical.

Values for the comproportionation constant, K_6, are seen to fall well below those observed for reductions by V^{II}, in accord with the findings of Muller[2d,13d] that this reaction is strongly affected by polyvalent metal ions. Note also that the calculated molar absorbances of the 1e product in the Ti^{III} systems are 2−3 times as great as those observed in the In^I and V^{II} reactions and are nearly invariant with $[H^+]$, indicating that this radical is substantially converted to a Ti^{III} adduct by the excess reductant used.

Rate constants for successive 1e reductions of riboflavin (protonation levels RbH^+ and Rb) and its radical ($RbH_2^{\bullet+}$ and $RbH^{\bullet}$), using the various metal centers, are summarized in Table 5. Since values for V^{2+} lie well above the substitution-controlled limit (20−40 M^{-1} s^{-1})[18] for this reductant, we infer a preponderant outer-sphere route in this case. A similar conclusion applies to $Ti(OH)^{2+}$, for which an inner-sphere maximum near 2×10^3 M^{-1} s^{-1} has been proposed.[19] However, the lower limit for Eu^{2+}, 10^3 times the V^{II} value, points to a bridged redox path (very probably involving an exocyclic C=O of the flavin), since outer-sphere reductions by this lanthanide ordinarily proceed only about one-third as rapidly as those by V^{2+}.[6c]

For the substitution-inert cation, $Ru(NH_3)_6^{2+}$, a bridged path is ruled out. Given sufficient driving force, this center would be expected to react 2−3 times as rapidly as V^{2+}.[6c] Its very slow reduction of RbH^+ may then be attributed to its weakly reducing formal potential ($E° = +0.067$ V),[20] a value only slightly below that estimated for the protonated flavin (0.22 V).[3a]

The marked kinetic reversal observed for In^+ ($k_{RbH^+} > k_{RbH_2^{\bullet+}}$) when compared with the corresponding steps for the outer-sphere reductions by V^{2+} and $Ti(OH)^{2+}$ constitutes strong evidence for an important inner-sphere path for RbH^+−In^+, but leaves open the question for $RbH_2^{\bullet+}$−In^+.[21] However, the rate of the latter component ($k = 2.0 \times 10^3$ M^{-1} s^{-1}) in contrast to the recognized[4,11] sluggishness of In^+ as an outer-sphere reductant indicates a predominant bridged route for this step as well.

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(15) See, for example: (a) Orhanovich, M.; Earley, J. E. *Inorg. Chem.* **1975**, *14*, 1478. (b) Martin, A. H.; Gould, E. S. *Ibid.* **1976**, *15*, 1934. (c) Chandra, S. K.; Paul, P. C.; Gould, E. S. *Ibid.* **1997**, *36*, 4684.

(16) Brunschwig, B. S.; Sutin, N. *Inorg. Chem.* **1979**, *18*, 1731.

(17) Kinetics cannot differentiate between the proposed path and the stoichiometrically equivalent route involving the protonated reductant, $Ti^{III}(aq)$, and the deprotonated oxidant, "Rb". Since protonation most generally activates the oxidant but deactivates the reductant, the alternate path is taken to be negligible. More complex treatments, assuming added contributions by the pairs $[Ti^{III}(aq) + RbH^+]$ and $[Ti(OH)^{2+} + Rb]$ yielded rate constants indistinguishable from zero for such routes.

(18) See, for example: (a) Sutin, N. *Acc. Chem. Res.* **1968**, *1*, 225. (b) Hicks, K. W.; Toppen, D. L.; Linck, R. G. *Inorg. Chem.* **1972**, *11*, 310.

(19) Ram, M. S.; Martin, A. H.; Gould, E. S. *Inorg. Chem.* **1983**, *22*, 1003.

(20) See, for example: Brown, G. M.; Krentzien, H. J.; Abe, M.; Taube, H. *Inorg. Chem.* **1979**, *18*, 3374.

(21) See, for example: Gould, E. S. *Inorg. Chem.* **1979**, *18*, 900.

Table 5. Kinetic Parameters for Successive One-Electron Reductions of Riboflavin with Metal Centers[a]

reductant	$k_{\mathrm{RbH^+}}$[b]	k_{Rb}[b]	$\mathrm{p}K^{\mathrm{A}}_{\mathrm{RbH^+}}$	$k_{\mathrm{RbH_2^{\bullet +}}}$[c]	$\mathrm{p}K^{\mathrm{A}}_{\mathrm{RbH_2^{\bullet +}}}$	$10^{-3}\epsilon_{\mathrm{RbH_2^{\bullet +}}}$, M^{-1} cm^{-1} [d]	$10^{-3}\epsilon_{\mathrm{RbH^{\bullet}}}$, M^{-1} cm^{-1} [d]
In^+	6.2×10^4		0.9	2.1×10^3	2.3	8.2	2.9
V^{2+}	9×10^4	5×10^3		1.0×10^3	2.3	9.0	2.5
$Ti(OH)^{2+}$	4×10^4		0.7	1.6×10^6	0.9[e]	14[e]	
Eu^{2+}	$>8 \times 10^7$						
$(NH_3)_6Ru^{2+}$	<0.016						

[a] Reactions at 21 °C. Reaction conditions listed in Tables 2−4. Rate constants in M^{-1} s^{-1}. [b] Reductions of the protonated (RbH^+) and deprotonated (Rb) forms of riboflavin. [c] Reductions of the protonated ($RbH_2^{\bullet +}$) form of the radical intermediate. [d] Extinction coefficient of the protonated and deprotonated radical. [e] $\mathrm{p}K_{\mathrm{A}}$ and extinction coefficient of the $Ti(OH)^{2+}$-bound radical.

The inversion of relative rates for the successive In^+ reductions is also in keeping with the apparent absence of autocatalysis in this system. Because reduction of $RbH_2^{\bullet +}$ is so much slower than its formation, at no time in the progress of the reaction do substantial quantities of the flavin and its 2e product coexist. Indeed, even when the comproportionation reaction 6 thought to lead to catalysis with V^{2+} and $Ti(OH)^{2+}$ is included in the computerized simulation, the kinetic profile for the In^+ system remains virtually unchanged.

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