

Figure 1. Electron spin resonance spectra of propanediol and the diol dehydrase- B_{12} coenzyme complex. For the rapid freeze experiment (1A), an aliquot of preformed holoenzyme (58 units) was mixed in 3 msec with an equal volume of 0.2 M propanediol to give a total volume of 0.4 ml. The frozen material was packed into a quartz esr tube. Conditions for obtaining the spectrum were: scan length, 1000 G; scan time, 4 min; time constant, 0.3 msec; modulation amplitude, 10 G; modulation frequency, 100 kHz; microwave frequency, 9.232 GHz; microwave power, 20 mW; relative gain, 4×10^3 ; temperature, -130° . For the manually mixed reaction (1B), an aliquot of preformed holoenzyme (55 units) was treated with propanediol for 15 sec prior to freezing in isopentane at -140° . The final propanediol concentration was 0.1 M. Conditions for obtaining the esr spectrum were identical with Figure 1A except that the gain setting was 2.5×10^3 .

Table I. Summary of Data from Rapid Freeze and Manual Mixing of ESR Experiments^a

Sample	Signal (G)	g value	(Total spins)/(mole enzyme)	% conversion to radical
[1,1- ² H ₂]DL-1,2-Propanediol (3 msec) (rapid freeze)	3000	2.22	1.23	61.5 ^b
	3230	2.05		
	3387	1.96		
[1,1- ² H ₂]DL-1,2-Propanediol (15 sec) (manual mix)	3000	2.22	1.40	70.5
	3230	2.05		
	3385	1.96		

^a Data were calculated from spectra described in Figure 1. (Total spins)/(mole of enzyme) was calculated from double integration of the over-all signals in the field range 2850–3500 G, using $Cu^{II}SO_4$ -EDTA (10^{-5} M) as a reference standard. "Conversion to radical" was determined using 2.0 as the maximum value. The copper standard was prepared by manually freezing the sample in isopentane at -140° . Differences in sample packing between manually and rapidly frozen samples are included in the calculations.⁸ ^b Conversion of DBCC to radical species at other times in rapid freeze experiments was: 14.7 msec, 65.5%; 36.5 msec, 54.5%; 74.5 msec, 55.1%; 202 msec, 52.5%.

tional resonance at 3275 G, which appears in the rapid freeze experiment.⁹ This resonance also appears in the spectra (not shown) of reactions of intermediate times (15–200 msec). Table I shows that the integrated intensities of these two spectra are identical within the error of the double integration method. This result

(9) This peak is probably an artifact since temperature studies show that it disappears after warming to -80° and recooling to -130° .

implies that the steady state concentration of radical intermediates is attained in less than 10 msec (3 msec reaction time + 7 msec maximum dead time) implying a $t_{1/2} < 3$ msec. At 37° the first-order rate constant for propionaldehyde formation is 250–300 sec^{-1} . Using the Arrhenius plots for this reaction,¹⁰ one may estimate that, at 5° , the rate should be decreased by a factor of 5–6. This rate corresponds to a $t_{1/2}$ of 11–14 msec. The result of this experiment is that the rate of radical formation, at least, is two to three times faster than the overall rate.

In order to confirm the calculation of spins per mole of enzyme, the optical spectrum of the enzyme-coenzyme complex was examined in the presence of propanediol. The coenzyme spectrum was immediately converted to that of $B_{12}(r)$. Calculations indicate that about 50% conversion to $B_{12}(r)$ occurred, in accord with the results in Table I.

In conclusion, the evidence presented in this study indicates that radical formation by diol dehydrase, in the conversion of 1,2-propanediol to propionaldehyde, is kinetically competent and provides strong evidence for a radical mechanism in B_{12} -catalyzed rearrangements of this type.^{11,12} A more detailed description of the spectra will follow in a later communication.

(10) P. Frey, Ph.D. Thesis, Brandeis University, 1968.

(11) Babior and coworkers have recently demonstrated that 2-amino-1-propanol, a poor substrate for ethanolamine deaminase, produces radical species with this enzyme at a kinetically significant rate. Information concerning this observation was kindly provided by Dr. Babior prior to publication.

(12) While this manuscript was in preparation, R. L. Blakely, *J. Biol. Chem.*, **249**, 2338 (1974), reported that $B_{12}(r)$ appears at a kinetically competent rate with ribonucleotide reductase, a B_{12} -dependent enzyme which does not catalyze a rearrangement reaction.

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Electron Transfer Quenching of the Luminescent Excited State of Tris(2,2'-bipyridine)ruthenium(II). A Flash Photolysis Relaxation Technique for Measuring the Rates of Very Rapid Electron Transfer Reactions

Sir:

Quenching processes involving the luminescent excited states of tris(2,2'-bipyridine)ruthenium(II), $Ru(bipy)_3^{2+*$, and related metal complexes are of considerable current interest. For example, it has been demonstrated that $Ru(bipy)_3^{2+*$ can be quenched in electronic energy transfer processes by both organic and transition metal ion quenchers.^{1–3} It has also been suggested that quenching of $Ru(bipy)_3^{2+*$ can occur by electron transfer quenching.^{4,5} Gafney and Adam-

(1) J. N. Demas and A. W. Adamson, *J. Amer. Chem. Soc.*, **93**, 1800 (1971).

(2) N. Sabbatini and V. Balzani, *J. Amer. Chem. Soc.*, **94**, 7587 (1972).

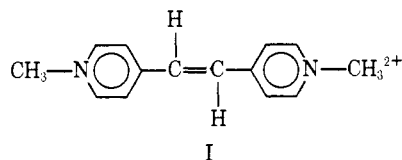
(3) M. Wrighton and J. Markham, *J. Phys. Chem.*, **77**, 3042 (1973).

(4) H. Gafney and A. W. Adamson, *J. Amer. Chem. Soc.*, **94**, 8238 (1972).

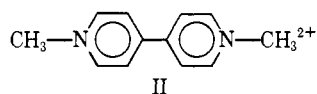
(5) J. N. Demas and A. W. Adamson, *J. Amer. Chem. Soc.*, **95**, 5159 (1973).

son⁴ have proposed that electron transfer quenching occurs in the deactivation of Ru(bipy)₃^{2+*} by a series of cobalt(III) complexes giving Co²⁺ and Ru(bipy)₃³⁺. However, the recent work of Natarajan and Endicott⁶ suggests that the quenching and redox processes occur separately and that quenching occurs largely by energy transfer. In this communication we present evidence that electron transfer quenching of Ru(bipy)₃^{2+*} is an allowed process for both transition metal ion oxidants and electron-deficient organic molecules.

The deactivation of Ru(bipy)₃^{2+*} by the organic cations *trans*-1,2-bis(*N*-methyl-4-pyridyl)ethylene (I,



trans-4,4'-BPE²⁺) and 1,1'-dimethyl-4,4'-bipyridine²⁺ (II, paraquat) and by the transition metal ion



complexes Ru(NH₃)₆³⁺ and Fe(H₂O)₆³⁺ has been studied by emission quenching. Stern-Volmer constants and second-order rate constants for the various quenching reactions are given in Table I. In all four

Table I. Quenching Constants and Reduction Potential Data for Reactions Involving the Quenching of Ru(bipy)₃^{2+*}

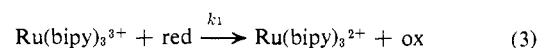
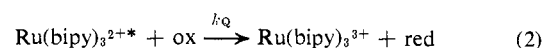
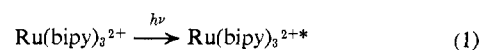
Quencher	K_{SV} (M^{-1}) ^a	E (V) ^b	k_q ($M^{-1} \text{ sec}^{-1}$)
II	2.12×10^3 ^d	-0.446 ^d	$2.5 \pm 0.2 \times 10^9$
I	2.06×10^3 ^d	-0.49 ^{d, 13}	$2.4 \pm 0.2 \times 10^9$
Fe(H ₂ O) ₆ ³⁺	1.80×10^3 ^e	0.74 ^e	$\sim 3 \times 10^9$ ^c
Ru(NH ₃) ₆ ³⁺	1.58×10^3 ^f	0.06 ^h	$\sim 2 \times 10^9$ ^c

^a Stern-Volmer constants for emission quenching were obtained from plots of Φ_0/Φ vs. [quencher]. In a typical experiment, solutions containing 5×10^{-6} M Ru(bipy)₃²⁺ and concentrations of quencher between 0 and 1×10^{-3} M in a 1.0-cm fluorescence cuvette fitted with a serum cap were deaerated with a stream of purified argon for 20 min. The emission intensities of the solutions were recorded on a Hitachi Perkin-Elmer MPF-2 spectrophotometer. ^b Reduction potential data for the couples: paraquat^{2+/+}, *trans*-4,4'-BPE^{2+/+}, Ru(NH₃)₆^{3+/2+}, and Fe(H₂O)₆^{3+/2+}. ^c Assuming $\tau = 0.6$ μsec .¹⁴ ^d CH₃CN solutions containing 0.10 M (CH₃CH₂)₄NClO₄ or 0.10 M (*n*-C₄H₉)₄NPF₆. E values are vs. the standard calomel electrode or the saturated sodium chloride calomel electrode at $25 \pm 2^\circ$. ^e Aqueous 1.0 M HClO₄ solutions. ^f Aqueous 1.0 M CF₃COOH solutions. ^g W. C. Schumb, M. S. Sherril, and S. B. Sweetser, *J. Amer. Chem. Soc.*, **59**, 2360 (1937). ^h From data of T. J. Meyer and H. Taube, *Inorg. Chem.*, **7**, 2369 (1968), extrapolated to 1.0 M HClO₄.

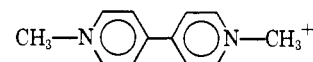
cases, flash spectroscopic studies⁷ have shown that electron transfer is the immediate observable consequence of the quenching processes. The initially observed products are Ru(bipy)₃³⁺ and the reduced form of the quencher. In all four cases the initial quenching reaction is followed by a rapid dark reaction which gives back Ru(bipy)₃²⁺ and the oxidized form of

the quencher. The overall pattern of reactions, where ox and red are the oxidized and reduced forms of the quencher, respectively, is given in Scheme I.

Scheme I



Quenching of Ru(bipy)₃^{2+*} by paraquat must occur by an electron transfer process. The triplet energy of paraquat (71.5 kcal/mol⁹) is too high for efficient quenching of Ru(bipy)₃^{2+*} ($E_T = 49$ kcal/mol)¹⁰ by triplet energy transfer, yet the quenching rate constant is essentially diffusion limited (Table I). Selective excitation of Ru(bipy)₃²⁺ by flash photolysis in a solution 5×10^{-3} M in paraquat and 2×10^{-5} M in Ru(bipy)₃²⁺ gives transient bleaching of the absorbance at 450 nm, indicating loss of Ru(bipy)₃²⁺. Transient absorbance maxima are also observed at 603 and 393 nm, corresponding to the λ_{max} values for the one-electron reduced form of paraquat¹¹



The transient intermediates are produced in a 1:1 ratio, and they decay with the same second-order rate constant.

The olefin quencher *trans*-4,4'-BPE²⁺ has a triplet energy of ~ 50 kcal/mol¹² and should, therefore, be able to accept electronic energy from Ru(bipy)₃^{2+*} at reasonable rates.³ With *trans*-4,4'-BPE²⁺ as the quencher, a competition between energy transfer and electron transfer quenching might be expected to exist. Flash photolysis of Ru(bipy)₃²⁺ in the presence of this quencher efficiently produces the one-electron-reduced form of *trans*-4,4'-BPE²⁺.¹³ Significant bleaching of the Ru(bipy)₃²⁺ absorbance was not observed because of the absorption of the radical in the same spectral region.¹³ The cis isomer of *trans*-4,4'-BPE²⁺, which has been shown to arise exclusively from the triplet excited state of *trans*-4,4'-BPE²⁺,¹² is also produced in the quenching process. However, the sensitized trans \rightarrow cis isomerization is very inefficient. From the intercept of a Stern-Volmer plot, a limiting quantum yield of 0.005 was obtained. The limiting value can be shown to be the product $\gamma\alpha\phi_{\text{et}}$ where $\gamma \approx 1$ is the intersystem crossing efficiency of Ru(bipy)₃²⁺,¹⁴ $\alpha \approx 0.6$ ¹² is the fraction of excited triplets of *trans*-4,4'-BPE²⁺ giving the cis isomer, and ϕ_{et} is the fraction of Ru(bipy)₃^{2+*} quenched by triplet energy transfer. ϕ_{et} can be estimated to be 0.008. This is an important result since it indicates that for *trans*-4,4'-BPE²⁺ electron transfer quenching is more efficient than energy transfer quenching by about two orders of magnitude.

(9) A. Ledwith, *Accounts Chem. Res.*, **5**, 133 (1972).

(10) J. N. Demas and G. A. Crosby, *J. Amer. Chem. Soc.*, **93**, 2841 (1971).

(11) E. M. Kosower and J. L. Cotter, *J. Amer. Chem. Soc.*, **86**, 5524 (1964).

(12) A. R. Gutierrez, J. W. Happ, and M. T. McCall, unpublished results.

(13) J. W. Happ, J. A. Ferguson, and D. G. Whitten, *J. Org. Chem.*, **37**, 1485 (1972).

(14) F. E. Lytle and D. M. Hercules, *J. Amer. Chem. Soc.*, **91**, 253 (1969).

(6) P. Natarajan and J. F. Endicott, *J. Phys. Chem.*, **77**, 971, 1823 (1973).

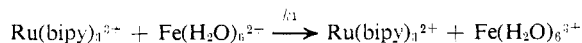
(7) The apparatus and techniques used have been previously described.⁸

(8) D. G. Whitten, P. D. Wildes, and C. A. DeRosier, *J. Amer. Chem. Soc.*, **94**, 7811 (1972).

Both paraquat and *trans*-4,4'-BPE²⁺ are relatively weak oxidants (Table I). Since their rates of electron transfer quenching of Ru(bipy)₃²⁺* are probably diffusion controlled, the net redox reactions involved, Ru(bipy)₃²⁺* + ox → Ru(bipy)₃³⁺ + red, must be favored thermodynamically. If Δ*G* ≤ 0, an upper limit for the reduction potential for the excited state couple, Ru(bipy)₃³⁺ + e → Ru(bipy)₃²⁺*, can be estimated as *E* ≤ -0.49 V in acetonitrile *vs.* the saturated sodium chloride calomel electrode.

Aqueous deaerated solutions of Ru(bipy)₃²⁺ at room temperature were flashed in the presence of Fe(H₂O)₆³⁺ (in 1.0 *M* HClO₄) and in the presence of Ru(NH₃)₆³⁺ (in 1.0 *M* CF₃COOH). In both cases, a very rapid initial bleaching was followed by slower but complete regeneration of the Ru(bipy)₃²⁺ absorbance at 450 nm (see Scheme I). The reaction between Ru(bipy)₃²⁺* and Fe(H₂O)₆³⁺ clearly involves *net* electron transfer quenching (see below), but the intimate details of the quenching process are not certain. Direct photolysis of acidic solutions of Fe(H₂O)₆³⁺ with 254-nm light is known to give hydroxy radicals.¹⁵ The excited state energy of Ru(bipy)₃²⁺* is apparently sufficient to sensitize the production of hydroxy radicals.¹⁶ In principle, the appearance of Ru(bipy)₃³⁺ could be accounted for by a secondary redox reaction between Ru(bipy)₃²⁺ and OH formed in the Ru(bipy)₃²⁺ sensitized oxidation of water by Fe³⁺(aq). However, this mechanism can be ruled out. Flash photolysis studies (Ru(bipy)₃²⁺ = 2.0 × 10⁻⁵ *M*; Fe(H₂O)₆³⁺ = 1.5 × 10⁻³ *M*) were carried out in the presence of relatively high concentrations of the known OH scavengers Fe(H₂O)₆²⁺ (6 × 10⁻³ to 4 × 10⁻⁴ *M*) and isopropyl alcohol (up to 60% by volume). In all cases, the initial yield of Ru(bipy)₃³⁺ remained essentially the same even under conditions where complete scavenging of hydroxy radicals would have occurred. It thus seems likely that quenching of Ru(bipy)₃²⁺* by Fe(H₂O)₆³⁺ occurs by direct electron transfer (reaction 2).

When taken together the series of reactions given in Scheme I constitute a flash photolysis relaxation technique for measuring the rates of very rapid electron transfer reactions. Under conditions where the quenching step is made rapid, the dark reaction (eq 3) involving back-electron transfer between the ground state ions becomes rate determining. Under conditions where electron transfer is the slow step, the rate of regeneration of Ru(bipy)₃²⁺ by Fe(H₂O)₆²⁺ was shown to be first order in Fe(H₂O)₆²⁺ and in Ru(bipy)₃³⁺ and independent of Fe(H₂O)₆³⁺ indicating that the regeneration reaction is



The calculated second-order rate constant, *k*₁ = 1.0 × 10⁶ *M*⁻¹ sec⁻¹, is in good agreement with the value found by Braddock and Meyer¹⁷ at 25°, *k* = 6.41 ± 0.38 × 10⁵ *M*⁻¹ sec⁻¹, by stopped-flow techniques. The rates of regeneration of Ru(bipy)₃²⁺ in solutions initially containing Ru(NH₃)₆³⁺, paraquat, and *trans*-4,4'-BPE²⁺ were also studied (Table II). In all three

(15) G. Adamson, D. L. Baulch, and F. S. Dainton, *Trans. Faraday Soc.*, **58**, 1388 (1962).

(16) Fe³⁺(aq) + H₂O → Fe²⁺(aq) + OH + H⁺ in 1.0 *M* acid, Δ*G*⁰ = 46 kcal/mol.

(17) J. N. Braddock and T. J. Meyer, *J. Amer. Chem. Soc.*, **95**, 3158 (1973).

Table II. Rate and Equilibrium Constant Data for Electron Transfer Reactions Involving Ru(bipy)₃³⁺

Reaction	<i>k</i> , <i>M</i> ⁻¹ sec ⁻¹	<i>K</i>
Ru(bipy) ₃ ³⁺ + Fe(H ₂ O) ₆ ²⁺ → Ru(bipy) ₃ ²⁺ + Fe(H ₂ O) ₆ ³⁺	1.0 × 10 ^{6a}	6.1 × 10 ^{8a}
Ru(bipy) ₃ ²⁺ * + Ru(NH ₃) ₆ ²⁺ → Ru(bipy) ₃ ²⁺ + Ru(NH ₃) ₆ ³⁺	3.68 ± 0.60 × 10 ^{9b}	1.9 × 10 ^{20a}
Ru(bipy) ₃ ³⁺ + paraquat ⁺ → Ru(bipy) ₃ ²⁺ + paraquat ²⁻	(8.3 ± 1.6) × 10 ^{9c}	2.5 × 10 ^{29c}
Ru(bipy) ₃ ³⁺ + <i>trans</i> -4,4'-BPE ⁺ → Ru(bipy) ₃ ²⁺ + <i>trans</i> -4,4'-BPE ²⁺	~5 × 10 ^{9c}	1.2 × 10 ^{30c}

^a In 1.0 *M* aqueous HClO₄ at room temperature. ^b In 1.0 *M* aqueous CF₃CO₂H at room temperature. ^c In acetonitrile containing 0.1 *M* tetra-*N*-ethylammonium perchlorate at room temperature.

cases the rate of appearance of Ru(bipy)₃²⁺ follows second-order (equal concentration) kinetics indicating that the dark electron transfer reaction (eq 3) was being studied.

As indicated by the rate and equilibrium constant data in Table II, the flash photolysis relaxation technique is remarkable in that reactions which are highly favored thermodynamically can be measured.¹⁸

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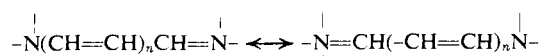
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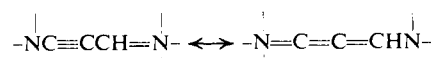
A New Resonance System. Ethnological Amidinium Compounds. Acetylenic Analogs of Cyanine Dyes

Sir:

Numerous examples of compounds that contain the amidinium resonance system are known. We should



like to report the first synthesis of analogs that contain a triple bond as part of the conjugated chain. The simplest such resonance system may be represented as follows.



Compounds containing such a functionality could be considered to be the oxidation products of the corresponding amidinium compound, in which two hydrogen atoms have been replaced by a pair of electrons in a π-orbital orthogonal to that of the conjugated chain. Such a transformation would have important electronic and steric implications. In contrast to amidinium compounds, the extreme structures representative of such a resonance system cannot be identical. The resulting asymmetric electronic distribution might be expected to produce a hypsochromic shift in electronic absorption relative to that of the corresponding amidinium compound, since progressive increase in electronic asymmetry in the amidinium system produces cor-