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### Prolongation of the Lifetime of the <sup>2</sup>E State of Tris(2,2'-bipyridine)chromium(III) Ion by Anions in Aqueous Solution<sup>1</sup>

Sir:

Compared to the emission lifetimes of <sup>2</sup>E states of other octahedral N-bonded Cr(III) complexes in aqueous solution ( $\tau < 2 \mu\text{s}$ ),<sup>2,3</sup> the <sup>2</sup>E state<sup>4</sup> of Cr(bpy)<sub>3</sub><sup>3+</sup> is remarkably long-lived ( $\tau = 63 \mu\text{s}$  at 22 °C in N<sub>2</sub>-purged solutions).<sup>5</sup> The Cr(bpy)<sub>3</sub><sup>3+</sup> <sup>2</sup>E state is readily quenched by O<sub>2</sub> and by a wide variety of electron-transfer agents (including N<sub>3</sub><sup>-</sup> and I<sup>-</sup>)<sup>5-8</sup> resulting in a diminution in the intensity of the <sup>2</sup>E → <sup>4</sup>A phosphorescence bands at 695 and 727 nm and a concomitant decrease in the <sup>2</sup>E lifetime. In neutral and basic aqueous solution, Cr(bpy)<sub>3</sub><sup>3+</sup> is photochemically active, undergoing ligand loss to form Cr(bpy)<sub>2</sub>(OH)<sub>2</sub><sup>2+</sup> and free bpy. The <sup>2</sup>E state is formed with a quantum yield of ~1 and is believed to be the direct precursor of the photoaquation intermediates.<sup>9</sup>

We have been examining the effect of changing the solution medium (solvent, salts) on the <sup>2</sup>E state of Cr(bpy)<sub>3</sub><sup>3+</sup> by monitoring the excited-state absorption spectrum (flash photolysis), lifetime (decay of the <sup>2</sup>E absorption), and phosphorescence spectrum (spectrofluorimetry). Substitution of D<sub>2</sub>O for H<sub>2</sub>O effects no change in the <sup>2</sup>E lifetime. Similarly, in nonaqueous solvents (CH<sub>3</sub>OH, CH<sub>3</sub>CN, DMF, ethylene glycol), the profiles of excited-state emission, excitation, and absorption spectra, as well as the excited-state lifetime, remain unchanged. However, in aqueous solution in the presence of high salt concentrations (>1 M), the lifetime is increased while all the other excited-state parameters remain unchanged. This general effect has been observed for NaCl, NaClO<sub>4</sub>, NaHSO<sub>4</sub>, and KNO<sub>3</sub>; the same effect is observed when HClO<sub>4</sub> is used in place of NaClO<sub>4</sub>. We have examined this phenomenon in detail for ClO<sub>4</sub><sup>-</sup> in order to establish the effect of anion on the radiative, nonradiative, and reactive (photochemical) pathways.

In dilute aqueous solution, the quantum yield of phosphorescence is very small ( $\Phi_{\text{rad}} < 10^{-3}$ )<sup>5</sup> indicating that  $k_{\text{rad}}$ , the radiative rate constant, is small compared to the sum of the nonradiative and reactive rate constants,  $k_{\text{nr}} + k_{\text{rx}}$ . In concentrated ClO<sub>4</sub><sup>-</sup> solutions,  $k_{\text{rad}}$  is still negligible.<sup>10</sup> The effect of ClO<sub>4</sub><sup>-</sup> on  $\Phi_{\text{rx}}$ ,  $k_{\text{rx}}$ ,  $k_{\text{nr}}$ , and  $\tau$  is shown in Table I. Perchlorate ion decreases both the nonradiative and reactive rate constants but has a greater effect on the latter. This is in marked contrast to the behavior of the <sup>2</sup>E state of Cr(en)<sub>3</sub><sup>3+</sup> which is unaf-

Table I. Effect of NaClO<sub>4</sub> on <sup>2</sup>E State of Cr(bpy)<sub>3</sub><sup>3+</sup>

	No added salt	5 M NaClO <sub>4</sub>
$\Phi_{\text{rx}}^a$	0.16	0.009
$\tau_{\text{air}}^b$	$4.8 \times 10^{-5} \text{ s}$	$7.1 \times 10^{-5} \text{ s}$
$k_{\text{rx}}^c$	$3.4 \times 10^3 \text{ s}^{-1}$	$1.2 \times 10^2 \text{ s}^{-1}$
$\tau_0^d$	$6.3 \times 10^{-5} \text{ s}$	$2.9 \times 10^{-4} \text{ s}$
$k_{\text{nr}}^e$	$1.3 \times 10^4 \text{ s}^{-1}$	$0.3 \times 10^4 \text{ s}^{-1}$

<sup>a</sup> Quantum yield of photochemical reaction ( $\lambda = 313 \text{ nm}$ ) at 22 °C measured in air-saturated solution at pH 9.6 relative to  $\Phi_{\text{rx}}$  of 0.11 under the same conditions at 11 °C.<sup>9</sup> <sup>b</sup> Lifetime of <sup>2</sup>E state for conditions, given above, under which  $\Phi_{\text{rx}}$  is determined. <sup>c</sup> Rate constant for the reactive pathway  $k_{\text{rx}} = \Phi_{\text{rx}}\tau_{\text{air}}^{-1}$ , estimated uncertainty  $\pm 25\%$ . <sup>d</sup> Lifetime in N<sub>2</sub>-purged solution at 22 °C;  $\tau_0^{-1} = k_{\text{nr}} + k_{\text{rx}} + k_{\text{rad}}$ . <sup>e</sup> Rate constant for nonradiative pathway calculated from  $k_{\text{nr}} = \tau_0^{-1} - k_{\text{rx}}$ , estimated uncertainty  $\pm 30\%$ .

ected<sup>12</sup> by the presence of 5.2 M MgCl<sub>2</sub> in aqueous solution at 25 °C.

The photochemical reaction of Cr(bpy)<sub>3</sub><sup>3+</sup> is believed to proceed through a seven-coordinate intermediate formed by the addition of a water molecule to the Cr(III) metal center.<sup>9</sup> By analogy to Fe(phen)<sub>3</sub><sup>2+</sup>, Cu(phen)<sub>3</sub><sup>2+</sup>,<sup>13</sup> and closely related Cr(II) complexes,<sup>14</sup> pockets undoubtedly exist between the bpy ligands large enough to accommodate small molecules such as H<sub>2</sub>O; in the presence of 5 M ClO<sub>4</sub><sup>-</sup>, where ion pairing would be extensive,<sup>15</sup> some of the interligand and solvation sphere water molecules would be expected to be replaced by ClO<sub>4</sub><sup>-</sup>. In addition, in concentrated salt solutions a considerable amount of bulk solvent is bound up in solvation of the ions,<sup>16</sup> thereby lowering the activity of water and the rate of formation of the photochemical intermediate,  $k_{\text{rx}}$ .

The lack of a solvent isotope effect on  $k_{\text{nr}}$  is indicative of the lack of direct vibrational coupling between the metal-centered <sup>2</sup>E state of Cr(bpy)<sub>3</sub><sup>3+</sup> and the solvent; a similar lack of a D<sub>2</sub>O isotope effect has been reported<sup>17</sup> for Cr(CN)<sub>6</sub><sup>3-</sup>. However,  $k_{\text{nr}}$  may be affected by changes in solvent polarity owing to electronic dipole perturbation of the <sup>2</sup>E state.<sup>18</sup> Solvent-dependent lifetimes have been reported for other Cr(III) complexes;<sup>16,17,19</sup> in the case of Cr(CN)<sub>6</sub><sup>3-</sup>, the <sup>2</sup>E lifetime has been shown to be a function of solvent polarity.<sup>17</sup> The lack of a solvent effect on the lifetime of the <sup>2</sup>E state of Cr(bpy)<sub>3</sub><sup>3+</sup> indicates that changes in solvent polarity do not cause sufficient perturbation to alter  $k_{\text{nr}}$  (within experimental uncertainty). In addition, the <sup>2</sup>E and <sup>4</sup>T states in Cr(bpy)<sub>3</sub><sup>3+</sup> are effectively isolated.<sup>9</sup> As a result, despite the Laporte and spin forbiddenness of the <sup>2</sup>E → <sup>4</sup>A transition in all Cr(III) complexes, the <sup>2</sup>E states of other Cr(III) complexes are substantially shorter lived than the <sup>2</sup>E state of Cr(bpy)<sub>3</sub><sup>3+</sup>.

Nonradiative decay of the <sup>2</sup>E state of Cr(bpy)<sub>3</sub><sup>3+</sup> occurs by transformation of electronic energy into vibrational energy; the ligand may act as both the perturbation (an oscillating dipole) and as the energy acceptor.<sup>18</sup> The anion effect on  $k_{\text{nr}}$  can be seen as arising from extensive ion pairing and placement of anions in interligand pockets, thereby decreasing both vibrational freedom of the ligands and the efficiency of energy transfer. This model suggests that increasing the rigidity of the bpy ligands should cause a decrease in the rate of energy transfer and a corresponding increase in the <sup>2</sup>E state lifetime. Such an increase in rigidity, accomplished by substituting phen for bpy, is reflected in the low energy metal–nitrogen vibrational modes which are at a higher frequency for tris(phen) complexes than for tris(bpy) complexes.<sup>20</sup> Thus, the <sup>2</sup>E state of Cr(phen)<sub>3</sub><sup>3+</sup><sup>21</sup> is expected to be longer lived than the corresponding state of Cr(bpy)<sub>3</sub><sup>3+</sup>. Furthermore, the effect of anions on the <sup>2</sup>E state lifetime should be less pronounced for Cr(phen)<sub>3</sub><sup>3+</sup> than for Cr(bpy)<sub>3</sub><sup>3+</sup> because the more rigid phen ligands would be less susceptible to perturbation by the interligand anions.

Both these predictions have been borne out experimentally. We find that the lifetime ( $\tau_0$ ) of the  ${}^2E$  state of  $\text{Cr}(\text{phen})_3^{3+}$  in  $\text{N}_2$ -purged dilute aqueous solution at 22 °C is 0.36 ms (compared to 0.063 ms for  $\text{Cr}(\text{bpy})_3^{3+}$ ). The anion effect on  $\text{Cr}(\text{phen})_3^{3+}$  and  $\text{Cr}(\text{bpy})_3^{3+}$  is seen by comparing these lifetimes with those in concentrated (11.7 M)  $\text{HClO}_4$ :  $\tau_0 = 0.53$  and 0.67 ms for  $\text{Cr}(\text{bpy})_3^{3+}$  and  $\text{Cr}(\text{phen})_3^{3+}$ , respectively. Thus, the maximum effect we have seen is almost a factor of 10 for  $\text{Cr}(\text{bpy})_3^{3+}$  but only a factor of 2 for  $\text{Cr}(\text{phen})_3^{3+}$ .

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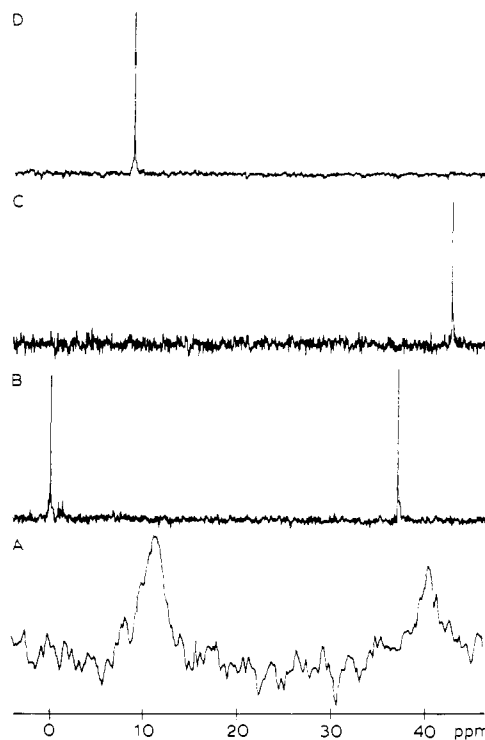
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## ${}^{19}\text{F}$ Nuclear Magnetic Resonance Investigation of the Ternary Complex Formed between Native Thymidylate Synthetase, 5-Fluoro-2'-deoxyuridylate, and 5,10-Methylenetetrahydrofolate

Sir:

The enzyme thymidylate synthetase catalyzes the reductive methylation of 2'-deoxyuridylate (dUMP) to form thymidylate (dTMP) while employing 5,10-methylenetetrahydrofolate ( $\text{CH}_2\text{FH}_4$ ) as a coenzyme. The proposed involvement of de novo synthesis of thymidylate as a rate-determining factor in



**Figure 1.**  ${}^{19}\text{F}$  NMR spectra of the ternary complex (A),  $\text{F}_2\text{dUMP}$  (B), II (C), and III (D) (all spectra were recorded at 20 °C using 18-mm sample tubes in our homemade 18-mm probe;<sup>22</sup> spectra B-D were recorded under conditions of full proton decoupling): (A) ternary complex comprising 0.4 mM thymidylate synthetase, 0.8 mM  $\text{F}_2\text{dUMP}$ , and 4 mM  $\text{CH}_2\text{FH}_4$  in 0.1 M Tris- $\text{SO}_4$ , 50 mM  $\text{MgSO}_4$ , 20 mM  $\beta$ -mercaptoethanol, pH 7.3; (B) 0.010 M  $\text{F}_2\text{dUMP}$  in 0.1 M Tris- $\text{SO}_4$ , pH 7.3; (C) 0.005 M II in 0.1 M Tris- $\text{SO}_4$ , pH 7.3; (D) 0.005 M III in 0.1 M Tris- $\text{SO}_4$ , pH 7.3.

DNA synthesis and cell division has resulted in many attempts to elucidate the mechanism of action of thymidylate synthetase. Foremost among these efforts has been the identification and isolation of stable ternary complexes<sup>1-7</sup> between native thymidylate synthetase, its coenzyme  $\text{CH}_2\text{FH}_4$ , and the inhibitor 5-fluoro-2'-deoxyuridylate (FdUMP). We report here  ${}^{19}\text{F}$  NMR data that provide direct physical evidence for the structure of this ternary complex formed with native enzyme isolated from amethopterin resistant *Lactobacillus casei*. The impact of these data with respect to previous studies involving model compounds<sup>8</sup> and fragments of the complex obtained by proteolytic degradation<sup>9-10</sup> is also discussed.

Numerous studies have sought to elucidate the structural features in the ternary complex, with particular emphasis on the coordination of FdUMP and  $\text{CH}_2\text{FH}_4$  to the enzyme. Danenberg et al.<sup>9</sup> and Pogolotti et al.<sup>10</sup> have reported procedures for the enzymatic degradation of the ternary complex to yield a small peptide fragment to which both FdUMP and  $\text{CH}_2\text{FH}_4$  moieties remain bound, and Bellisario et al.<sup>11</sup> have presented evidence which suggests that the FdUMP is linked to a cysteinyl residue in a related peptide. These studies were based on radiolabeling techniques; hence there is no specific bonding information available to account for the association. Spectroscopic techniques,<sup>12-15</sup> used in attempts to determine the molecular nature of the binding of these moieties, provided inferences of the structural features. Danenberg and Heidelberg<sup>16</sup> have reported evidence based on chemical degradation which indicates that the point of covalent attachment for FdUMP to thymidylate synthetase occurs through nucleophilic attack of a cysteine sulfhydryl group on the pyrimidine ring. It is now generally accepted that the catalytic mechanism is initiated by attack of an active site nucleophile, most likely a cysteinyl sulfhydryl group, on carbon 6 of the pyrimidine ring to generate a carbanion which subsequently attacks  $\text{CH}_2\text{FH}_4$