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Metal–Nitroxyl Interactions. 12. Nitroxyl Spin Probes in the Presence of Tris(oxalato)chromate(III)

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$Cr(ox)_3^{3-}$ is more effective than the commonly used $Fe(CN)_6^{3-}$ in broadening the EPR spectra of nitroxyl spin probes. The extent of broadening of the EPR spectrum of 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxy by $Cr(ox)_3^{3-}$ depends on pH, the nature of the buffer, and the concentration of the nitroxyl as well as on the identity and concentration of the metal species.

Introduction

Many examples have been reported of interactions via collisions between nitroxyl radicals and paramagnetic transition-metal ions in solution. It is well-known that EPR signals are broadened when the concentration of the observed (or some other) paramagnetic species is increased. Much of the work in this area is summarized in a recent review.[1] Among the applications of metal–nitroxyl collision interactions, two warrant attention within the context of the present work. By use of charged and neutral metal complexes, the existence of charged groups near a spin-labeled site on a protein can be shown.[1-3] The broadening effect of paramagnetic metals on nitroxyl EPR spectra has also been used to "subtract" overlapping spectra in studies of membranes and cell interiors. With the nitroxyl spin label partitioned, e.g., between the interior region of the cell and the surrounding solution, a paramagnetic metal in the solution broadens the EPR signal of the nitroxyl outside the cell, facilitating observation of the EPR spectrum of the nitroxyl inside the cell.[1,4,5] $K_3Fe(CN)_6$ has been used in many of these studies, and its advantages relative to Ni(II) as a broadening agent for extracellular nitroxyl radicals have been emphasized.[5]

In this paper we report that $Cr(ox)_3^{3-}$ is more effective than $Fe(CN)_6^{3-}$ in broadening nitroxyl spin probes. The dependence of the broadening effectiveness on some of the solution conditions likely to be relevant to anticipated applications is also presented.

Experimental Section

Potassium ferricyanide, 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxy (tempamine), and the buffer materials are commercially available. Potassium tris(oxalato)chromate(III) trihydrate, $K_3Cr(ox)_3 \cdot 3H_2O$, was prepared as reported in the literature[6] and characterized by its visible spectrum[7] and EPR g value.[8] Refrigerated aqueous solutions of buffer and tempamine appeared to be stable over a period of several months. However, $Cr(ox)_3^{3-}$ in aqueous solution is not stable over long periods of time. Stability is greater near pH 7 and lower at pH >10. No change in broadening effectiveness was observed over a period of a few days near pH 7. A 1 month old solution of $Cr(ox)_3^{3-}$ in 50 mM Tris buffer at pH 8.1 exhibited only 75% of the broadening effectiveness of a freshly prepared solution. At pH >10 the solution turns bright green within 0.5 h.

EPR spectra were obtained on a Varian E-9 spectrometer with the use of an E-231 cavity containing the quartz dewar of the E-257 variable-temperature accessory. The spectrometer was interfaced to a Varian 620L/103 computer permitting data manipulation by use of the CLASS language.[9] Total intensity of the EPR signals was obtained by double integration of the first-derivative spectra. EPR spectra were run on freshly prepared solutions in ca. 2-mm o.d. quartz tubes. Solutions were not degassed. EPR spectra were taken at power levels below saturation. All comparisons of spectral amplitudes were based on data which had been corrected for differences in modulation amplitude, power level, and gain.

Solution pH was measured with a Radiometer 26 or a Corning 109 pH meter. Final pH was adjusted with small amounts of HCl or NaOH. All reported pH values were measured on the same solutions on which the EPR spectra were taken.

The pK_b of 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxy was measured by titration of an aqueous solution of the nitroxyl radical with standardized aqueous HCl solution with the use of a pH meter to monitor the pH changes. The pK_b of the amino nitroxyl was 5.2.

Computer simulations to obtain the line widths of the broadened nitroxyl spectra were done on a Burroughs B6700 computer by use of a first-order treatment of the electron–nitrogen hyperfine coupling.

Repeat measurements of the signal amplitude of the EPR signal for tempamine under the same conditions of concentration and instrument settings were reproducible to ±6%. Much of this uncertainty is thought to be due to variations in the diameter of the commercially available quartz tubing.

Results and Discussion

The interaction of a paramagnetic transition-metal ion with a nitroxyl radical in solution results in a broadening of the nitroxyl lines in the EPR spectrum and a resulting decrease in the amplitude of the first-derivative spectrum. Figure 1 shows the line shape and amplitude changes which occur when $Cr(ox)_3^{3-}$ is added to a 10 mM solution of tempamine in 5 mM phosphate buffer at pH 7.5. Note that in order to clearly display the line shape changes, the instrumental amplification of the spectra in parts B and C of the figure are 40 and 253 times, respectively, the amplification used part A. In the discussion of the use of $Fe(CN)_6^{3-}$ as a broadening agent, the relative amplitude of the mid-field peak was used as a measure of the extent of broadening.[5] However, as seen in Figure 1, when the nitroxyl signal is sufficiently broadened, the three nitrogen hyperfine components are no longer distinguishable lines. Thus all the comparisons in this paper are based on measurements of the overall amplitude of the first-derivative spectrum. Percent amplitude remaining is defined as the overall amplitude at a particular metal ion concentration divided by the overall amplitude with no metal ion present but all other concentrations the same and taking into account differences in instrumental settings. The term percent amplitude remaining is used instead of percent signal remaining[5] since the total intensity (second integral) remains constant while the line broadening causes a decrease in the amplitude of the first-derivative spectrum. The use of a 100% value at the same conditions except for the absence of metal ion is necessary since the nitroxyl line width varies slightly with pH, concentration, and buffer.

The EPR spectrum of $Cr(ox)_3^{3-}$ in aqueous solution is a broad, asymmetric line with a crossover point near $g = 3$ as shown in Figure 2A. However, over the narrow scan ranges and with the lower instrument settings typically used to record the nitroxyl EPR spectrum, the contribution to the EPR spectrum from $Cr(ox)_3^{3-}$ is only a slightly sloping baseline, as shown in Figure 2B.

Factors Influencing Broadening Effectiveness. Tempamine Concentrations. The effectiveness of $Cr(ox)_3^{3-}$ as a broadening agent for the nitroxyl radical tempamine is independent of tempamine concentration when the tempamine concentration is below about 2 mM. At higher tempamine concentrations

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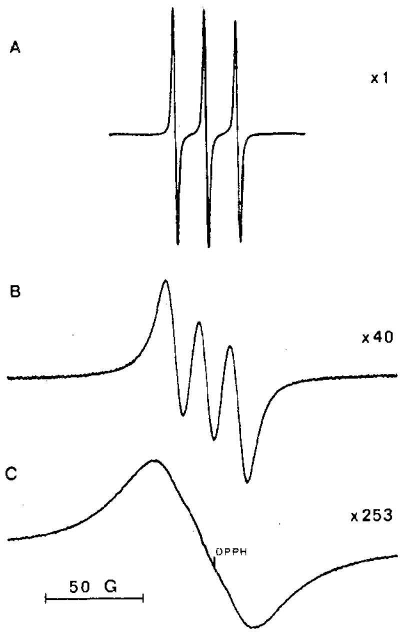

Figure 1. X-Band EPR spectra of 10 mM tempamine in pH 7.5 solutions containing 5 mM phosphate buffer and the following concentrations of $K_3Cr(ox)_3$: (A) 0 mM $Cr(ox)_3^{3-}$, gain 100, modulation amplitude 0.4 G, 1.0 mW; (B) 20 mM $Cr(ox)_3^{3-}$, gain 1.25 × 10^3, modulation amplitude 0.4 G, 10 mW; (C) 80 mM $Cr(ox)_3^{3-}$, gain 10^3, modulation amplitude 3.2 G, 10 mW. All spectra are presented with the same magnetic field axis scale, but the differences in gain, modulation amplitude, and power result in relative signal height amplifications of 1, 40, and 253 for (A), (B), and (C), respectively.

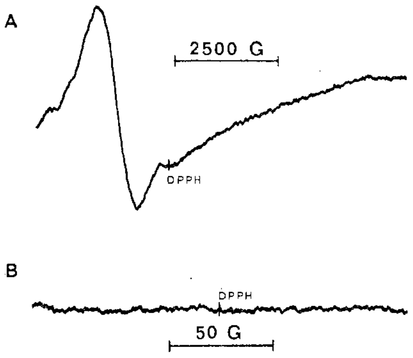

Figure 2. X-Band EPR spectrum of 0.1 M $K_3Cr(ox)_3$ in H_2O, gain 6.3 × 10^3, modulation amplitude 10 G, 40 mW: (A) 10 000-G scan and (B) 200-G scan of the same region as in Figure 1. The differences in gain, modulation amplitude, and power result in (B) being amplified 40 times Figure 1C and 10^4 times Figure 1A.

the effectiveness of a given concentration of $Cr(ox)_3^{3-}$ decreases as the tempamine concentration increases such that in 50 mM Tris buffer at pH 7.5, 10 mM $Cr(ox)_3^{3-}$ results in a 9% signal amplitude remaining for 16 mM tempamine but only a 4% signal amplitude remaining for 2 mM tempamine. However, increasing $Cr(ox)_3^{3-}$ concentration has a greater impact on signal amplitude than does increasing tempamine concentration such that at a constant ratio of $Cr(ox)_3^{3-}$ to tempamine the percent amplitude remaining is smaller at higher concentrations. For example in 50 mM Tris buffer at pH 7.5 and a 1.25:1 ratio of $Cr(ox)_3^{3-}$ to tempamine, the percent amplitude remaining is 3% at a $Cr(ox)_3^{3-}$ concentration of 20 mM and 6% at a $Cr(ox)_3^{3-}$ concentration of 10 mM. Thus even at relatively high tempamine concentrations, $Cr(ox)_3^{3-}$ is highly effective in broadening the nitroxyl EPR signal.

pH Dependence. The effectiveness of $Cr(ox)_3^{3-}$ as a broadening agent is also pH dependent. It must be emphasized that the pH values cited in this discussion are values measured with a pH meter on the actual solutions studied, in contrast to other conventions used in some of the biochemical literature. Some confusion may attend the use of the data in a previous report[5] concerning the effectiveness of $Fe(CN)_6^{3-}$ in which the pH value cited was the pH of the buffer solution used in making up the solution and not the pH of the final solution. Since tempamine is a relatively strong base ($pK_b = 5.2$), the pH of a solution of tempamine in a buffer is dependent on the concentration of tempamine and the concentration of buffer. A 1.0 mM solution of tempamine in 5 mM pH 7.5 phosphate buffer has a pH of 9.6. Thus the results in ref 5 are at a pH of about 9.6 rather than the pH 7.5 reported for the buffer solution.

The pH of the final solution is important as it affects the fraction of the tempamine which is present in the protonated form and therefore affects the extent of ion-pair formation. At pHs where the tempamine is largely in the protonated form, $Cr(ox)_3^{3-}$ is substantially more effective as a broadening agent than at pHs where there is relatively little protonated tempamine present. For example, for 1.0 mM tempamine in 50 mM Tris buffer in the presence of 20 mM $Cr(ox)_3^{3-}$, the percent amplitude remaining is 1% at pH 5 and at pH 7.5 but 3% at pH 10. Similarly for 10 mM tempamine in 5 mM phosphate buffer in the presence of 20 mM $Cr(ox)_3^{3-}$, the percent amplitude remaining is 2% at pH 7.5 and 8% at pH 9.6.

Influence of Buffer. As the concentration of phosphate buffer is increased, the effectiveness of the $Cr(ox)_3^{3-}$ as a broadening agent decreases. For example, at pH 7.5 with 10 mM tempamine in the presence of 20 mM $Cr(ox)_3^{3-}$ the percent amplitude remaining is 2% in 5 mM phosphate, 3% in 50 mM phosphate, and 5% in 100 mM phosphate. The effectiveness is also dependent on the nature of the buffer. For 10 mM tempamine at pH 7.5 in the presence of 10 mM $Cr(ox)_3^{3-}$, the percent signal remaining is 7% in 50 mM Tris buffer and 11% in 50 mM phosphate buffer.

Comparison of $Cr(ox)_3^{3-}$ and $Fe(CN)_6^{3-}$. For the determination of the relative effectiveness of $Cr(ox)_3^{3-}$ and $Fe(CN)_6^{3-}$ as broadening agents for nitroxyl EPR spectra the percent amplitudes remaining were compared for tempamine in the presence of the metal ions under a variety of conditions. In 50 mM Tris buffer containing 10 mM tempamine at pH 7.5, 0.9% amplitude remained in the presence of 80 mM $Fe(CN)_6^{3-}$ and 0.3% in the presence of 80 mM $Cr(ox)_3^{3-}$. Alternatively stated, 40 mM $Cr(ox)_3^{3-}$ was slightly more effective in broadening the nitroxyl signal than 80 mM $Fe(CN)_6^{3-}$. Similarly, for 1 mM tempamine at pH 7.5 in 50 mM Tris buffer containing 80 mM $Fe(CN)_6^{3-}$ or 80 mM $Cr(ox)_3^{3-}$, the respective percent amplitudes remaining are 0.5% and 0.3%. Also, in 5 mM phosphate containing 10 mM tempamine at pH 7.5, addition of 80 mM $Fe(CN)_6^{3-}$ or 80 mM $Cr(ox)_3^{3-}$ resulted in 0.9% and 0.3% remaining, respectively. Thus at 80 mM metal ion concentration, the $Cr(ox)_3^{3-}$ is 2–3 times more effective than $Fe(CN)_6^{3-}$ in broadening the nitroxyl EPR spectrum. At lower metal ion concentrations, the $Cr(ox)_3^{3-}$ is generally about twice as effective as $Fe(CN)_6^{3-}$. It should also be noted that Cr^{3+} has considerably less tendency to undergo redox reactions which also makes $Cr(ox)_3^{3-}$ more useful than $Fe(CN)_6^{3-}$ for many biological applications.

The nitroxyl EPR spectra in 5 mM phosphate buffer at pH 7.5 for 10 mM tempamine as a function of metal ion concentration were computer simulated to obtain the line widths of the peaks. The results are consistent with the observations based on the peak amplitudes. The line widths in the presence of $Cr(ox)_3^{3-}$ are consistently larger than in the presence of $Fe(CN)_6^{3-}$. For example, in the presence of 20 mM $Cr(ox)_3^{3-}$ and 20 mM $Fe(CN)_6^{3-}$ the line widths assuming 100%

Lorentzian line shapes are 10.2 and 7.5 G, respectively. At 80 mM Cr(ox)$_3^{3-}$ and 80 mM Fe(CN)$_6^{3-}$ the line widths are 33 and 14 G, respectively. In all cases the integrated intensity of the nitroxyl spectrum was independent, within experimental uncertainty, of the concentration of the metal ion added.

Conclusion

Cr(ox)$_3^{3-}$ is a more effective broadening agent for the tempamine nitroxyl radical than is Fe(CN)$_6^{3-}$. The optimum conditions for use in aqueous solutions are pH <8 and low buffer concentrations. It is more effective in Tris buffer than in phosphate buffer. The background signal for Cr(ox)$_3^{3-}$ near $g = 2$ is small compared to the nitroxyl signals.

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Registry No. Cr(ox)$_3^{3-}$, 15054-01-0; Fe(CN)$_6^{3-}$, 13408-62-3; tempamine, 14691-88-4.

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High-Pressure Phase Transformation of Platinum Sulfide

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PtS was found to undergo a high-pressure phase transformation at 30 kbar to a phase isomorphous with PdS. The ambient and high-pressure phases are diamagnetic semiconductors with electrical band gaps of 0.32 and 0.19 eV, respectively. The extent of solid solution of NiS in both phases of PtS was found to be less than 10 atom %.

Introduction

PtS, the mineral cooperite, adopts a tetragonal structure (Figure 1), space group $P4_2/nmm$, in which each Pt atom forms four coplanar bonds to bridging sulfur atoms.[1] The Pt–S bonds form the sides of large open channels that extend along the *a* directions. These channels are not retained in the closely related but less symmetric structure of PdS,[1] space group $P4_2m$ (Figure 2). A more compact structure for PdS is also indicated by a comparison of packing densities: 1 formula unit/34.1 Å^3 for PdS and 1 formula unit/36.8 Å^3 for PtS. In addition, the mineral braggite, (Pt, Pd, Ni)S, for which the Pt content is greater than that of Pd and Ni crystallizes in the PdS structure.[2] These observations suggested that PtS would under high pressure transform to a phase with the PdS structure. It was also of interest to determine the extent of substitution of other transition metals, i.e., Pd, Ni, Co, and Cr, for Pt and the effect of these substitutions on the phase transformation.

Experimental Section

Sample Preparation. All samples were prepared by the direct reaction of high-purity (>99.99%) elements in evacuated silica tubes at 750 °C. These samples were heated for at least 130 h with several intermittent grindings. After each heat treatment, they were cooled to room temperature at a rate of 100 °C/h. Pt$_{1-x}$M$_x$S (M = Ni, Cr, Co, Cu) reacted samples were subjected to pressures between 25 and 50 kbar for $^1/_2$ h at a temperature of 1000 °C in a belt apparatus of the type described by Hall.[3]

When a sample of NiS was cooled to room temperature at the cooling rate employed above, the millerite phase was obtained. Quenching to room temperature yielded NiS with the NiAs structure as reported previously.[4] The two modifications of NiS are a high-temperature phase with the NiAs structure and the millerite phase which is stable below 380 °C.

Sample Characterization. X-ray Crystallography. Powder patterns were recorded with a Norelco X-ray diffractometer equipped with a high-intensity copper source (λ(Cu Kα_1) = 1.5405 Å) and with a graphite monochromator located in the diffracted beam. Scan rates of 1 and $^1/_4$°/min were employed to obtain patterns for phase identifications and to collect data for lattice parameter determinations, respectively. The lattice parameters reported for each sample were determined from a least-squares refinement using 2θ angles between 50 and 140° corrected with reference to an Si internal standard.

Density Measurements. Densities were measured by a hydrostatic technique previously described by Adams.[5] Perfluoro(1-methyldecalin) calibrated with a high-purity silicon crystal (ρ = 2.328 g/cm^3) was employed as the density fluid.

Magnetic Measurements. Magnetic susceptibilities were determined over a temperature range of 77–300 K with a Faraday balance described by Morris and Wold.[6] No corrections were applied for core diamagnetism.

Electrical Measurements. Resistivity measurements were performed by the four-probe technique developed by van der Pauw.[7] Ohmic contacts were made with indium using an ultrasonic soldering device (12-W generator, Sonobond Corp.).

Results

PtS transformed completely to a high-pressure phase, PtS(p), at 30-kbar pressure and 1000 °C in $^1/_2$ hour. At 25 kbar this transformation was incomplete. Comparison of the powder patterns of PtS(p) and PdS confirmed that PtS(p) is isomorphous with PdS. Lattice parameters and densities for the two phases are given in Table I. Also listed for comparison are the lattice parameters of PtS, PdS, and braggite reported previously.[2,8,9]

Both the ambient and high-pressure phases of PtS are diamagnetic. The measured susceptibility at room temperature for PtS prepared at ambient pressure is −0.15 emu/g. At liquid nitrogen temperature the susceptibility is slightly field-strength dependent. Grønvold et al. also observed this behavior and reported a room-temperature diamagnetic susceptibility equal to that given here.[8] For PtS(p) similar results were obtained; the diamagnetic susceptibility measured at room temperature is −0.11 emu/g.

PtS and PtS(p) are narrow band gap semiconductors. A plot of log ρ vs. $10^3/T$, where ρ is sample resistivity, is shown in Figure 3 for these phases. For PtS, measurements were made on a cold-pressed bar sintered at 975 °C; the PtS(p)

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