highly conducting copper complexes.

Experimental Section

Preparation of $(TTF)_{4/3}CuI_2$. When a methanol solution of tetrabutylammonium iodide (0.15 mmol/2 mL) was added to a mixture of copper(II) acetate hydrate (0.07 mmol) and TTF (0.15 mmol) in methanol (3.5 mL) with stirring under a nitrogen atmosphere, a dark purple powder separated out. After the resulting mixture was stirred for ca. 1 h, the precipitate was collected on a filter, washed with methanol, and dried under vacuum. Anal. Calcd for $(C_6H_4S_4)_{4/3}CuI_2$: C, 16.29; H, 0.91; I, 43.03; Cu, 10.8. Found: C, 16.23; H, 1.06; I, 43.09; Cu, 9.7. Single crystals were grown by diffusing a methanol solution containing $Cu(CH_3CO_2)_2 \cdot H_2O$ and TTF (1:2) and a methanol solution of tetrabutylammonium iodide. Very thin needles were formed with the typical dimensions 0.05 × 0.05 × 2 mm.

Physical and Spectroscopic Measurements. The XPS spectrum was obtained by the use of a Vacuum Generators ESCALAB MK II spectrometer with Al Kα X-rays (1486.6 eV). The binding energies were calibrated by assuming that carbon 1s electrons of a graphite powder, which was mixed with the sample, had a binding energy of 284.6 eV. The IR spectrum was recorded for Nujol mulls with a Perkin-Elmer 1420 spectrometer. The powder conductivity was determined by van der Pauw's four-probe method.[11] The crystal conductivity was measured along the needle axis by the standard four-probe method. Electrical contacts were made with Aquadag. The magnetic susceptibility was determined with the aid of a Faraday balance.[12] The molar susceptibility was corrected for diamagnetic contributions (10^{-6} emu mol^{-1}) from TTF (−99)[13] and I^- (−52).[14]

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Vanadyl(IV)–Thallium(I)-205,203 Superhyperfine Coupling in Complexes with Pyruvate Kinase

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Pyruvate kinase is one of several enzymes that are known to require specific activation by monovalent cations.[1] Although K^+ is the likely physiological activator for pyruvate kinase, several other species of monovalent cation bring about varying degrees of activation.[2,3] The relative positions of the monovalent cation and the enzyme-bound divalent cation that is also required for activity of pyruvate kinase have been studied by NMR relaxation methods. These studies have been carried out with monovalent cations that have nuclear spins in conjunction with paramagnetic divalent metal ions such as Mn(II).[4-9] The paramagnetic contributions to the longitudinal relaxation rates ($1/T_{1p}$) have indicated that in the presence of the substrate, phosphoenolpyruvate, the monovalent cation binds within 4.5–5.5 Å from the divalent cation at the active site of the enzyme. Thallous ion is a good activator of pyruvate kinase,[2] and Tl^+ has been useful as an NMR probe of the monovalent cation site because of the favorable magnetic properties of the two stable isotopes (^{205}Tl, $I = {}^1/_2$, 70.48%; ^{203}Tl, $I = {}^1/_2$, 29.52%).

Reuben and Kayne[5] suggested that the substantially larger paramagnetic contribution to the transverse relaxation rate ($1/T_{2p}$) in measurements with $^{205}Tl(I)$ and Mn(II) was possibly due to an appreciable (A_{iso} = 0.85 MHz) scalar superhyperfine coupling between the unpaired electrons of Mn(II) and the ^{205}Tl nucleus. Vanadyl(IV) binds at the divalent cation site of pyruvate kinase,[10] and the narrow EPR signals for enzyme–VO^{2+} complexes made it feasible to investigate superhyperfine coupling interactions between VO^{2+} and nuclear spin of monovalent cations by EPR spectroscopy. A resolved superhyperfine coupling between the unpaired electron spin of VO^{2+} and the nuclear spins of Tl^+ has been observed with several different substrates or substrate analogues bound at the active site. Markham and Leyh[11] have independently observed a VO^{2+}–$^{203,205}Tl$ superhyperfine coupling of a similar magnitude in complexes with the enzyme *S*-adenosylmethionine synthetase.

Materials and Methods

Pyruvate kinase was prepared by the method of Tietz and Ochoa.[12] Solutions of the enzyme were equilibrated in a buffer containing 50 mM 4-(2-hydroxyethyl)-1-perazineethanesulfonic acid/tetramethylammonium hydroxide, pH 7.5, and 85 mM tetramethylammonium nitrate (buffer A) by gel filtration over a column (100 × 4 cm) of Sephacryl S-200 that was equilibrated and eluted with buffer A. Solutions of the protein were concentrated by using a collodion bag apparatus from Schleicher and Schuell. A stock solution of vanadyl sulfate was made up from the trihydrate (Aldrich Gold Label 99.999%). An aliquot from the stock solution was added to solutions of enzyme and substrate in buffer A that contained 1 mM dithiothreitol to minimize oxidation of the vanadyl ion. The amplitudes of the EPR signals for such solutions were stable for several hours. Spectra were recorded at 9.1 and 35 GHz with Varian spectrometers. Components of the **g** tensor and the ^{51}V hyperfine tensor were obtained from the measured resonance fields by an iterative procedure based on the second-order expressions given by Chasteen.[13]

Results

Oxalate, a competitive inhibitor with respect to phosphoenolpyruvate[14] binds at the active site of the enzyme as a bidentate chelate with VO^{2+}.[10] In buffer containing $(CH_3)_4N^+$ as the monovalent cation, the EPR spectrum for solutions of the enzyme–VO^{2+}–oxalate complex consists of a well-resolved powder pattern with $g_\parallel = 1.926$, $g_\perp = 1.969$, $A_\parallel(^{51}V) = 175 \times 10^{-4}$ cm^{-1}, and $A_\perp(^{51}V) = 67 \times 10^{-4}$ cm^{-1}. Addition of Tl(OAc) (see Figure 1) to a sample containing enzyme, oxalate, and vanadyl ion in the $(CH_3)_4N^+$ buffer results in the appearance of well-resolved, satellite signals spaced symmetrically about the original signals. The separation of the satellite signals measured 34 ± 1 G (32×10^{-4} cm^{-1}) both for the parallel and for the perpendicular lines in the powder pattern. Splitting of precisely the same magnitude was observed in the spectra obtained at 35 GHz. These observations indicate that the satellite signals are due to a scalar superhyperfine coupling between the unpaired electron spin of VO^{2+}

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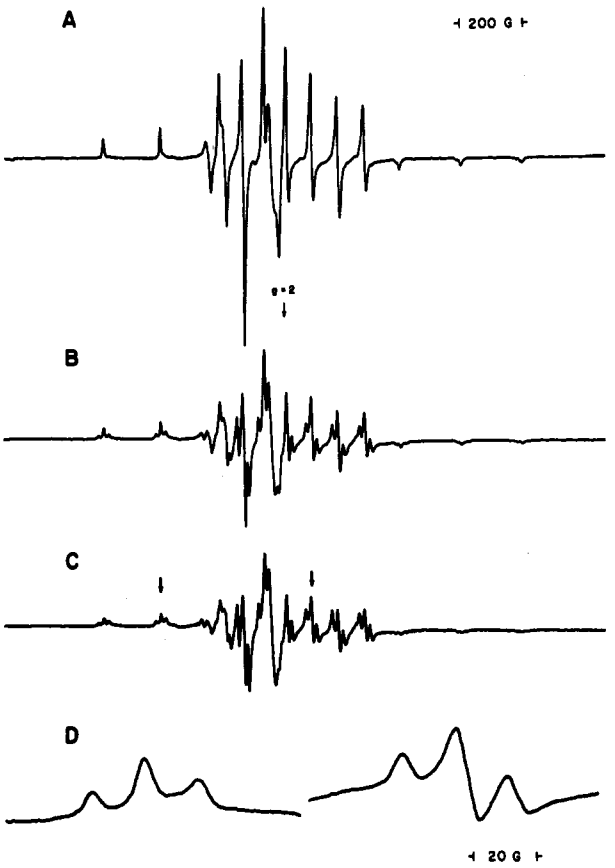

Figure 1. EPR spectra for solutions of pyruvate kinase–VO^{2+}–oxalate with and without Tl(OAc) present. Solution components were Buffer A, [enzyme sites] = 3 mM, [VO^{2+}] = 1.8 mM, and [oxalate] = 6.6 mM. Spectra are single scans at 9.1 GHz. Sample temperature was 0 °C. Key: A, [Tl^+] = 0; B, [Tl^+] = 10 mM; C, [Tl^+] = 27 mM; D Expansions of the $-^5/_{2\parallel}(^{51}V)$ and $+^3/_{2\perp}(^{51}V)$ hyperfine components from spectrum C (marked by vertical arrows).

and the nuclear spins of Tl^+. The similar magnitudes of the magnetogyric ratios for ^{205}Tl and for ^{203}Tl preclude observation of a separate set of satellite signals from each isotope. Anisotropy in the superhyperfine coupling (assessed from the magnitude of the coupling in the parallel and perpendicular lines in the spectrum) was within the experimental error (±1 G).

The amplitudes of the satellite signals increased with increasing concentrations of Tl(OAc). An approximate dissociation constant for Tl^+ of 12 mM was obtained from a titration. Addition of KNO_3 to the samples (30 mM [K^+]; 15 mM [Tl^+]) mM) diminished the amplitudes of the satellite signals and restored amplitude to the central features. Additional Tl(OAc) reversed the influence of KNO_3. These observations indicate that K^+ and Tl^+ compete for common sites.

The $^{205,203}Tl^+$ superhyperfine splitting is also evident in complexes of VO^{2+} and Tl^+ with other substrates and substrate analogues (e.g., pyruvate, phosphoenolpyruvate, acetopyruvate, and 3-fluoropyruvate). The magnitudes of the superhyperfine splittings (G) are as follows: pyruvate, 28 ± 1; 3-fluoropyruvate, 28 ± 1; phosphoenolpyruvate, 31 ± 1; acetopyruvate 26 ± 2. The dissociation constant for Tl^+ from the species giving rise to the superhyperfine splitting was on the order of 10–40 mM for all of the complexes examined. Optimal activation of the normal reaction by Tl^+ depends on the concentration and species of divalent cation in the assay mixture.[2] With Mg^{2+} in the assay mixture optimal activation occurs at 3–10 mM [Tl^+].[2] The relatively high dissociation constants for Tl^+ indicate the site that gives rise to the coupling may not be the highest affinity site for the monovalent cation. The sites that give rise to superhyperfine splitting are, however, competitive with respect to K^+ in the range of [K^+] that is normally required for activation of the physiological reaction of the enzyme. EPR measurements with the enzyme–

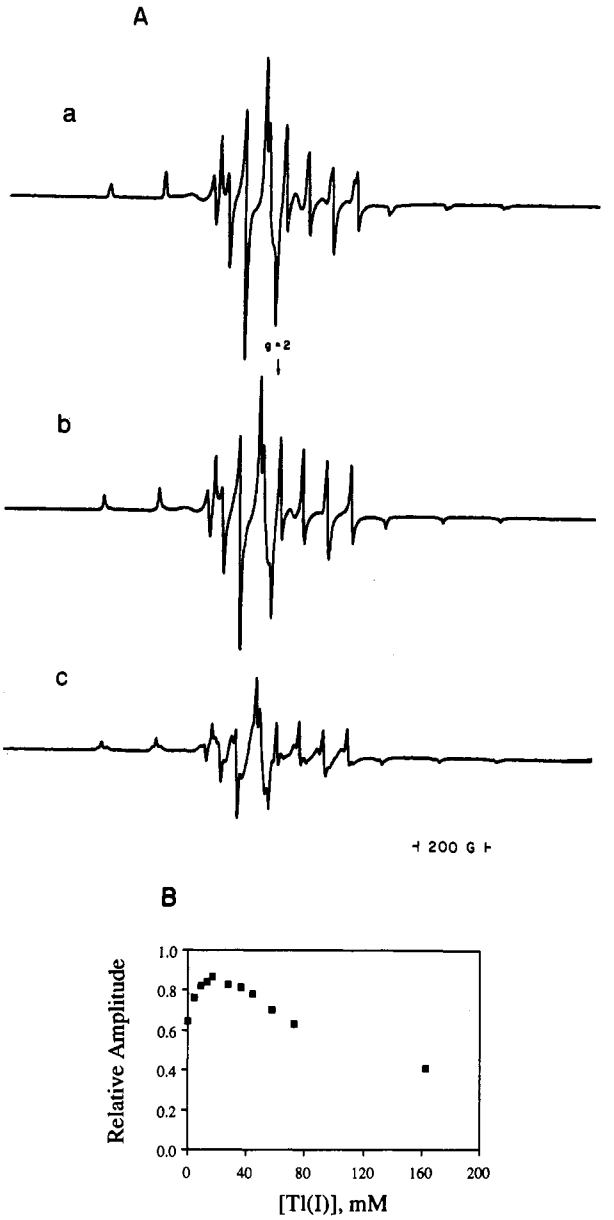

Figure 2. (A) EPR spectra for solutions of pyruvate kinase–VO^{2+}–pyruvate with and without Tl(OAc). Solution components were Buffer A, [enzymes sites] = 3.7 mM, [VO^{2+}] = 2.5 mM, and [pyruvate] = 22 mM. Key: a, [Tl(OAc)] = 0; b, [Tl(OAc)] = 17 mM; c, [Tl(OAc)] = 98 mM. Spectra are single scans at 9.1 GHz. Sample temperature was 0 °C. (B) Amplitude of the signal for the central feature of the $+^7/_{2\perp}$ line in the spectrum for VO^{2+} vs. [Tl^+].

VO^{2+}–pyruvate species provide further evidence for multiple sites for monovalent cations. EPR spectra for the complex enzyme–VO^{2+}–pyruvate differ with $(CH_3)_4N^+$ and with K^+ present because in $(CH_3)_4N^+$ alone there are two distinct sets of signals. At high [K^+] the spectrum shows only one set of signals. Titration of a solution of the enzyme–VO^{2+}–pyruvate complex in buffer A with Tl(OAc) shows a biphasic dependence on [Tl^+] (see Figure 2). At low concentrations, Tl^+ causes changes in the spectrum for bound VO^{2+} that are analogous to those observed with K^+ (i.e., conversion from two sets of overlapping signals to a single set of signals). These changes result in an apparent increase in the amplitudes of the EPR signals (Figure 2B). The satellite signals (Figure 2A) appear at much higher concentrations of Tl^+ and reduce the amplitudes of the central features.

Discussion

The Tl nucleus is especially sensitive to the presence of unpaired electron spin density in orbitals of the ion. For example, the calculated isotropic hyperfine coupling constant for $^{205}Tl^0$ is 15 040 G.[15] Hence, a small extent of overlap between the spin-bearing

d orital of VO^{2+} and atomic orbitals of Tl^+ or a superexchange mechanism involving bridging ligand groups could account for the magnitude[16] of the scalar coupling.

Strong scalar superhyperfine coupling between the unpaired electron spin of VO^{2+} and magnetic isotopes of Sn has been reported previously in EPR studies of VO^{2+} doped into a host crystal of SnO_2.[17] In the SnO_2 crystal, two sets of superhyperfine couplings were observed. From the crystal structure it was deduced that the strongest couplings (168 G) were from Sn nuclei that were located 3.18 Å from the vanadyl(IV) center between O ligands in the equatorial plane. This geometry maximizes interaction with the unpaired electron in the nonbonding d orbital of the vanadyl ion.[17] If the VO^{2+}–Tl^+ coupling occurs as a result of direct orbital overlap, the relatively large magnitude of the coupling would be most consistent with a geometry in which Tl^+ was positioned peripheral to and between equatorial ligands in the coordination sphere of the VO^{2+}. The carboxylate groups of the substrates and analogues could also act a bridging ligands between VO^{2+} and Tl^+, and the bridging ligand could mediate a superexchange interaction. Furthermore, a bridging carboxylate could also position Tl^+ for direct interaction with the nonbonding d orbital of VO^{2+}. The strong VO^{2+}–Tl^+ superhyperfine coupling is certainly compatible with a very close spacing of the monovalent and the divalent cation sites on the enzyme.

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Interaction of $AlCl_3$ with Tetrahydrofuran. Formation and Crystal Structure of $[AlCl_2(THF)_4][AlCl_4]$

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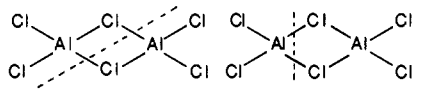
It is well-known that aluminum trichloride forms neutral donor–acceptor complexes with bases through a symmetrical dissociation of the dimer (**1**). Less common is the unsymmetrical



1 **2**

cleavage (**2**), which produces the ionic species $[AlCl_2(base)_n][AlCl_4]$. We have previously reported complexes of the latter type, which were formed by the reaction of aluminum trichloride with crown ethers in toluene. In the cation, the $AlCl_2^+$ ion fits into the crown cavity (for 15-crown-5[1]) or perches on the crown such that there is a strong interaction with four crown oxygen atoms for 12-crown-4 and 18-crown-6.[2] In the former the chlorine atoms adopt a trans configuration, whereas in the latter a cis geometry is seen. Our studies on bidentate oxygen donors such as dimethoxyethane (DME) showed that the related $[AlCl_2(DME)_2]^+$ was formed.[3] Since these ionic species exhibit interesting two-phase liquid behavior in aromatic solvents,[4] it was of interest to extend the work to bases with only one donor oxygen atom. The $AlCl_3$/THF system was a desirable candidate for study since a number of investigations had already been carried out. The presence of an ionic moiety in the workup of a saturated solution of $AlCl_3$ in THF was deduced from a vibrational spectroscopic study by Derouault and Forel,[5] but Cowley and co-workers found only the molecular $AlCl_3$·2THF in a related system.[6] We report here the isolation and structural characterization of Derouault's compound $[AlCl_2(THF)_4][AlCl_4]$ as the only product of the reaction of stoichiometric quantities of the reactants in toluene.

Table I. Calculated and Reported X-ray Diffraction Data for 1:2 $AlCl_3$/THF Compounds

d_{calcd},[a] Å	d_{obsd},[b] Å	d_{obsd},[c] Å
12.0	7.12	12.0
9.78	6.31	9.80
8.81	5.39	8.92
8.20	5.18	8.22
7.43		
7.29	4.90	7.31
7.16		
6.04	4.72	6.05
5.90		
5.54	4.57	5.51
	4.30	5.24
4.89	4.18	4.92
4.67	4.00	4.65
	3.87	4.34
4.13	3.71	4.10
3.81	3.43	3.91
3.78	3.26	3.75
3.57		
3.54	3.19	3.50
3.51		

[a] Calculated *d* spacings based on the single-crystal data of this study. [b] $AlCl_3$·2THF.[6] [c] $[AlCl_2(THF)_4][AlCl_4]$.[5]

Results and Discussion

The species formed upon the interaction of aluminum trichloride with tetrahydrofuran is seen to be dependent upon the state of the $AlCl_3$ and perhaps upon the solvent as well. Derouault and Forel[5] obtained $[AlCl_2(THF)_4][AlCl_4]$ from a THF solution of Al_2Cl_6, while Cowley and co-workers prepared molecular $AlCl_3$·2THF from the dissolution of $(Me_2N)_3SiCl$·$AlCl_3$ in THF. Our approach was different since we sought the ionic complex in the presence of the aromatic solvent. The identity of the title complex has been established by a single-crystal X-ray diffraction study. The compound reported by Derouault and Forel[5] has been shown to be $[AlCl_2(THF)_4][AlCl_4]$ by a comparison of the powder diffraction data reported in the 1977 study with the single-crystal data. The results are given in Table I.

One of the two independent cations is shown in Figure 1. There is no significant difference between the crystallographically independent ions, cations or anions. Important bond lengths and angles are given in Table II. In the cations, the average Al–Cl distance, 2.230 (9) Å, is slightly longer than those found in *cis*-$[AlCl_2(donor)_4]^+$: 2.204 (2) Å in $[AlCl_2(DME)_4]^+$,[3] 2.201 (1) Å in $[AlCl_2(12\text{-crown-4})]^+$,[2] and 2.18 (1) Å in $[AlCl_2(18\text{-crown-6})]^+$.[2] The latter values are not significantly different from the 2.200 (3) Å value in the trans cation $[AlCl_2(15\text{-crown-5})]^+$ in which the aluminum atom is seven-coordinate.[1] In the anions, the Al–Cl distances average 2.10 (2) Å, in keeping with literature values.

Interestingly, the Al–O bonds average 1.94 (1) Å in the title complex, compared to 1.96 (2) Å for the 12-crown-4 analogue.

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