ordered oxygen overlayer. These perturbations are also manifest in the observed π-bonding of molecular ethylene at 200 K, as opposed to the di-σ-bonded molecular ethylene that is observed on the Ru(001) surface.

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Superhyperfine Coupling between Metal Ions at the Active Site of *S*-Adenosylmethionine Synthetase

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S-Adenosyl-L-methionine synthetase (ATP:L-methionine *S*-adenosyltransferase) is one of numerous enzymes which require monovalent cations for significant catalytic activity.[1-3] Although *S*-adenosyl-L-methionine (AdoMet) synthetase from *Escherichia coli* has been shown to bind a single monovalent cation on each of the four identical subunits,[3] it had not been determined whether this cation binds at the active site. Like many other monovalent cation activated enzymes for which the physiological cation activator is probably K^+, the Tl^+ ion activates to ~80% of the level of K^+ and binds to the enzyme with higher affinity than other monovalent cation activators.[3,4] In a continuation of our EPR studies using the VO^{2+} ion to probe the structure of the divalent metal ion binding site at the active site of AdoMet synthetase,[5] we have observed superhyperfine coupling between the nuclear spin of the Tl^+ ion and the electron spin of VO^{2+}. The results reported herein demonstrate that the monovalent cation activator binds at the active site and suggest the formation of a metal ion cluster at the active site of *S*-adenosylmethionine synthetase.

S-Adenosylmethionine synthetase was purified to homogeneity from the *E. coli* strain DM25pK8[3,6] and was prepared for EPR spectroscopy as described.[5] All samples were prepared in D_2O solutions of 50 mM Hepes/$(CH_3)_4N$ buffer (Hepes = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH meter reading 7.6 (uncorrected for deuterium effect on the pH electrode), since narrower spectral lines are obtained in D_2O.[5] EPR spectra were recorded on a computer-interfaced Varian E-109 spectrometer operating at 9.14 GHz. Spectra were recorded in solution at 1 °C, and were generally the average of 4-10 scans. Spectra were analyzed by computer simulation, using the program QPOW of Belford and co-workers.[7] The reported g values and hyperfine coupling constants are apparent values since the rigid limit values are partially averaged due to slow rotation of the macromolecular complex. Uncertainties in the reported hyperfine coupling constants are estimated to be ±1 MHz and uncertainties in g values to be ±0.003.

Figure 1 compares EPR spectra for complexes of *S*-adenosylmethionine synthetase with VO^{2+}, methionine, and the ATP analogue 5'-adenylylimidodiphosphate (AMPPNP) in the presence of K^+ and Tl^+. The spectra, which are essentially rigid-limit powder spectra due to the slow rotation of the 168 000-dalton protein complex, show that the g values and ^{51}V hyperfine coupling constants for the axially symmetric spectra are identical for the two monovalent cations ($g_\parallel = 1.923$, $g_\perp = 1.975$; $A(^{51}V)_\parallel = 540$ MHz, $A(^{51}V)_\perp = 208$ MHz). However, in the presence

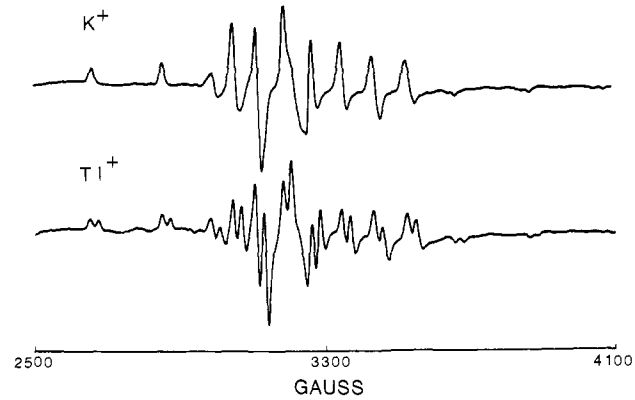

Figure 1. EPR spectra of VO^{2+} complexes of *S*-adenosylmethionine synthetase. Solutions contained 1.0 mM AdoMet synthetase active sites, 1.0 mM $VOSO_4$, 5.0 mM methionine, 1.0 mM AMPPNP, and either 50 mM KNO_3 or 2 mM $TlNO_3$.

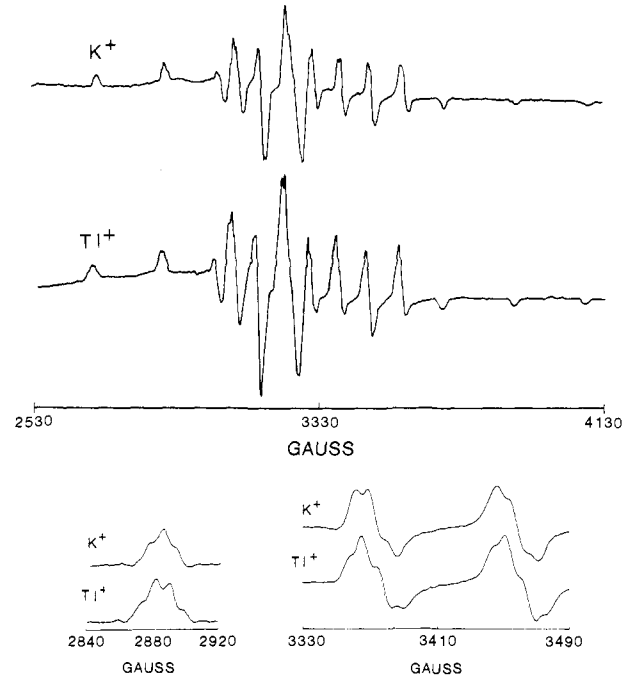

Figure 2. EPR spectra of complexes of AdoMet synthetase with VO^{2+}, PPi, AdoMet, and either K^+ or Tl^+. Solutions contained 1.0 mM enzyme subunits, 1.0 mM $VOSO_4$, 1.3 mM AdoMet, 1.0 mM PPi, and either 50 mM KNO_3 or 2 mM $TlNO_3$.

of Tl^+, each ^{51}V hyperfine line is split into a doublet due to a coupling of the electron spin to the nuclear spin of the thallous ion (both thallium isotopes, ^{203}Tl (30% abundant) and ^{205}Tl (70% abundant), are spin $^1/_2$ and have magnetic moments within 1% of each other yielding equivalent splittings within the resolution of these spectra). The superhyperfine coupling is 67 MHz (ca. 23 G) for both the parallel and perpendicular orientations of the complex in the magnetic field. Since the coupling is isotropic, it arises from delocalization of the unpaired electron spin into the Tl^+ orbitals. The superhyperfine coupling constant is equivalent to 0.16% of the unpaired electron spin density being located in a thallium 6s orbital.[8] The maximal anisotropy of the interaction is 3 MHz, which would have been undetectable at these line widths; in a point dipole approximation,[8] a 3-MHz anisotropy would reflect a distance of 3.6 Å between V(IV) and Tl^+, yielding a lower limit for the separation of the ions.

The other complexes of AdoMet synthetase with VO^{2+} and substrates were examined for coupling to the Tl^+ ion. The addition of Mg(II) to the above complex (which allows catalysis to occur

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to yield a enzyme–VO^{2+}–AdoMet–PPNP–Mg(II)–Tl^+ complex, PPNP = imidotriphosphate (O_3P–O–PO_2–NH–PO_3)) did not alter the coupling to Tl^+, whereas if either methionine or AMPPNP were omitted, coupling to Tl^+ was not observed.

In complexes formed with enzyme and products of the AdoMet synthetase reaction, coupling to Tl^+ was observed when both AdoMet and PPi were present in an enzyme–VO^{2+}–AdoMet–PPi–Tl^+ complex, with or without added Mg^{2+} (Figure 2). The spectra of complexes containing PPi also exhibit superhyperfine coupling to the two ^{31}P nuclei of the bidentate vanadyl ligand PPi, yielding the triplet structure seen in the spectrum of the K^+ complex.[5] Computer simulations indicate that the superhyperfine structure in the spectra of the Tl^+ complex is due to a 24-MHz isotropic superhyperfine coupling to a spin-$^1/_2$ nucleus, in addition to the ^{31}P couplings of 22 MHz (additional parameters are $g_\parallel$ = 1.936, $g_\perp$ = 1.979; $A(^{51}V)_\parallel$ = 532 MHz, $A(^{51}V)_\perp$ = 198 MHz). Spectra identical with those shown in Figure 2 were obtained for the enzyme–VO^{2+}–ATP–methionine complexes with K^+ and Tl^+. Superhyperfine couplings to Tl^+ were not observed for either the enzyme–VO^{2+}–PPi–Mg^{2+} complex, for which the VO^{2+} binding site structure is extremely similar as indicated by the g values, ^{51}V and ^{31}P hyperfine couplings,[5] nor in the enzyme–VO^{2+}–AdoMet complex. Apparently both substrate binding sites must be occupied in order for the thallium ion to be positioned with respect to VO^{2+} in such a fashion as to allow superhyperfine coupling.

The observation of coupling between the thallium nuclear spin and the unpaired electron spin of VO^{2+} at the active site of AdoMet synthetase is a rare case of superhyperfine coupling between V(IV) and another metal ion; other cases are pyruvate kinase[9] and a coupling between V(IV) and tin in SnO_2 doped with vanadium.[10] In the latter case vanadium couples to two types of Sn; crystallographic data show that these Sn atoms are located 3.1 and 3.7 Å from the vanadium and both lie in the plane of the vanadium orbital in which the unpaired electron is localized; the isotropic superhyperfine splittings for these Sn are 168 and 28 G, respectively. Although isotropic superhyperfine coupling is commonly mediated by a ligand that is shared between the two species involved,[11] the tin superhyperfine coupling is thought to result from direct orbital overlap between metal ion orbitals rather than being mediated by the oxygen ligands.[10] In the AdoMet synthetase complexes, the large spatial distribution of the thallium orbitals also leaves a distinct possibility that direct overlap between the Tl^+ and VO^{2+} orbitals, rather than an intervening ligand, mediates the interaction. ^{205}Tl NMR studies have indicated that enzyme-bound Tl^+ does not directly coordinate the substrates.[12]

In addition to the divalent metal ion binding site which VO^{2+} has been used to probe, a second divalent metal ion is required for catalytic activity of AdoMet synthetase.[5] Magnetic coupling between Mn(II) bound to both divalent metal ion binding sites has been reported, demonstrating not only that the two divalent metal ions bind extremely closely together but that there is orbital overlap between them, consistent with formation of a metal cluster structure.[5] The finding that there is also orbital overlap between the monovalent cation and one of the divalent cations suggests that AdoMet synthetase may assemble a trimetallic cluster at the active site. Elucidation of the detailed structure of the metal ion binding sites will undoubtedly play an important role in understanding of the mechanism of catalysis.

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Activation of Allylic Carbon–Carbon Bonds by Gas-Phase Copper(I)

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It is now established that certain gas-phase transition-metal ions activate C–C and C–H bonds of hydrocarbons,[1-8] whereas metal complexes in solution are much less reactive.[9] The first evidence that Fe^+, Co^+, and Ni^+ oxidatively add alkanes was reported by Ridge and co-workers.[1,2] In a later study, Armentrout and Beauchamp[4] proposed that Co^+ oxidatively adds allylic C–C bonds. Transfer of a β-H atom and reductive elimination produces a bis(olefin) complex. Fe^+ and Ni^+ also activate allylic C–C bonds of alkenes by the same mechanism as was reported in ref 4–8.

We wish to report here that Cu^+ also activates the allylic C–C bonds of alkenes. Evidence from two different experimental strategies points to a copper ion/alkene reactivity that phenomenologically mimics that of the other reactive metal ions. The first strategy is collisional activation (CA) of Cu(olefin)$^+$ adducts formed in a high-pressure mass spectrometer ion source. Copper ions were produced by electron ionization of copper(II) acetyl acetonate.[10] In the second approach, Cu^+ was formed by laser ionization of a metal target[11] and reacted with olefins in a FT mass spectrometer.[12]

As illustrated in Figure 1, the collisionally activated decomposition (CAD) spectrum of Cu(1-pentene)$^+$ is very similar to that of Fe(1-pentene)$^+$.[5] $TiCl^+$ was previously reported to react with 1-pentene to eliminate ethylene,[13] and the spectrum of TiCl(1-pentene)$^+$ is also similar to that of Fe(1-pentene)$^+$. All complexes decompose principally by eliminating C_2H_4.

To be certain that the CAD spectra are consistent with published results from other experimental approaches, the CAD spectra of all the first-row transition-metal ions (except Sc^+) complexed with 1-pentene were obtained (Table I). The results are consistent with the known reactivities of metal ions with other hydrocarbons and serve as controls to ensure there are no peculiarities with our experimental approach.

The reactivities of different M^+ with 1-pentene were also compared by using FTMS. Relative rate constants (Table I) for disappearance of M^+ were obtained. Dehydrogenation reactions of Ti^+ and V^+ are the most rapid, whereas Fe^+, Co^+, Ni^+, and Cu^+ react slightly slower with 1-pentene to give the same array of products as in Table I. As expected,[2] Cr^+ and Mn^+ do not activate, but do condense slowly to form $MC_5H_{10}^+$. Zn^+ charge exchanges with 1-pentene, in accord with the difference in ionization energies.[14]

The survey results demonstrate that Cu^+ and $TiCl^+$, in addition to Fe^+, Co^+, and Ni^+, activate allylic C–C bonds. The reactivity

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