of enzymes is of considerable agricultural and medicinal importance.

**Acknowledgment**. Work was supported by a grant from the Herman Frasch Foundation.

## Evidence for an Externally Bound Fe$^+$–Buckminsterfullerene Complex, FeC$_{60}$$^+$, in the Gas Phase

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*Received May 10, 1991*

One of the most intriguing aspects of C$_{60}$[1] is its ≃7-Å-diameter cavity, which may be impregnated by other elements and perhaps even by small molecules, thus altering its chemical and physical properties. Even before a macroscopic synthesis of C$_{60}$[2] was available, Smalley and co-workers demonstrated that metal-included species could be generated in the gas phase by growing them in a supersonic expansion source following laser desorption from a graphite target dosed with various metals, M = La, K, and Cs.[3,4] Also formed are a variety of less symmetrical MC$_n$ complexes. These species, in particular MC$_{60}$ and its singly charged counterpart, are highly stable. In all cases the complexes were predicted to have the metal atom either wholly or partially surrounded by a shell of carbons. Supporting this hypothesis was the fact that the MC$_{60}$$^+$ ions fragmented only under the extreme activation conditions of multiphoton absorption at high ArF excimer laser fluence and, then, only by sequential C$_2$ loss.[4] Further loss of C$_2$ ceased at some critical even number of carbon atoms depending on the metal, such as C$_{44}$La$^+$ (possibly C$_{42}$La$^+$), C$_{44}$K$^+$, and C$_{48}$Cs$^+$. Again, these results are in accord with expectations of a central metal enclosed in an inert carbon cage. While these MC$_{60}$ species were grown in situ in the supersonic expansion, the question arises as to whether a metal ion interacting with a preformed fullerene will attach externally or internally. Looking at a space-filling model for CsC$_{60}$ and KC$_{60}$,[5] for example, one would predict external attachment. Recently, potassium-doped C$_{60}$ has shown a superconducting transition; the metal ions are externally bound.[6]

Although our initial attempts to generate a LaC$_{60}$$^+$ species failed due to the rapid reaction of La$^+$ with background gas to form LaO$^+$,[7] FeC$_{60}$$^+$ was formed in our Nicolet FTMS-2000 Fourier transform mass spectrometer via the following multistep sequence:[8] (1) Fe$^+$ was generated by laser desorption from an Fe target in a source external to the solenoid magnet;[9] (2) the Fe$^+$ was permitted to react with pentane at 1 × 10$^{-6}$ Torr, generating Fe(C$_n$H$_{2n}$)$^+$ ($n$ = 2–5); (3) these ions then underwent ligand-exchange reactions with preformed C$_{60}$ and C$_{70}$ heated at 350 °C off a solids probe to generate FeC$_{60}$$^+$ and FeC$_{70}$$^+$; and (4) after a total reaction time of 300 ms, C$_{60}$$^+$, C$_{70}$$^+$, and other, lower mass ions were ejected by double-resonance techniques,[10] Figure 1A. The FeC$_{60}$$^+$ and FeC$_{70}$$^+$ were not observed to react further with background pentane.

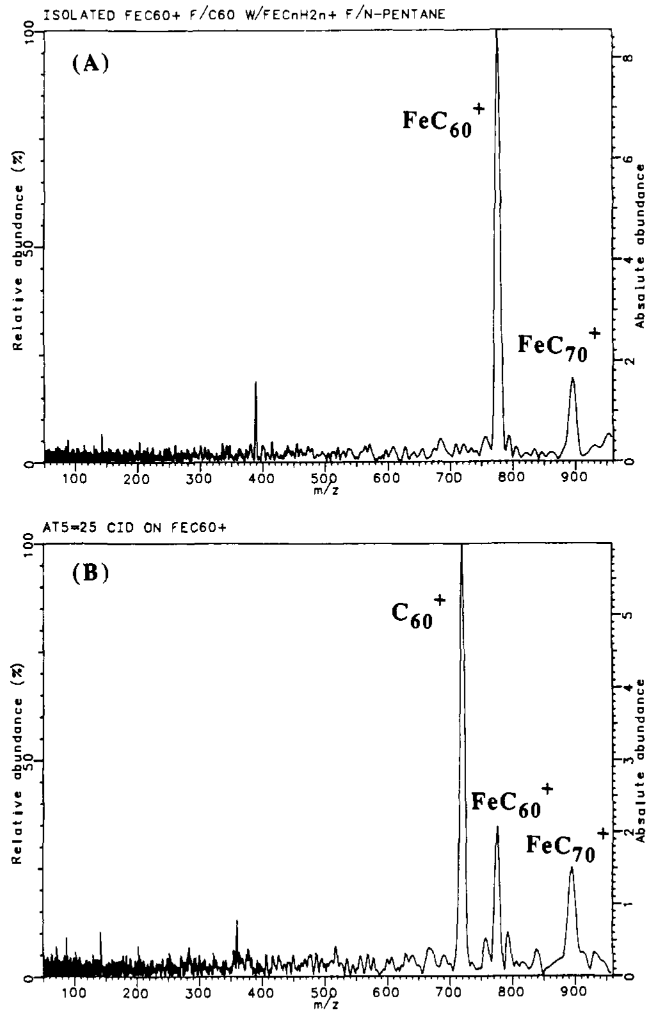

**Figure 1.** (A) Isolated FeC$_{60}$$^+$ and FeC$_{70}$$^+$. The peak at $m/z$ 388 is attributed to a harmonic. (B) Collision-induced dissociation of FeC$_{60}$$^+$ at 76 eV in laboratory energy, and 3.7 eV in center-of-mass energy. Both spectra were obtained using 16K data points.

Collision-induced dissociation of FeC$_{60}$$^+$ was performed by rf irradiation of the ion at its resonant frequency in the presence of Ar at a pressure of 3 × 10$^{-6}$ Torr. As shown in Figure 1B, C$_{60}$$^+$ is the sole product ion observed. This result is consistent with IP(Fe) =7.9024 eV[11] > IP(C$_{60}$) =7.61 eV[12] and contrasts with the results of the earlier studies on MC$_{60}$$^+$ species in requiring relatively little activation energy (12–241 eV in laboratory energy and 0.6–11.8 eV in center-of-mass energy[13]) and in generating the intact C$_{60}$$^+$ rather than losing C$_2$ molecules. Thus, either the

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0002-7863/91/1513-6298$02.50/0

Fe is externally bound or it readily passes into and out of the $C_{60}$. This latter possibility is unlikely, given the radius of $Fe^+$ of $\simeq$0.96 Å and that of Fe of $\simeq$1.24 Å.[14] Furthermore, these results also provide strong support for internally bound "eggshell" $MC_{60}^+$ (M = La, K, Cs) species reported earlier, "grown" in a supersonic expansion following laser desorption from a metal-impregnated graphite target, since these latter species are highly stable and lose $C_2$ molecules when sufficiently activated.[3,4] In summary, if Fe is indeed bound externally, generation of $MC_{60}^+$ in this way with other metal ions such as $La^+$ should result in externally bound isomeric complexes.[7] We are currently attempting to generate this new family of ions.

The ligand displacement reactions imply $D^\circ(Fe^+{-}C_nH_{2n}) \simeq$ 40 kcal/mol[15] $< D^\circ(Fe^+{-}C_{60})$. Similar displacement reactions should provide a bracket for $D^\circ(Fe^+{-}C_{60})$ and, likewise, for other metal cations. Finally, reactions other than displacement may occur with these externally bound species and are also being investigated in our laboratory.

**Acknowledgment**. This work was supported by the National Science Foundation (CHE-8920085) and the Division of Chemical Sciences in the Department of Basic Energy Sciences in the United States Department of Energy (DE-FG02-87ER13766).

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## Heteroatom Requirements for Substrate Recognition by GTP-Binding Protein Methyltransferase

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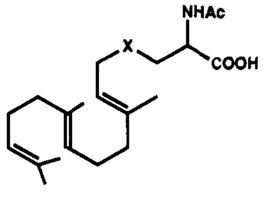
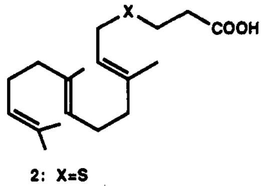
Signal transducing G proteins are posttranslationally modified by isoprenylation.[1-7] This process occurs at protein carboxyl termini ending in a CAAX motif (C = cysteine, A = aliphatic amino acid, and X = any amino acid).[1-3,8] The cysteine residue is enzymatically farnesylated or geranylgeranylated at sulfur in an isoprenyltransferase-mediated process using an isoprenyl pyrophosphate as substrate.[9-11] This reaction is followed by a protease-mediated cleavage that occurs after the modified cysteine, and finally by a *S*-adenosylmethionine- (SAM-) linked methylation at the newly generated cysteine carboxyl terminus.[12-18]

In studies on retinal rod outer segment GTP-binding protein methyltransferase, it was found that the enzyme can process relatively simple substrate molecules. For example, it can methylate *N*-acetyl-*S*-farnesyl-L-cysteine[14] (AFC, **1**) and *S*-farnesylthiopropionic acid (FTP, **2**), with $K_M$ values of 23 and 14 $\mu$M, respectively.[19] In both cases all remnants of the protein backbone are gone, suggesting that the enzyme recognizes only the 3-farnesylthiopropionic acid moiety, and not the carrier protein. In this communication we further investigate the nature of the specificity requirements for the enzyme and show the importance of the unmodified sulfur atom in defining whether a molecule is a substrate or inhibitor of the G protein methyltransferase.



1: X=S
3: X=S=O
4: X=Se
6: X=O



2: X=S
5: X=Se
7: X=O
8: X=NH

To probe the role of the sulfur in **1**, the sulfoxides **3** (1:1 mixture of diastereomers) were studied. These sulfoxide analogues were previously shown to be completely inactive as substrates for the enzyme[19] and are shown here to be potent competitive inhibitors of it (Figure 1), with a $K_i$ of 13.2 $\mu$M for the mixture. Moreover, no stereoselection at sulfur was observed. The diastereomeric sulfoxides were separated by HPLC on silica.[20] The less polar diastereomer was equipotent with the diastereomeric mixture as an inhibitor. These observations show that the nucleophilic sulfur atom is crucial for substrate activity, but not for binding to the enzyme. The nature of the sulfur requirement was further explored by studying the racemic selenocysteine analogue **4**. This molecule proved to be a substrate for the enzyme (Table I). The $K_M$ was close to that of **1**, assuming stereospecificity, but the $V_{max}$ was substantially reduced. In addition **5**, the selenopropionate analogue of **2**, was also a substrate for the enzyme, but had a larger $K_M$ and a smaller $V_{max}$ than found with **2** (Table I).

Several attempts were made to synthesize *N*-acetyl-*O*-farnesyl-L-serine (**6**) with little success due to the great lability of this molecule. However, **7**, the oxo analogue of **2**, proved to be an exceedingly weak substrate for the methyltransferase, but bound to the enzyme approximately as well as the other propionate analogues (Table I). *Thus, the major differences between the S, S=O, Se, and O substrates appear primarily in the* $V_{max}$ *term, rather than in the* $K_M$ *term.*

One possible mechanism to account for the observations described above is one in which initial full or partial transmethylation occurs between SAM and the heteroatom of the methyltransferase

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