stable in the mass spectrometer. Catalytically active dehydroquinase was mixed with 3-dehydroquinate immediately prior to injection into the mass spectrometer. By using a large excess of dehydroquinate (10000:1 substrate:enzyme) it was possible to observe a second series of signals of lower intensity (Figure 2). These correspond to a species of molecular weight 27618 ± 7.4, which is that expected for **4**, the imine adduct between the enzyme and the product (calculated molecular weight 27621.9).

The low intensity of the imine adduct observed in Figure 2 is probably a consequence of the extreme conditions the liganded enzyme encounters upon injection into the spectrometer.[13,15] The observation of the imine adduct with the product rather than the substrate is consistent with the result from the borohydride trapping experiment and suggests that **4** is the major covalently bound intermediate on the enzyme. This is reasonable as the equilibrium constant for the dehydration is 15.[16]

In summary, the use of electrospray mass spectrometry has allowed direct observation of an imine intermediate in an enzyme reaction. The molecular weight of both the imine intermediate and the adduct formed after reduction with sodium borohydride suggests that the major covalent enzyme adduct is the imine with the product 3-dehydroshikimate. The inactivation experiment also showed the enzyme does not exhibit half-sites reactivity. These experiments provide a powerful demonstration of the use of electrospray mass spectrometry to study enzyme mechanisms.

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(15) The acidity of the carrier stream in the mass spectrometer (pH 3)[13] will protonate the substrate (increasing K_m) and the active site lysine on the enzyme inhibiting imine formation. Furthermore acetate is a competitive inhibitor of dehydroquinase.[12]

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Externally Bound Metal Ion Complexes of Buckminsterfullerene, MC_{60}^+, in the Gas Phase

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Shortly after their discovery of the special stability of the fullerenes,[1] Smalley and co-workers demonstrated that metal–fullerene species could be generated in the gas phase by growing them in a supersonic expansion source following laser desorption from a graphite target impregnated with various metals, M = La, K, and Cs.[2,3] Cox and co-workers made similar observations, but did not consider these to be fullerene derivatives.[4,5] As with the fullerenes themselves, the metallated species were detected mass spectrometrically and their structures probed by multiphoton photodissociation. These complexes, and in particular MC_{60} and its singly charged counterpart, MC_{60}^+, were found to be highly stable, requiring significant laser fluence to cause fragmentation by sequential C_2 loss.[3] 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 $C_{48}Cs^+$. These results led Smalley and co-workers to predict metal-included structures in which the central metal is enclosed in an inert carbon cage. If such species could be synthesized in macroscopic amounts, as demonstrated for C_{60} and C_{70},[6] they could exhibit important chemical and physical properties. Most notably to date is the observation that preformed C_{60} doped with potassium exhibits a superconducting transition,[7] although an externally bound metal–fullerene association has been demonstrated.[8] In this work, we extend our initial study[9] on FeC_{60}^+ to several other metal ions and demonstrate unequivocally that metals added to preformed C_{60} generate externally bound complexes. In addition, externally bound LaC_{60}^+ is observed to exhibit dramatically different properties than its metal-included isomer.

MC_{60}^+ (M = Fe, Co, Ni, Cu, Rh, La, and VO) species were formed in a Nicolet FTMS-2000 Fourier transform mass spectrometer via a multistep sequence[10] initiated by laser desorption to generate M^+ from the pure metal targets.[11] The next step depended on the metal ion, but the majority of the ions including Co^+, Ni^+, Cu^+, Rh^+, and La^+ were then permitted to react directly with a background pressure of C_{60} heated off a solids probe at 350 °C. Using a system temperature of 250 °C, charge transfer to background potassium was found to be a problem, although K^+ was not observed to form KC_{60}^+ under these conditions. Reducing the temperature to 150 °C, however, removed the potassium background and permitted longer trapping periods to make up for the reduced C_{60} pressure. Under these conditions, the metal ions formed MC_{60}^+ by direct attachment together with varying amounts of C_{60}^+ by charge transfer. V^+ and La^+ form very strong bonds with oxygen[12] and rapidly reacted with the background gas ($\sim 2 \times 10^{-7}$ Torr using the solids probe) to form VO^+ and LaO^+. Only VO^+ was observed to directly attach to C_{60} to form the fullerene complex, but conditions probably exist favorable to the formation of $LaOC_{60}^+$. In order to generate LaC_{60}^+, it was necessary to suppress LaO^+ formation. This was accomplished by encasing the external elements of the solids probe with a flexible plastic sheath through which Ar flowed. Finally, Fe^+ reacts with C_{60} predominantly by charge transfer and, thus, a background of *n*-pentane at about 4×10^{-8} Torr was added which yields $Fe(C_nH_{2n})^+$ (n = 2–5) species.[13] These ions undergo ligand exchange reactions with C_{60} to form FeC_{60}^+. A similar procedure could be used on selected metal ions to enhance MC_{60}^+ intensities over that observed by direct attachment.

After a total reaction period, which varied from about 0.3 to 10 s depending upon the system and the conditions, the MC_{60}^+ was isolated by double resonance ejection pulses[14] and subjected to low-energy collision-induced dissociation (CID)[15] using Ar as the target gas at about 2×10^{-6} Torr. The collision energies ranged from 30 to 130 eV, laboratory frame. CID on FeC_{60}^+, CoC_{60}^+, NiC_{60}^+, and CuC_{60}^+ yields C_{60}^+, while LaC_{60}^+ and VOC_{60}^+ yield La^+ and VO^+, respectively, and RhC_{60}^+ yields a mixture of C_{60}^+ and Rh^+, with the Rh^+ predominating. Figure 1 for LaC_{60}^+ illustrates the type of data obtained.

Given the ionization potentials[16] of IP(Fe) = 7.87 eV, IP(Co) = 7.86 eV, IP(Cu) = 7.726 eV, IP(Ni) = 7.635 eV, IP(Rh) =

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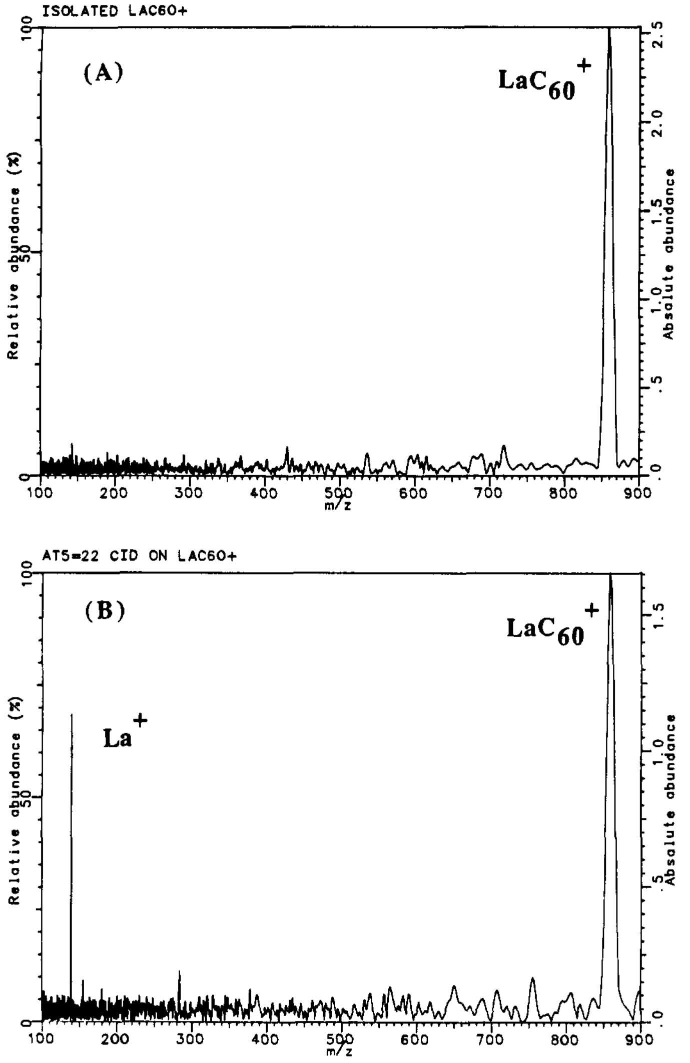

Figure 1. Plot of relative abundance vs *m/z*: (A) isolated LaC_{60}^+; (B) collision-induced dissociation of LaC_{60}^+ (105 eV Laboratory, 4.7 eV center of mass energy) yielding La^+.

7.46 eV, IP(VO) = 7.23 eV (Weisshaar, J. C., private communication) and IP(La) = 5.577 eV, the CID results indicate IP(Fe, Co, Ni, Cu) > IP(C_{60}) ~ IP(Rh) > IP(VO, La), which is in agreement with a recently reported value of IP(C_{60}) = 7.61 ± 0.11 eV.[17] Furthermore, these results provide strong support for internally bound "egg shell" MC_{60}^+ species (particularly for M = La) reported earlier grown in a supersonic expansion, since these latter species are highly stable and lose C_2 molecules when sufficiently activated. In direct contrast, the externally bound isomers, presumably always formed by adding the metal to the preformed C_{60}, require relatively little activation energy to cleave the metal from the complex, leaving an intact C_{60} species. Although the type of activation used in the current study (collisional) and that reported in the previous study (photoexcitation) can yield different fragmentation processes,[18] it is likely that some metal loss would be observed for an externally bound complex regardless of the method of excitation employed.

Studies are underway to synthesize other members of this new family of ions and to characterize their subsequent chemistry with other reagent gases. It should be possible to synthesize virtually any metal ion–C_{60} complex, as well as an endless variety of ligated metal ion–C_{60} complexes, in the gas phase. Determination of the M^+–C_{60} bond energies will also be possible by monitoring ligand-exchange reactions using reference ligands.

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Surface Electrostatics, Reduction Potentials, and the Internal Dielectric Constant of Proteins

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Electrostatic contributions by surface charges are presumed to play important roles in dictating the affinity and selectivity of numerous protein–macromolecule and protein–small molecule interactions. Although numerous modeling and theoretical efforts have attempted to predict the detailed electrostatic fields in and around globular proteins,[1] there have been only a few systematic efforts to experimentally map the electrostatic potential surface of a protein in solution and correlate this with simplified theories.[1h] In this communication we demonstrate that site-directed mutagenesis of surface charges together with high-resolution electrochemical measurements can be used to quantitate the boundary-value electrostatic potentials of soluble proteins.

Prosthetic group reduction potentials[2] and amino acid side chain ionization constants can be affected by surface-charged residues.[3] Here we show that precise measurement of the shift in the reduction potential for a heme prosthetic group upon alteration of surface charge can be used to test the validity of theoretical calculations of macromolecular electrostatic fields and, in a simple two-continuum dielectric model, to estimate the effective bulk internal dielectric constant of a protein. By using amino acid replacements at numerous sites on the surface, we find that a single distance-dependent dielectric will not fit experimental data, but rather the detailed shape of the macromolecule must be taken into account.

We have chosen rat liver cytochrome b_5 for these investigations, since previous studies have shown that the protein is structurally rigid to surface mutations.[4] The heme redox potential was measured by direct electrochemical methods using a cysteine-modified gold working electrode.[5] For these investigations we

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