EVALUATION OF SELECTIVE METAL-ION BINDING TO PHOSPHORYLATED BIOMOLECULES

Robert Hettich, Paul Schnier, and Michelle Buchanan

Chemical and Analytical Sciences Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6365

Metal ions play an important role in biology, and are essential for the structural stability and function of many biological complexes. In most cases, metal-ion binding to biomolecules is remarkably selective for not only the identity of the metal species but also its oxidation state (i.e. Fe^{2+} vs. Fe^{3+}). Development of mass spectrometry technology to exploit this metal ion specificity in gas phase studies of biomolecules should provide a powerful method of obtaining detailed structural information about these important molecules. The specific objective of this report is to describe research focused on the determination of whether iron can be used as a selective probe in the gas phase for phosphorylated biomolecules, in particular oligonucleotides and modified peptides. The presence of the transition metal ion alters the reactivity and fragmentation of the resulting complexes, thereby providing additional structural information about the biomolecule as well as details about the site of metal ion attachment.

Experiments were conducted with a Fourier transform ion cyclotron resonance mass spectrometer equipped with a 6-Tesla superconducting magnet and a nitrogen laser (337 nm radiation). Samples were prepared by mixing a small amount of analyte and aqueous iron salt solution ($FeCl_3$ or $FeSO_4$) with an excess of matrix compound (either 2,5-dihydroxybenzoic acid or 3-hydroxypicolinic acid), and then drying onto a metal disk (either stainless steel or aluminum). CAD experiments were conducted with argon as the target gas.

MALDI-FTICR revealed abundant (M+Fe-xH) ions for small oligonucleotides prepared by the experimental method discussed above. High resolution mass measurement of the $(M+Fe-xH)^-$ ions revealed the presence of both $(M+Fe-4H)^-$ and $(M+Fe-3H)^-$, suggesting that both Fe^{3+} and Fe^{2+} are present in these species. The appearance of iron-matrix adducts such as $(Fe_3DHB_4)^-$ in the mass spectra indicates that the organic acid matrix compound effectively chelates the iron ions, and assists in their transfer to the oligonucleotide. This mechanism is supported by the observation of $(\text{oligonucleotide + iron+DHB})^-$ ions in the mass spectra. The number of iron atoms which can be added to a small oligonucleotide (dimers - decamers) appears to correlate with the number of phosphate groups. For example, Figure 1 illustrates that the tetranucleotide pd(AGCT) can attach up to three iron ions, presumably one to each of the non-deprotonated phosphate groups in the negative ion. The insert in Figure 1 is an expansion of the $(M+2Fe-6H)^-$ ion, revealing the presence of an (Fe^{3+}, Fe^{2+}) species at m/z 1359 and an (Fe^{3+}, Fe^{3+}) species at m/z 1358. In contrast, the related tetranucleotide d(AGCT) only coordinates up to two iron ions, which might be expected since it possesses only two non-deprotonated phosphate moieties in the negative ion. The formation of the (M+Fe-xH) ions appears to be independent of the identity of the iron salt, as $FeCl_3$, $FeSO_4$, and $Fe(NH_4)_2(SO_4)_2$ all function with similar efficiency. This suggests that the pH of the aqueous matrix solution (which changes during the desolvation drying process) controls the identity of the iron cations regardless of identity of the original iron salt. The (M+Fe-xH) ions (either positive or negative) are readily formed with either DHB or HPA matrices, indicating that both of these organic acids can assist in the chelation of ionic iron

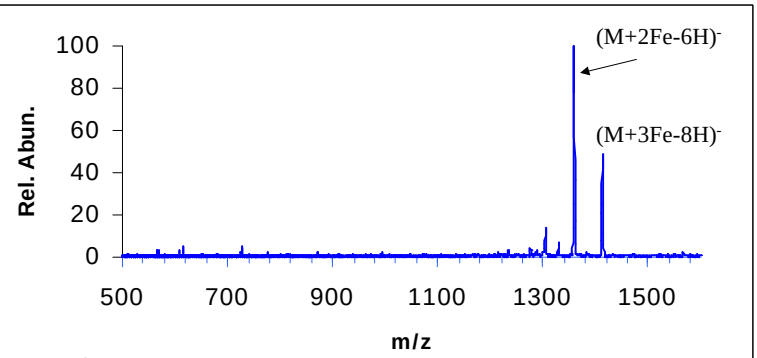

Figure 1

and subsequent transfer to the oligonucleotides.

Collisional dissociation of $(M+Fe-xH)^-$ ions for a series of tetraoligonucleotides was undertaken to examine how the presence of the iron atom would affect the fragmentation, and whether this information could be used to identify the site of metal ion attachment to the biomolecule. CAD of $(M+Fe-3H)^-$ for d(ACGT) revealed abundant fragment ions corresponding to (w_2+Fe) and (w_3+Fe) as well as elimination of neutral thymine. CAD of the $(M+Fe-3H)^-$ ion for the sequence isomer of d(AGCT) also revealed an abundant (w_3+Fe) fragment ion; however, no (w_2+Fe) ion was observed in this case. Rather, fragment ions corresponding to (a_3-B_3+Fe) and (a_3-B_3+Fe+HPO_3) were observed. These results imply that the iron ion appears to favor localization at or near the guanine nucleic base. The (a_3-B_3+Fe+HPO_3) ion is somewhat unexpected, and indicates that the iron species may be able to assist in a phosphate migration concomitant with a deoxynucleoside elimination. Similar results were obtained for the CAD of the $(M+Fe-4H)^-$ for pd(ACGT), which contains a terminal phosphate group. The presence of a strong (w_3+Fe+HPO_3) fragment ion was observed in this case, providing further support that the iron ion can assist in a phosphate migration process.

To expand the study of the selectivity of iron for phosphorylated biomolecules, the experimental procedure discussed above was applied to the examination of phosphorylated peptides. Development of selective probes to determine post-translational modifications such as protein phosphorylation would significantly enhance the rapid characterization of these important compounds. A non-phosphorylated peptide decamer of sequence **gly-lys-ala-lys-val-thr-gly-arg-trp-lys** (Mr = 1130) was examined under MALDI-FTICR conditions ($FeCl_3$ added to sample). No (M+Fe-xH) ions were observed for either positive or negative ions. A phosphorylated peptide decamer of the same sequence, except that the threonine was phosphorylated (M_r = 1210), was then examined under the same conditions. While the positive ion mass spectra was unchanged, the negative ion mass spectra contained an abundant $(M+Fe-3H)^-$ ion, as shown in Figure 2. Higher mass resolution measurements of this ion, shown in Figure 3, reveal that the ion at "nominal" m/z 1264 consists of two components. Evident is a minor amount of $(M+Fe-4H)^-$ at m/z 1263 and a higher abundance of $(M+Fe-3H)^-$ at m/z 1264, suggesting the presence of both Fe^{3+} and Fe^{2+} in this species. A variety of small peptides of different amino acid sequences were examined to probe the generality of the iron-attachment process. No iron-peptide adducts were observed for other non-phosphorylated peptides or sulfonated peptides; however, the presence of a cysteine residue or two adjacent glutamic acid residues in a peptide enhanced the production of iron-peptide complexes. Further work is in progress to examine these species more closely.

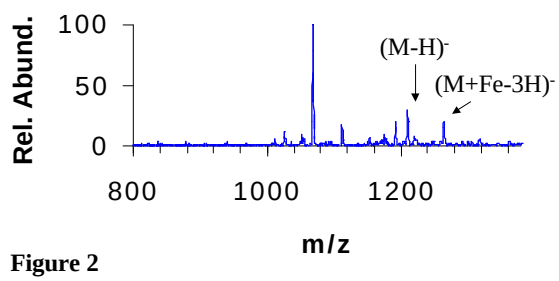

Figure 2

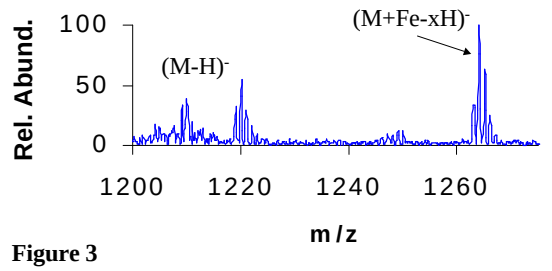

Figure 3

These studies indicate that abundant iron-biomolecule adducts (containing both Fe^{3}+ and Fe^{2+}) can be generated for oligonucleotides and phosphorylated peptides by adding iron salts such as $FeCl_3$ to MALDI matrix. For small oligonucleotides, the number of iron atoms which can be added correlates with the number of phosphate groups of the oligomer. CAD of the iron-oligonucleotide species reveals the strong affinity of the iron atom for the phosphate groups, which is manifest in the fragment ions observed. Future work will be directed

at examination of possible fragmentation differences between the $Fe^{3}+$ and Fe^{2+} adducts of these biomolecules.

Research at the Oak Ridge National Laboratory sponsored by the Office of Biological and Environmental Research, U.S. Department of Energy, under contract No. DE-AC05-96OR22464 with Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp.