Conversion of Methanol to Formaldehyde Following CID of a Gas-Phase Zinc Complex Ion


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1. Introduction

The active site of the metalloenzyme liver alcohol dehydrogenase (LAD) contains a zinc ion coordinated by histidine and cysteine residues and catalyzes the conversion of alcohols to aldehydes. A complete understanding of the function of this and other zinc metalloenzymes requires a detailed picture of the role of geometric and electronic structure in the activity of the metal ion. We are exploring the viability of an approach that uses electrospray ionization to “synthesize” gas-phase zinc complexes ions that mimic the coordination structure and composition of the active sites of zinc metalloenzymes and for which intrinsic chemistry might be studied using ion trap mass spectrometry. As we show here, multiple-stage CID of several monopositive zinc complex cations causes the conversion of coordinated methanol and methoxide ligands to formaldehyde. The formation of formaldehyde is confirmed using extensive isotope labeling.

2. Methods

Mass spectrometry experiments were conducted on a Finnigan LCQ-Decca electrospray ionization, ion-trap mass spectrometer. Complex ions were generated from spray solutions containing zinc nitrate in deionized water and 3.5% methanol. Isotope labeling studies were conducted using commercially available CD3OH and and CH318OH as well as CH3OD which was generated in-situ using a spray solution composed of zinc nitrate and methanol in D2O. The CID parameters were the following: He collision gas, activation amplitudes of ~20-30% (of 5V), qe values of 0.3-0.35 and activation time of 30 msec.

3. Results

Utilization of electrospray ionization generated intense peaks corresponding to complex ions with the formulas, [(ZnNOx)(CH3OH)y] z/m/z 196 (figure 1), [(ZnOCH3)(CH3OH)] z and [(ZnOH)(CH3OH)] z from a solution consisting of zinc nitrate in H2O with 5% methanol. For each complex, multiple collision induced dissociation stages caused the step-wise elimination of methanol ligands to form [(ZnNO3)CH3OH]+ at m/z 161 (figure 1), [(ZnOCH3)CH3OH]+ and [(ZnOH)CH3OH] +. Subsequent CID of (ZnNO3)CH3OH]+ caused the elimination of 47 mass units. The neutral loss shifted by one mass unit at m/z 113 as seen in figure 1 when CD3OH was incorporated into the complex composition, suggesting that the compositions of the neutral and ion fragments were HNO2 and [(ZnOH)CH3O] z, respectively. Subsequent CID of the latter species caused the elimination of 30 mass units. The neutral loss shifted by 2 to 32 giving a m/z of 81 (figure 1) mass units when either CD3OH or CH318OH were incorporated into the complex ion, consistent with the formation and elimination of formaldehyde, CH3O. In particular, the elimination of the 18O label demonstrates the oxidation of the alcohol, rather than a mechanism involving the elimination, in part, of the oxygen atom remaining from the nitrate ligand. Following the elimination of formaldehyde, the final product ion generated corresponded in mass to [ZnOH] z: the mass to charge ratio of the ion shifted by one mass unit to [ZnOD] z when CH3OD was used to generate the initial complex. Use of the multiple forms of deuterium labeled methanol allowed the synthesis of an overall reaction pathway (figure 2) that involves the transfer of a methyl proton to the nitrate group, with subsequent elimination of nitrous acid. Generation of formaldehyde then continues by transfer of a hydrogen atom from the –OH group to the zinc-oxygen cation. Elimination of formaldehyde was also observed for CID of [(ZnOCH3)(CH3OH)] z, which ended in formation of [(ZnH)CH3OH] z. The elimination of 30 shifted to 32 for complexes containing CD3OH, and the final product ion generated was [ZnD(CD3OH)] z. For [(ZnOH)(CH3OH)] z, elimination of water competed with the elimination...
of methanol at low CID stages. CID of [(ZnOH)CH\textsubscript{3}OH]\textsuperscript{+} caused formation of two major final product ions: (a) [(ZnH)H\textsubscript{2}O]\textsuperscript{+} via the elimination of formaldehyde and (b) [ZnOCH\textsubscript{3}]\textsuperscript{+} via the elimination of water. Computational studies using Gaussian are being conducted to test the overall stability of the structures of our proposed mechanism shown in figure 2. Preliminary data indicates stable optimizations of several transition state species while others are stable as just reactants and products. To the best of our knowledge, these experiments are the first demonstration of gas-phase chemistry within a zinc complex that mimics the behavior of the LAD active site.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Proposed fragmentation mechanisms for both the zinc nitrate and zinc hydroxide complexes with deuterium labeled methanol. After the loss of all but one methanol each species goes through rearrangement to yield formaldehyde and the final product ion.}
\end{figure}

4. Conclusions

Isotope labeling experiments coupled with ESI-MS\textsuperscript{n} provided the data necessary to come to a plausible mechanism for the conversion of methanol to formaldehyde via two possible pathways as indicated above in figure 2. Computational studies of transition state species and their relative energies will provide further insight into the more energetically favorable pathway as well as probable transition states.

5. References


