Applications of Gas-Phase Ion Chemistry: Protein Identification in Pathogenic Microorganisms

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Mass spectrometry coupled with separations is a powerful approach for the identification of proteins in organisms. Traditionally, large numbers of proteins are separated using two-dimensional gel electrophoresis. Although large numbers of proteins can be separated with high resolution, the identification of any individual spot requires in-gel proteolytic digestion and tandem mass spectrometry analysis to generate a protein sequence tag which can be searched against a database for rapid identification. The major disadvantage of this approach is the time and effort required to perform two-dimensional gel electrophoresis, and proteolytic digestion on the protein samples/spots. Recently, we have developed ion/ion proton transfer chemistry in the quadrupole ion trap for separation of complex protein mixtures. This technique utilizes proton transfer reactions to reduce the charge states generated by electrospray ionization, thereby decompressing the mass scale and separating a complex mixture directly in the mass analyzer without the use of chromatographic equipment. The time scale for the ion/ion reaction separation is on the order of hundreds of milliseconds, as compared to two days for most two-dimensional gel electrophoresis separations. In addition, we have demonstrated that ion/ion proton transfer reactions are an effective means for reducing the charge states of product ions derived from intact proteins. This extends the utility of the ion dissociation process to highly charged, high mass species where multiple charging can lead to a richer array of structurally informative product ions, thus simplifying the spectral interpretation process by reducing the product ion charge states to predominantly the +1 charge state.

In figure 1, is shown the post ion/ion reaction spectrum from the isolated +11 charge state from the coat protein of bacteriophage MS2. By mass isolating the individual charge state and reacting to +1, a significant increase in signal-to-noise ratio is observed as compared to ion/ion reactions for the entire charge state distribution. However, even when performing mass isolation with subsequent ion/ion reaction a small amount of chemical noise is still present. In the bottom half of figure 1 is shown the post ion/ion reaction spectrum from the MS/MS of the +11 charge state of the coat protein. High mass singly-charged b and y ions have been effectively separated from their +2 counterparts in the m/z 6000 range (high chemical noise region). By repeating the above mentioned experiment on different charge states, sufficient protein sequence information can be obtained to generate a sequence tag for protein database searches. By adjusting the ESI interface conditions to transmit a broader range of m/z values and subjecting the ion population to charge state reduction, evidence for the bacteriophage assembly protein (protein a) can be seen in figure 2. This protein is present at 1 copy per viral particle, and should have a concentration a factor of 180 lower than the viral coat protein.

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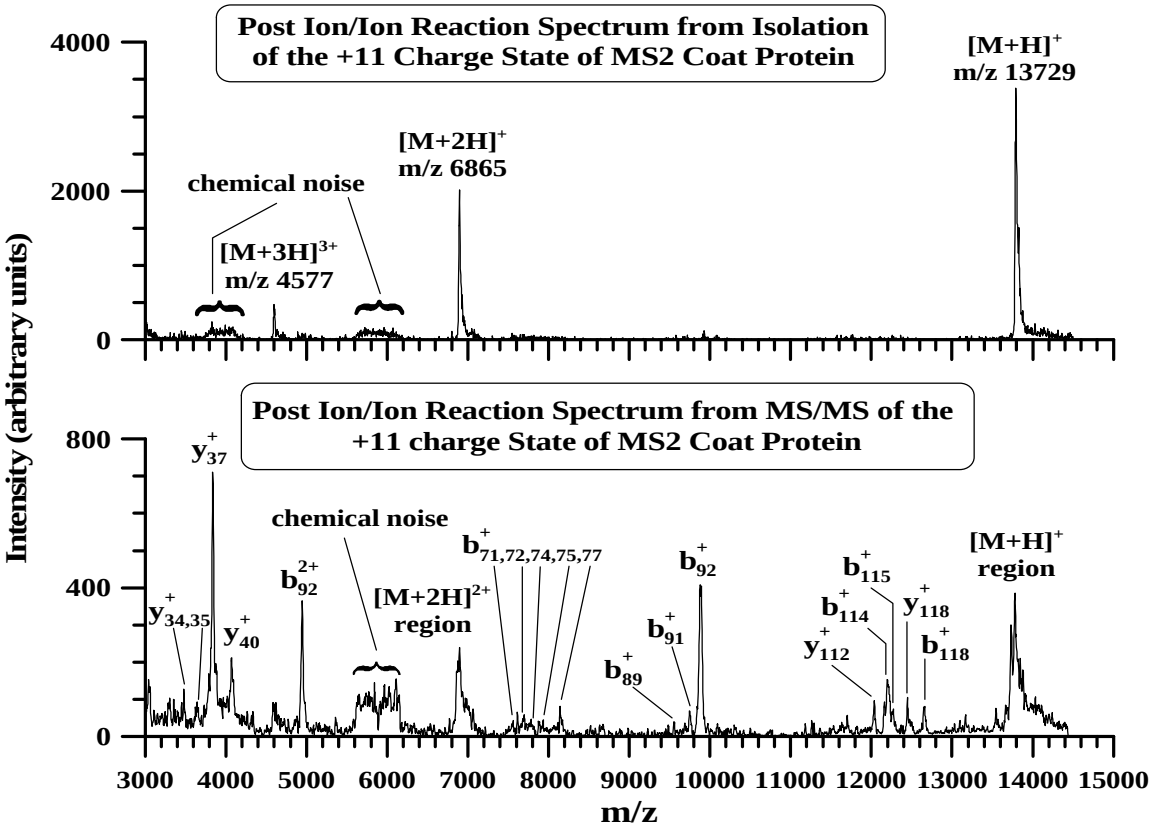

Figure 1. Ion/Ion reaction of isolated +11 charge state of bacteriophage MS2 coat protein, with subsequent post ion/ion MS/MS data of the same charge state.

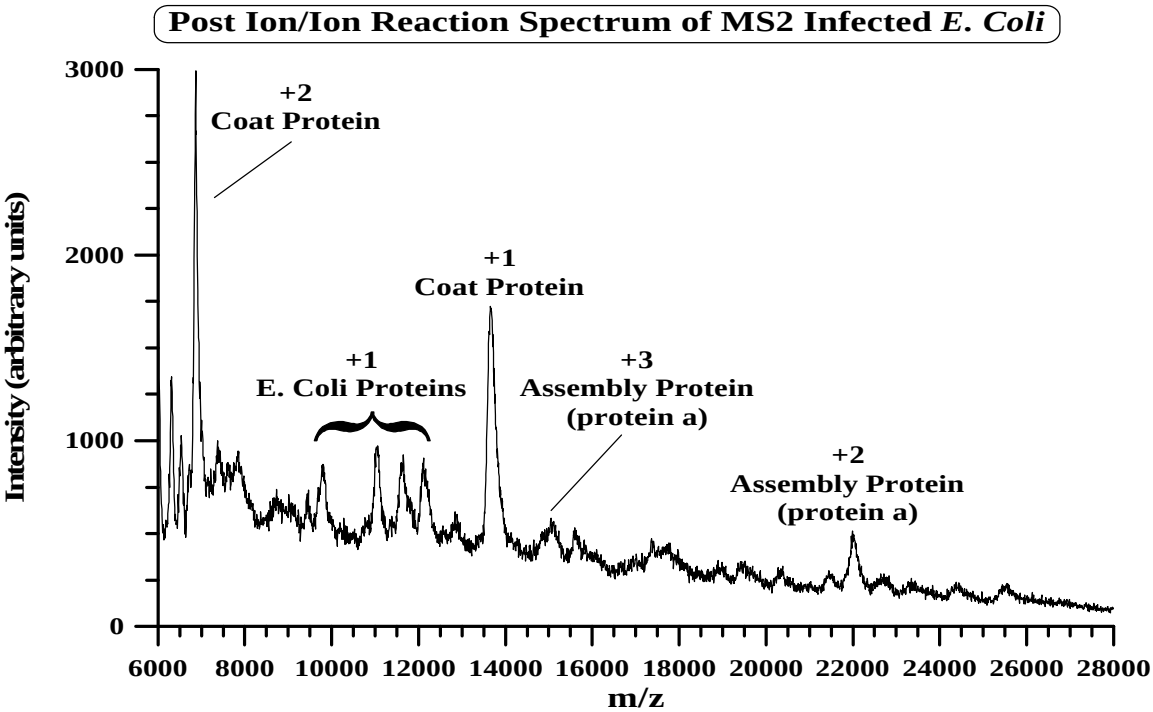

Figure 2. Ion/Ion reaction of MS2 infected E. coli. showing both the coat protein and the assembly protein (protein A) present at one copy per viral particle.