Probing the Gas Phase Folding Kinetics of Peptide Ions by IR Activated DR-ECD

Cheng Lin; Jason J. Cournoyer; Peter B. O'Connor

Boston University School of Medicine, Boston, MA 02118

Introduction

Electron capture dissociation (ECD) of protein ions sometimes produces poor sequence coverage due to tertiary structures that prevent fragment ion separation. These higher order structures result from gas phase folding, and could be broken down by ion activation.[1] The folding kinetics is often studied using IR-delay-ECD approach,[2] but only if the IR irradiation results in noticeable fragment ion yield increases. In smaller peptide ion ECD, the tertiary non-covalent interactions are so weak that all fragment ions are readily detected without ion activation. Double resonance (DR)-ECD can reduce the product ion yield by ejecting the fragment ion complexes before ion separation.[3] In combination with IR irradiation, DR-ECD provides a way to probe folding kinetics of peptide ions with weak intra-molecular interactions.

Methods

Experiments were carried out on a custom-built qQq-Fourier-transform ion-cyclotron-resonance (FT-ICR) mass spectrometer (MS) with an electrospray ionization source.[4] Ions of interest were isolated and accumulated in the front end quadrupole before entering the cell, where they were subjected to low energy electron irradiation from an on-axis indirectly heated dispenser cathode. Double resonance experiments were performed by resonantly ejecting the charge reduced molecular ions during the electron irradiation period. IR laser beam (10.6 μm) was introduced off-axis towards a mirror mounted on the cell end trapping plate, and the angle of the mirror was such that the reflected beam passed through the center of the cell. The IR laser intensity was adjusted to just below the dissociation threshold for maximum ion activation without fragmentation.

Results

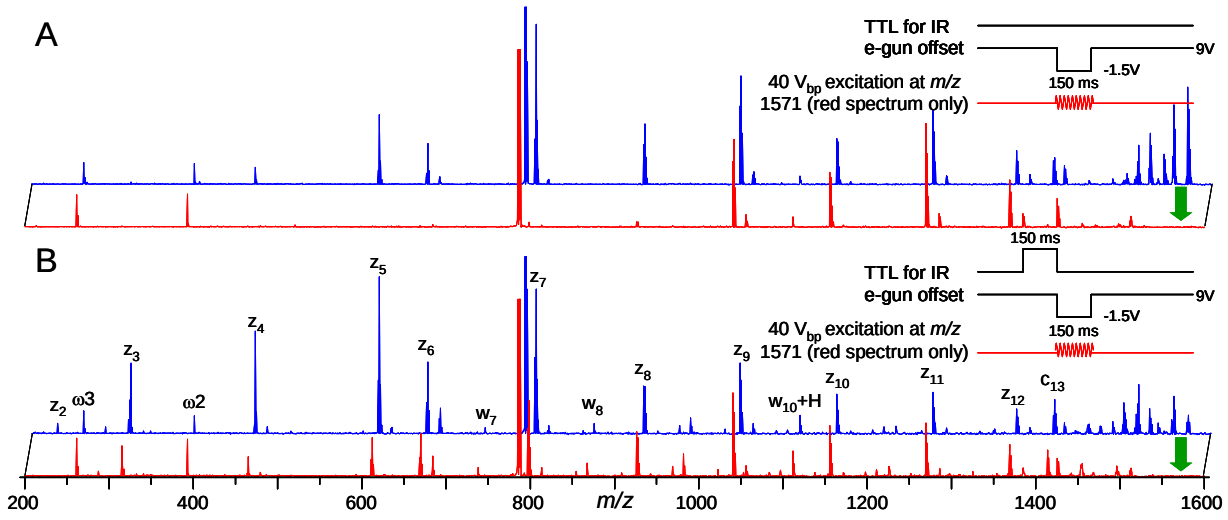

Figure 1. ECD (blue) and DR-ECD (red) spectra of fibrinopeptide B without (A) and with (B) IR irradiation before ECD event.

Fibrinopeptide B (EGVNDNEEGFFSAR) was chosen as the model peptide to study the effect of IR heating on peptide ECD, as well as the gas phase folding kinetics of peptide ions. Figure 1 shows its ECD spectra in blue, both without IR (top) and with 150 ms IR irradiation immediately before the ECD event (bottom); the corresponding DR-ECD spectra are shown in red. The IR-ECD spectrum contains many w-ions, which can be used to differentiate leucine and isoleucine residues in *de novo* sequencing.[5] There are also numerous internal fragments present in the IR-ECD spectrum, likely secondary fragments

produced by free radical initiated reactions, made possible with the excess energy provided by IR light to overcome the barrier, supporting the free radical cascade mechanism.[6] Interestingly, IR irradiation after ECD does not produce these fragment ions (spectrum similar to that in Figure 1A, not shown), possibly due to radical stabilization via radical rearrangement before IR heating.

The IR-ECD spectrum also shows very different z-ion distribution, which shifts towards lower mass, with z_2 ion appearing, and z_3-z_5 population increasing dramatically. The increased small z-ion yield upon IR irradiation cannot be solely attributed to faster fragment ion separation when heated, as post-ECD IR irradiation has little effect on z-ion yield. Rather, the improved sequence coverage is the result of the increased conformational heterogeneity of the peptide ion by IR heating. This correlates well with the low-temperature ECD studies, which displayed highly selective dissociations.[7]

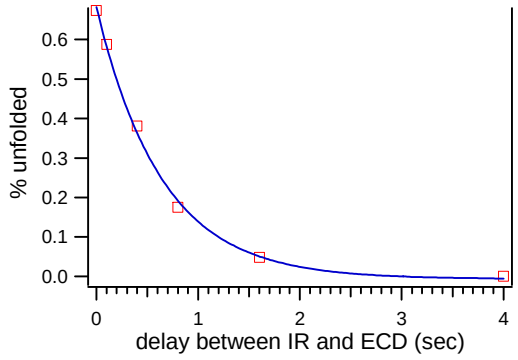

Figure 2. Percentage of the peptide ions that remain unfolded at a specific delay after IR, as calculated from z_7 ion abundance in DR-ECD.

Many fragment ions (e.g. z_3 to z_8) show appreciable drop in abundance in DR-ECD, suggesting that they are formed via long-lived intermediates, most likely c/z ion pair complexes. IR irradiation prior to ECD unfolds the precursor ions, breaking the hydrogen bonds, facilitating fragment ion separation, leading to greater fragment ion yields in DR-ECD. However, as the delay between the IR pulse and the DR-ECD event increases, these fragment ion yields will decrease, due to gas phase refolding of the precursor ions. At any given delay, from the abundance of the fragment ion detected, one can in principle calculate the percentage of the peptide ions that remain unfolded. Plotting this percentage against the delay between IR and ECD, the refolding kinetics constant can be obtained. Figure 2 shows one of such plots, using z_7 ion abundance to calculate the unfolded percentage. The fit gives a first-order reaction constant of 1.6 s^{-1}, corresponding to a half-second refolding time, much faster than what it takes for a protein ion to refold, which is typically several minutes.[2]

Finally, it is worth mentioning that fibrinopeptide B ECD produces both z and $z^{\bullet}$ ions, with the former being the product of intra-complex $H^{\bullet}$ transfer. Thus the changing $z^{\bullet}$/z ratio upon varying delay between IR and ECD can also be used to gauge the extent of refolding without resorting to the DR-ECD approach. Such convenience is not always afforded, as some peptide ions do not produce appreciable $c^{\bullet}$ or z ions in ECD.

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