Leveraging ion-ion and ion-photon reactions to improve the sequencing of proteins carrying multiple disulfide bonds: the human albumin case study

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ABSTRACT

Two-phase sequencing of large target proteins (e.g., 100 kDa) is an intensely challenging process, complicated by the presence of multiple charged protein ions which are prone to fragmentation and require isolation before detection. ETD is considered a standard technique to excise and fragment peptides, often achieving near-complete fragmentation of the target. A problem that limits ETD’s utility is the charge-reduced nature of many ETD fragments, which reduces the sequence coverage of naturally disulfide-bridged proteins. Here, we present the first study of ion-ion reactions between intact and fragmented recombinant human albumin (HSA) and its disulfide-bridged fragments using EThcD and protons. The results indicate that ion-ion reactions can increase the sequence coverage of these proteins.

INTRODUCTION

Mass spectrometry (MS) is the most powerful tool in chemical biology. In a single-shot experiment, MS can detect a complex mixture of thousands or even millions of species and their isobaric variants, as well as detect and characterize the chemical bond breaks that occur during fragmentation reactions that happen upon their collision with a high-energy electron (electron transfer dissociation—ETD) or laser (electrospray ionization—ESI). Such fragmentation methods not only break bonds such as covalent, hydrophobic, hydrogen, and disulfide bonds but also break the sites of post-translational modifications on proteins, allowing the detection of any binding interaction.

Mass spectrometry’s capability is also greatly enhanced by the ability to fragment and re-assemble ions, an approach known as ion-ion reactions via MS/MS. While collision-induced dissociation (CID) is one common example of ion-ion reactions, recently, there has been a surge in ion-ion reactions that use other excitation modes such as ETD and electron capture dissociation (ECD), some of which use photons.

Several groups have previously demonstrated that ion-ion reactions can improve the sequencing of proteins carrying multiple disulfide bonds. For example, Yan et al. showed that ion-ion reactions with ECD could improve the sequencing of disulfide-bridged proteins, but only when the ion-ion reactions were carried out at a lower energy. However, the sequencing enhancement of ion-ion reactions has not been demonstrated for proteins carrying multiple disulfide bonds.

Here, we present the first study of ion-ion reactions between intact and fragmented recombinant human albumin (HSA) and its disulfide-bridged fragments using EThcD and protons. The results indicate that ion-ion reactions can increase the sequence coverage of these proteins.

MATERIALS AND METHODS

Sample Preparation: Recombinant human albumin was purchased from Abnova Biotechnology and used without further purification. ECD and EThcD were employed as the fragmentation modes.

RESULTS

Figure 1: Experimental setup for EThcD and EThcD fragmentation spectra.

Figure 2: Comparison of ETD on HSA before and after EThcD fragmentation of disulfide bridges. A single charge state of HSA was isolated for EThcD. Additional experiments were performed on the 17 charge state of HSA in order to confirm the results seen in Figure 1. The fragmentation of HSA revealed that the intact protein was more fragmented than the fragmented protein.

Figure 3: Comparison of ETD of HSA before and after EThcD fragmentation of disulfide bridges. A single charge state of HSA was isolated for EThcD. Additional experiments were performed on the 17 charge state of HSA in order to confirm the results seen in Figure 1. The fragmentation of HSA revealed that the intact protein was more fragmented than the fragmented protein.

CONCLUSIONS

Our results demonstrate that ETD and the related EThcD can significantly improve the sequencing of disulfide-bridged proteins. The results from this study demonstrate that ion-ion reactions can be used to improve the sequencing of proteins carrying multiple disulfide bonds. Future work should focus on optimizing the ion-ion reactions for proteins carrying multiple disulfide bonds.

REFERENCES

