Disulfide bonds are essential for stabilizing the tertiary structure of many proteins, but the development of unambiguous methods for locating them is an ongoing challenge. Electron-based fragmentation methods cleave disulfide bonds preferentially, but can be challenging to achieve. We here demonstrate electron capture dissociation (ECD) resulting in disulfide bond cleavage and extensive backbone fragmentation in a commonly available quadrupole time-of-flight instrument. ECD fragmentation by this method can be usefully carried out at a millisecond time scale, making it compatible with LC-MS methods.

An Agilent 6545XT upgraded to include ECD capability was used for these experiments. An e-MSion ExD cell is installed between the quadrupole and a shortened collision cell of the Q-TOF instrument. The ExD cell uses a combination of electric and magnetic fields to trap low-energy electrons in the path of ions selected by the quadrupole. Electrons are produced within the cell by a rhenium filament.

Intact proteins were introduced by infusion or via the Agilent LC system. A parent ion was selected by the quadrupole using a targeted acquisition method and fragmented in the ExD cell. Data was processed using MASH Explorer® or LCMS Spectator® for fragment identification.

ECD of Apamin

The bee-venom peptide apamin contains 18 amino acids, two disulfide bonds, and three hydrogen bonds. In spite of its compact structure, using the ExD cell resulted of ECD cleavage of 15 of 16 (94%) of ECD- cleavable bonds.

ECD of Bovine Insulin

Insulin is composed of two polypeptide chains a and b, bridged by two disulfide bonds. In addition, there is an intramolecular disulfide in chain a. The mass of intact insulin confirms that it is fully oxidized. The +5 or +6 charge state of insulin was selected and subjected to ECD.

To calculate possible cleavages, dehydro-cysteine was used to model homolytic cleavage of the disulfide bond, and the mass of the uncleaved peptide was added to cysteine to model an intact disulfide. Combining results, cleavage was observed in 27 of a possible 29 bonds (93%) for chain b, and 14 of 20 bonds (70%) for chain a. c-type (blue) and z-type (red) ions are abundant and most intense, but complementary ion types are also observed.

Conflicts of Interest and Acknowledgements

JSR, VGV, and YVV are cofounders of e-MSion, Inc., which uses technology developed and licensed from Oregon State University. For employees of Oregon State University, a potential conflict of interest exists. This work was supported by the NIH (GM122131-02 and GM123855).

References


More references and information are available at https://e-msion.com/