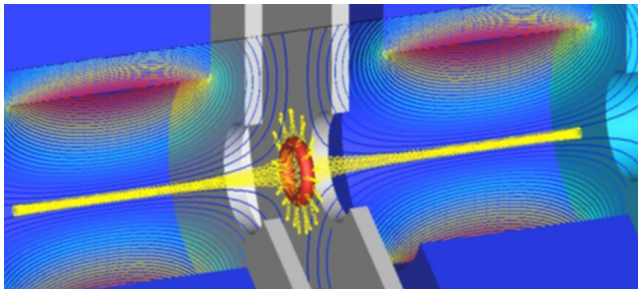
MSVision

Dedicated to Mass Spectrometry

èMSION

ECD for the Masses

Electron-Capture Dissociation - General Information



How ECD can help you?

Electron dissociation techniques such as electron-capture dissociation (ECD) and electron-transfer dissociation (ETD) are considered low-energy fragmentation techniques and therefore offer the ability to dissociate molecules in a manner that allows for the retention of more labile bonds. It is particularly useful when investigating larger peptides and proteins as the induced fragmentation gives better coverage than higher energy techniques such as collision-induced dissociation (CID) and has also been shown to retain post translational modifications (PTMs) allowing for a significantly improved understanding of the protein structure. Until recently, ECD was restricted first to FTMS instruments and then some newer non-FT based mass spectrometers.

Enhanced structural information

For proteins and peptides, ECD has the ability to indiscriminately cleave the backbone N-C_α bond, while leaving the more labile side-chain modifications intact. Combining ECD and CID spectra can potentially provide more complete data for de novo sequencing.

Because post translational modifications such as phosphorylation, carboxylation, glycosylation, and sulfation are less easily lost in ECD than in CID, ECD assignments of their sequence positions are far more specific. This is particularly useful when studying protein interactions or conducting structural biology experiments. Combining this with ion mobility adds still more potentially useful information.

„In summary, the ECD technique is set to become a central method in structural biochemistry, and its potential as a tool for biomolecular analysis is only just beginning to be realized."

Helen Cooper, Kristina Håkansson , Alan G. Marshall, Mass Spectrometry Reviews, 2005, 24, 201– 222

When aiming for structural information by H/D exchange, fragmentation by ECD – in contrast to CID – gives significantly less rise to hydrogen scrambling. This makes the analysis of the obtained data much simpler. Furthermore, ECD is able to efficiently fragment larger peptides than CID, thereby giving access to areas with only a few enzymatic cleavage sites, such as e.g. transmembrane domains, for H/D exchange analysis.

What does it give me that I can't already get?

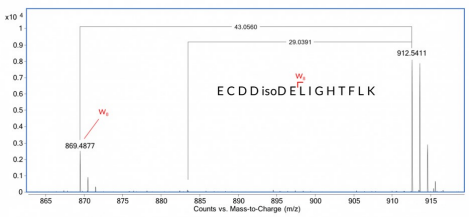
The ExD cell produces ExD fragmentation (covering ECD, EID and EIEIO) similar to FTICR-ECD, but on workhorse mass spectrometers like QTOFs and QExactives. The ability to perform simple electron-based fragmentation enables valuable new types of data and time-saving workflows. ECD produces mainly c- and z-type ions, and as a result the spectra produced are less congested thus making interpretation and top down sequencing simpler. The localized activation allows for the retention of PTMs and thereby enables the identification of the site of PTMs such as phosphorylation and glycosylation etc. especially when combined with native MS.

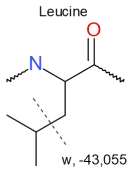
All of this can also be achieved in combination with HPLC, CE or IMS thus making the technique compatible with your standard workflows.

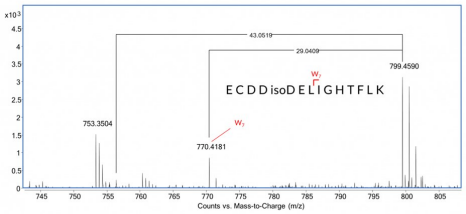
While ECD/ExD is similar to ETD, there are also some relevant differences. The three most relevant are that ExD, in comparison to ETD, can be tuned in the fragmentation energy whereas in ETD that is defined by the ETD reagent. As a consequence, ETD is of limited use for lipid or glycan analysis. The second difference is that ECD/ExD does not require any reagent at all, which also avoids issues with instrument contaminations or more frequent cleanings. The third important difference is the fact that ExD, unlike ETD, produces d– and w-type sidechain fragmentation in peptides and intraring cleavages in glycans. These ions are required for detailed structural analysis such as glycan connectivity analysis or the differentiation of Leucine and Isoleucine.

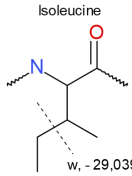
Leucine/Isoleucine Differentiation

Due to the generation of specific side chain fragments, ECD is - contrary to CID - able to differentiate between Leucine and Isoleucine. This allows for unequivocal identification of the amino acid as Isoleucine, which loses 29 Da to form a w-ion, or Leucine, which loses 43 when forming the w-ion.



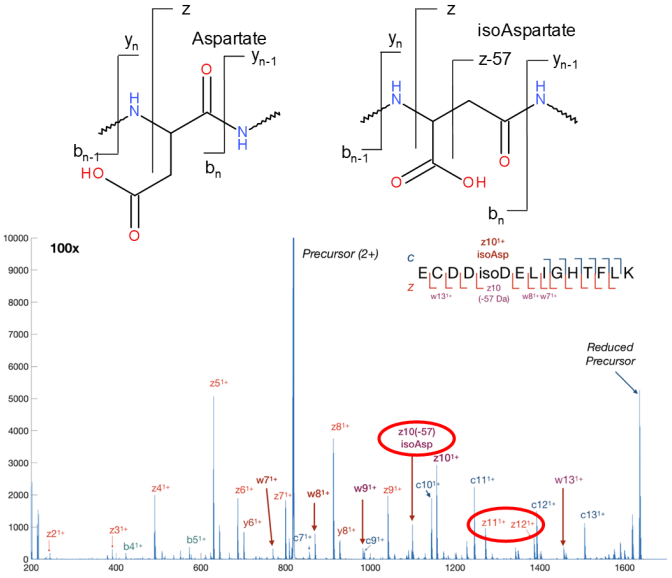






Asp/isoAsp differentiation

A common problem in biopharmaceutical analysis is protein aging. A frequent observation in this case is the deamidation of Asparagine residues resulting in a mixture of Aspartic acid and isoAspartic acid. As these two residues are identical in mass and share the same bonds with their neighbouring residues, they cannot be differentiated by CID. ECD enables you to directly identify the presence of isoAspartic acid by a characteristic z-57 fragment. In most cases this overcomes the time consuming need to generate reference peptide for analysis by LC-MS against the reference standards.



Glossary

As it can be somewhat confusing to talk about electronic activation techniologies, here is a summary of the various terms:

ECD — electron-capture dissociation; lower energy
EID — electron-impact dissociation; higher energy
EIEIO — electron-impact excitation of ions from organics; similar to EID, higher energy
ExD — used to summarize ECD, EID and EIEIO
ETD — electron-transfer dissociation; chemically driven!
CID — collision-induces dissociation