Learn about the nuances of peptide mapping within protein characterization from our Phenomenex biologics guru

Guest Author: M. Christina Malinao

Peptide mapping is a technique in protein characterization that reveals detailed information about the protein structure, including primary structure and post translational modifications. Often called “bottom up” characterization, this technique is driven by the enzymatic digestion of a protein of interest.

The enzyme utilized can vary depending on the overall goal, but the most common endopeptidase is trypsin. Cleavage by trypsin typically provides peptides of good size that contains +2 or +3 charge states, with the ability to accept a proton upon introduction to the mass spectrometer. These characteristics are observed because trypsin cleaves at the lysine and arginine residues in a sequence (except when they are followed by a proline). These are basic amino acids, so they can easily accept a proton in positive ion mode.

These amino acids are spaced along the amino acid backbone where they produce nicely sized peptide chains. Another commonly used digestion enzyme is Glu-C, a serine protease. This enzyme cleaves after aspartic acid and glutamic acid residues. These residues are also basic amino acids, but Glu-C is usually only used to when an orthogonal cleavage is desired.

Trypsin digestion can be a lengthy process. It involves an optional denaturation with a chaotrope coupled with reduction using dithiothreitol. The denaturation aids in disrupting the secondary and tertiary structures of the intact protein. The reduction clips the disulfide bonds, further linearizing the protein. Following these steps, an alkylation reagent is added cap the free thiols so that disulfide bonds are not reformed.

If a denaturing agent is used, buffer exchange to remove it is recommended. The amount of time that a digestion takes to complete varies widely, between 1 hour to overnight (approximately 17 hours). Usually, because of the steps preceding the addition of enzyme, a common method allows the digestion to occur overnight. When sequence coverage is the goal, a shorter incubation time can be beneficial because of minor missed cleavages. However, when checking the sequence coverage, it is always important to check the MS/MS fragmentation to confirm all the peptides that are identified are valid hits.

Whether or not to use a chaotrope while doing an enzymatic digestion is a hot topic. When using a chaotrope, it is important to understand the characteristics of the one that is used. For example, urea, a denaturing agent, cannot be brought to higher temperatures because it will introduce carbamylation adducts on the peptides. These adducts must be included in the search to correctly identify the peptides contained in the digest.

Guanidine is another denaturing agent that works better at higher temperatures without having adduct formation. However, trypsin is not active in higher concentrations of guanidine, so a buffer exchange is needed to purify it out. A chaotrope is needed for a reproducible peptide map. When a chaotrope isn't used, the seconodary interactions within a protein will cause an enzyme to miss cleavage sites, which can lead to poor sequence coverage. Because of the variables introduced when using a denaturing agent, method optimization must be done to determine whether a chaotrope is effective in the workflow of each lab.

Ammonium bicarbonate is an ideal buffer for trypsin digestion because it is volatile. When the dry-down process is initiated, after digestion is complete, the buffer will evaporate quickly, and will not leave residue behind. Another way to ensure a clean sample after

digestion and before the dry-down, is using some type of clean-up device, whether it be zip tips, SPE, or a spin column. However, the loss of the sample that occurs when using these devices mitigates the benefit of the cleanup. To minimize sample loss, on column desalting is recommended.

The importance of using MS-grade chemicals is paramount because lower grade chemicals can have impurities in them. Mass spectrometers are very sensitive, which means they can and will pick up these impurities, and it will further convolute the data in question. This high purity requirement also applies to glassware, pipettes, and even auto sampler vials. Sometimes, when a specific molecule is observed, +H, +Na and +K versions are seen. These salt additions can come from anywhere, so it's always in good practice to make sure to use the cleanest tools possible.

As you can see, peptide mapping is a required characterization tool with many intricacies that must be controlled in all protein analyses. There is no "ideal" method and experts in different labs will likely be adamant that their particular procedure is the "best" one. Whatever the debate, one thing is certain, ensuring consistency in your method is essential so that any variation in the data can be examined appropriately.

For future reference, turn to this condensed poster of Tips from our Protein Separation ZenMasters: Peptide Mapping

To learn more about peptide mapping, reach out to one of our Technical Experts through chat, nearly 24/7 around the world.

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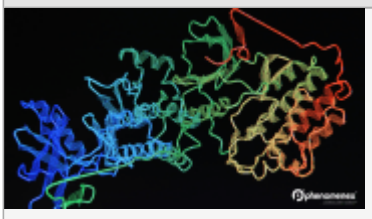
Summary



Article Name

What is the Best Method for Bottom Up Protein Characterization?

Description

Learn about the nuances of peptide mapping and protein characterization from our Phenomenex biologics guru who answers all your questions.

Author

M. Christina Malinao