ProDM™ – A kit for tryptic digestion monitoring for a successful shotgun proteomics

Successful shotgun proteomics depends on optimal digestion of proteins into peptides prior to LC/MS/MS. Current methods to determine the status of protein digestion are time consuming and/or require expensive reagents and equipment. We describe a method for determining the extent of protein digestion using ProDM™ kit and a basic spectrophotometer. Determining whether the protein is digested or not prior to LC/MS/MS will avoid wasting instrument time, samples and money.

Proteomics has advanced rapidly over the past decade. Depth of sampling, instrument sensitivity and scan speed have tremendously quadrupled, and is no longer major bottlenecks for most standard proteomic analysis[1]. However, sample preparation and validation of critical steps prior to mass spectrometric analysis of protein samples have not advanced equally.

An important objective in shotgun proteomics is the identification of as many individual proteins as possible in a single run. It is generally accepted that the more the number of proteins identified in a proteomic experiment the more the likelihood of finding meaningful proteins. One of the critical steps in shotgun proteomics is digesting the proteins into corresponding peptide fragments using a protease like trypsin. The number of unique proteins identified is primarily limited by the quality and completeness of the proteolytic digestion[2]. Sub-optimal digestion of proteins prior to LC-MS/MS negatively impacts shotgun proteomics by reducing the depth of sampling and overall protein sequence coverage. Considering that several factors including type of protein, complexity of protein mixture, presence of surfactants^3 and impurities can affect efficiency of tryptic digestion, verifying the quality and completeness of protein digestion prior to the LC-MS/MS step of shotgun proteomics will prevent wasting instrument/personnel time and samples.

Kutralanathan Renganathan[1], Stephen Russell[1], Steven Wolfe[1], Florentina Mayko[1], Stella B. Somiari[2], Richard I. Somiari[1]

[1]ITSI-Biosciences, Johnstown, PA, USA; [2]Windber Research Institute, Windber, PA, USA. *Correspondence: Dr Richard Somiari. Email: Richard@itsibio.com.

Until now, the way to determine if the protein has been digested by trypsin or test the completeness of digestion is to analyze the digest by SDS-PAGE, HPLC or mass spectrometry. These approaches are time/sample consuming and costly. There have been efforts for real-time monitoring of trypsin progress but they involve utilizing natural fluorophores e.g. Epicocconone[4] and costly fluorescence spectrophotometers.

ITSI-Biosciences (www.itsibio.com) has introduced a product for cost effective end-point monitoring of trypsin digestion. This unique product “Protein Digestion Monitoring Kit (ProDM™ – patent pending)” allows researchers to precisely determine the degree of protein digestion using a colorimetric reagent and any spectrophotometer that can read absorbance at 585nm. In this Application Note in-solution tryptic digestion of Bovine Serum Albumin (BSA) was monitored with ProDM kit and SDS-PAGE over a 24hr period to demonstrate the convenience and sensitivity of the approach.

In-solution Tryptic Digestion

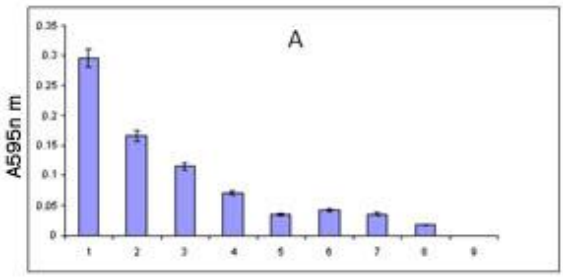
To demonstrate the usefulness and sensitivity of ProDM, we digested BSA over a 24h period and followed the extent of digestion with ProDM kit and SDS-PAGE. The ProDM kit contains Standard Buffer (Urea-Tris Buffer pH 8.5), Reaction Buffer (Tris-Buffer pH 8.5), Reaction Quencher (Buffered Phosphoric acid) and Colorimetric Reagent (modified ToPA reagent). For this study, 120ul of BSA solution (250ug) prepared with Standard Buffer was transferred to a microfuge tube and 0.36ul of 1M DTT was added. The tube was incubated for 20 minutes at room temperature to reduce the protein. Then 2.64ul of 500mM Iodoacetamide was added and the tube incubated

in the dark for 15 min at room temperature. The reduced BSA solution was diluted 8x with 840ul of Reaction Buffer. Then 25ul of trypsin solution containing 5ug of trypsin was added and the tube inverted several times to mix prior to incubation.

Tryptic Digestion Monitoring

BSA digestion by trypsin was monitored with ProDM using the manufacturer's protocol, and by SDS-PAGE using a 4%-20% gradient gel. Briefly, the reaction mixture was incubated at 37^{o}C and 20ul aliquots were removed from the master reaction mixture at zero, 0.5h, 1h, 1.5h, 2.0h, 2.5h, 3.0h, and 6h. A similar mixture was incubated at ambient room temperature and sampled after 24h. At the end of each incubation period, trypsin activity was immediately stopped by the addition of 2ul of the Reaction Quencher, 10ul of the reaction was removed and added to 10ul of 2x SDS-PAGE buffer and flash frozen for electrophoresis.

Figure 1 shows the ProDM (A) and SDS-PAGE (B) data obtained following the digestion of BSA with trypsin. As the results indicate the ProDM offers results that are indicative of the trypsin digestion progress but also supersedes the SDS-PAGE by demonstrating the presence of significant amount of undigested BSA, which is not obvious in the SDS-PAGE gel. For example, the SDS-PAGE gel image does not show any BSA band after 30min incubation (lane 2, Figure 1B) whereas the ProDM data indicates that only about 45% of the BSA has been digested after 30min incubation at 37oC (lane 2, Figure 1A). Because the actual assay can be completed in 10 min and it requires only 2ul-3ul of the digested sample mixture, ProDM proves to be the most cost and time effective solution for end-point monitoring trypsin digestion prior to LC-MS/MS.



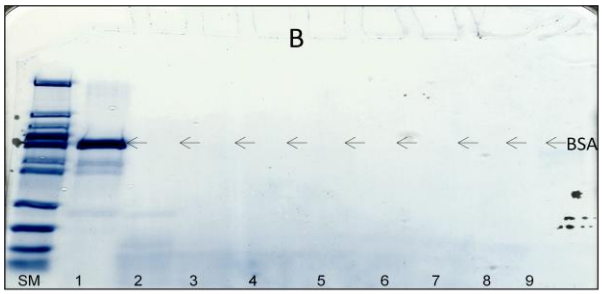


Figure 1: Trypsin digestion monitoring of BSA with ProDM (A) and SDS-PAGE (B). The numbers 1-9 represent samples analyzed at 0, 0.5, 1, 1.5, 2, 2.5, 3, 6 and 24h. The bars in Figure 1A represent 5% error. SM (Figure 1B) is size marker.

ProDM™ utilizes ready-to-use reagents, simple colorimetric reagent and UV spectrophotometer to rapidly monitor trypsin digestion. The kit is also suitable for monitoring any protease digestion, including digestion with chymotrypsin. The assay needs only about 3 microliters of the digestion mixture. It is useful for purified and complex protein mixtures, including plasma, serum or cell lysates. It has the added advantage to offer the ability to determine precise percentage (%) of proteins digested in the sample using a simple equation programmed in Microsoft Excel.
This kit will help generate better shotgun proteomics results by precisely determining the completeness of digestion, or determine when to stop the enzymatic digestion of proteins when partial digestions are needed. ProDM a) is more sensitive than SDS-PAGE b) can be completed in 10 min compared to the approximately 2 hours required for SDS-PAGE and c) cost over 90% less than SDS-PAGE.

References:

1. Mann M et al (2008*). Proc. Natl. Acad. Sci. U. S. A.* 105: 18132– 18138.
2. Klammer A and MacCoss M (2005). *Journal of Proteome Research* 5: 695–700.
3. Zhang & Li (2004). *Rapid Commun Mass Spectrom*. 18(8): 889-896.
4. Karuso P. *et al* (2008). *Journal of Proteome Research* 7: *361–366*