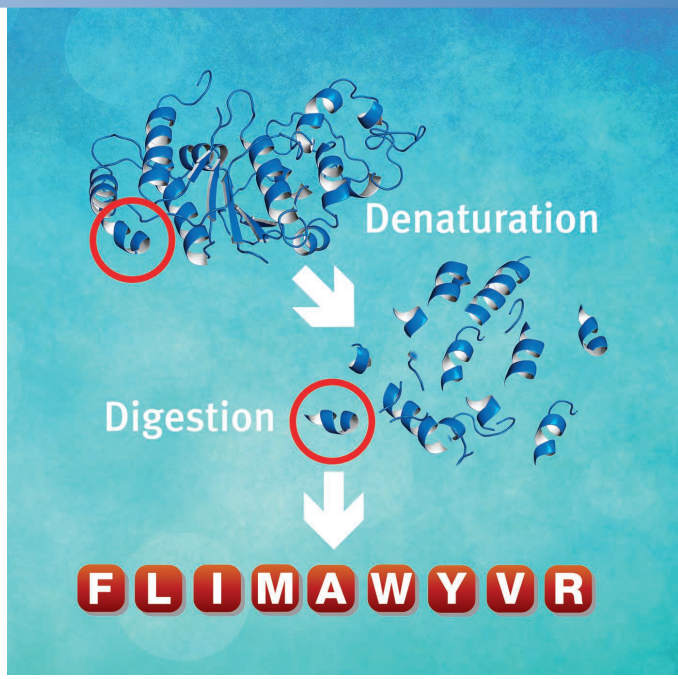
Increased Efficiency of Biomolecule Identification by Optimization of Trypsin Digestion Buffers

Phillip Humphryes, Valeria Barattini; Thermo Fisher Scientific, Runcorn, UK

Key Words
SMART Digest, peptides, monoclonal antibody, protein, proteomics, trypsin digestion, buffer, mAb, biomolecules, biotherapeutics, biopharmaceutical

Goal

To demonstrate how the Thermo Scientific™ SMART Digest™ kit removes the uncertainty associated with conventional solution-based tryptic digestion protocols, resulting in higher efficiency and sample characterization.



Introduction

Protein insolubility can be a major impediment to traditional bottom-up proteomic methods becauses it reduces the efficiency of enzymatic digestions and makes analysis problematic. Insolubility can be caused by a variety of factors, including the salt concentration, the presence of various organic solvents, temperature, or simply the nature of the protein itself. In some cases, this insolubility can be useful for purification purposes as is seen with immunoprecipitation or pre-pelleting protocols. However, in most cases, precipitation of the protein out of solution is undesirable.

The digestion itself can increase the solubility of a protein as the resulting peptides, due to their smaller size, are more soluble than their parent proteins or corresponding polypeptide chains. Protein solubility can be enhanced by the addition of small amounts of solvents, detergents, and/or chaotropes; however, many of these methods are also detrimental to trypsin digestion.

The SMART Digest buffer was developed to maintain protein solubility while increasing the rate of digestion. This was achieved by optimizing the solubility buffer then the digestion buffer.

Thermo SCIENTIFIC

Experimental

Digestion

- SMART Digest Kit (P/N 60109-101)

Chemicals

- Fisher BioReagents™ bovine serum albumin (BSA) (P/N 128516300)
- Thermo Scientific™ Pierce™ trypsin protease (P/N 13464189)
- Fisher BioReagents Tris buffered saline (TBS) (P/N 10648973)
- Fisher Scientific™ Optima™ LC-MS water (P/N 10095164)
- Fisher Scientific Optima acetonitrile (ACN) (P/N 10001334)
- Fisher Scientific Optima isopranol (IPA) (P/N 10091304)
- Fisher Scientific dimethyl sulfoxide (DMSO) (P/N 10500151)
- Fisher Scientific Optima methanol (MeOH) (P/N 10031094)
- Fisher BioReagents 2,2,2-trifluoroethanol (TFE) (P/N 10468733)
- Thermo Scientific Pierce guanidine HCl (P/N 11821365)
- Fisher BioReagents Tween 20 (P/N 10113103)
- Thermo Scientific Pierce octyl glucoside (P/N 28310)
- Fisher BioReagents deoxycholate (P/N 10346653)

Sample Handling Equipment

Heater/shaker equipped with PCR block and heated lid

Experiment 1: Solubility buffer comparison

Bovine serum albumin (BSA) was dissolved in the following buffers:

- Tris buffered saline (TBS)
- TBS with trypsin (1:5 trypsin to protein ratio)
- An optimized proprietary solubility buffer

Final concentrations of BSA of 1.25, 2.5, 3.75. 6.25, and 12.5 mg/mL were produced.

Experiment 2: Digestion buffer comparison

A 12.5 mg/mL solution of BSA in the optimized digestion buffer was prepared and compared to BSA in optimized digestion buffer with the organic solvents, chaotropes, surfactants, and detergent additives listed in Table 1. Samples were then digested using the SMART Digest kit at 1400 rpm and 70 °C using a range of different incubation times (30, 60, 90, and 210 minutes).

Table 1. List of additives and concentrations used to assess BSA solubility.

Additives	Concentrations
Optimized Buffer	Undiluted
ACN	1, 5, 10, 20%
IPA	1, 5, 10, 20%
DMSO	1, 5, 10, 20%
MeOH	1, 5, 10, 20%
TFE	1, 5, 10, 20%
Guanidine HCl	0.5M
Tween 20	0.005%, 0.05%
Octylglucoside	Undiluted
Deoxycholate	Undiluted

Results and Discussion

Experiment One: Solubility Buffer Comparison

Data in Figure 1 shows BSA solubility up to 1.25 mg/mL in the TBS solution, which further increased to 2.5 mg/mL with the addition of trypsin (Figure 2) for the ≥ 15 min time points. This increase in solubility on addition of trypsin can largely be attributed to the effect of digestion of the protein to its constituent peptides, which are generally more soluble than the parent protein.

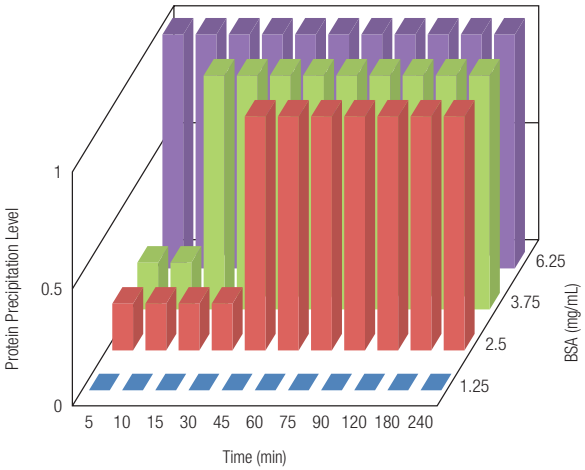


Figure 1. BSA solubility in TBS solution.

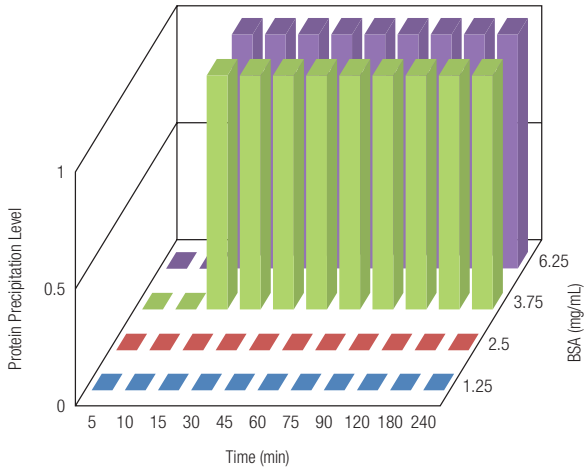


Figure 2. BSA solubility in TBS solution with trypsin.

Concentrations of BSA greater than 2.5 mg/mL in TBS/trypsin solutions (Figure 2) displayed precipitation after only 10 minutes of incubation. This can be attributed to the denaturation of the trypsin, due to the high temperature, limiting the extent to which digestion of the protein could be achieved.

By contrast, the optimized solubility buffer was capable of solubilizing all concentrations of BSA up to as high as 12.5 mg/mL (Figure 3). This is five times higher than the other approaches.

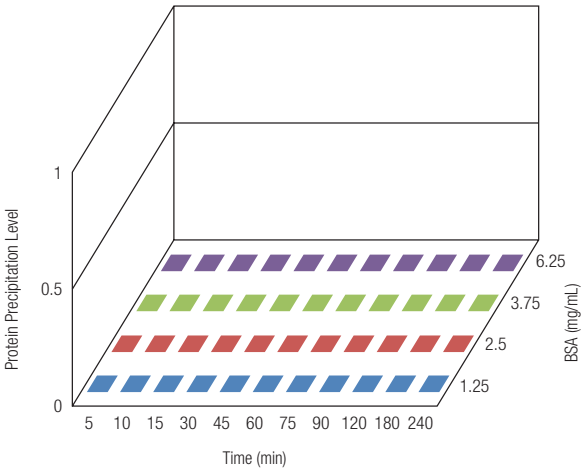


Figure 3. BSA solubility in optimized buffer.

Experiment Two: Digestion Buffer Comparison

The solubility of BSA in digestion buffer samples was assessed after digestion with the SMART Digest kit (Figure 4). The digestion buffer showed excellent solubility with incubation times ranging from 30 to 210 minutes.

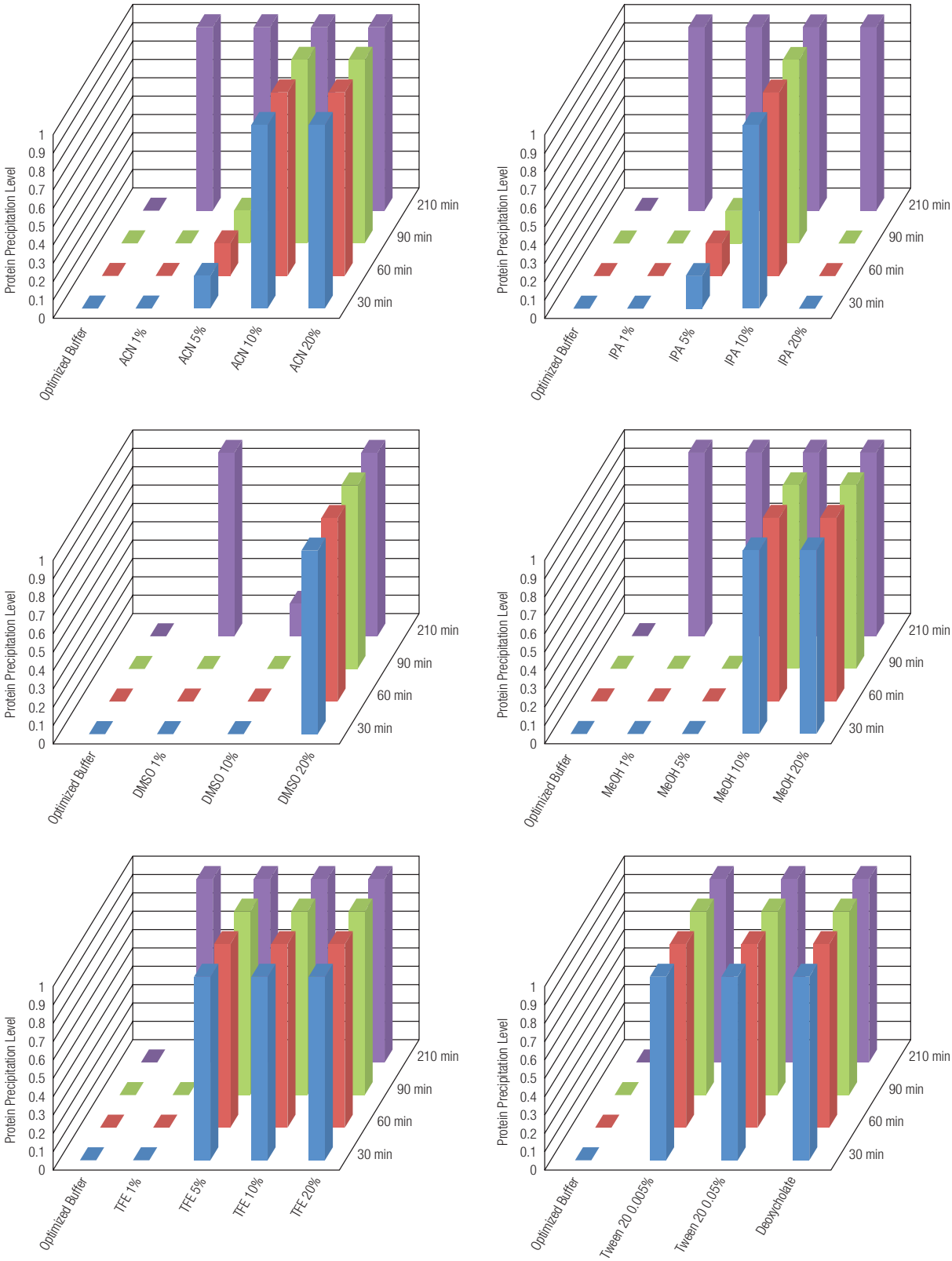


Figure 4. BSA solubility after digestion with SMART Digest kit.

Conclusion

Based on this analysis, the two most effective buffers were combined to create the SMART Digest buffer, which does the following:

- Enables BSA to be kept in solution, both with and without the addition of trypsin, at concentrations up to 12.5 mg/mL.
- Allows higher concentrations of proteins to be kept in solution for longer periods of time, than standard digestion buffers.
- Permits use with high-temperature (up to 70 °C) digestions, enabling digestion and denaturation of proteins to be performed in one step.

For Research Use Only

thermoscientific.com/SMARTdigest

USA and Canada +1 800 332 3331
Australia 1300 735 292 (free call domestic)
China 800 810 5118 (free call domestic)
400 650 5118
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
India +91 22 6742 9494
+91 27 1766 2352
Japan 0120 753 670 (free call domestic)
0120 753 671 (fax)
Korea +82 2 3420 8600
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
Singapore +65 6289 1190
All Other Enquiries +44 (0) 1928 534 050

Technical Support

For advice and support, please visit our website:
www.thermoscientific.com/chromexpert

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

AN21174-EN 0515S