Understanding protein solubility enables more effective digestion protocols

Protein insolubility can be a major impediment to analysis. Insolubility can be caused by a variety of factors: salt concentrations, the presence of various organic solvents, temperature, or simply the nature of the protein itself. While in some cases this can be utilized for purification, as is seen with immunoprecipitation or pre-pelleting protocols, in most cases precipitation of the protein out of solution is undesirable and inhibitory to digestion.

Protein solubility can be enhanced in a number of ways. The addition of small amounts of solvents, detergents, or chaotropes can be very useful to maintaining solubility. However, as seen in our previous application note – Effect of Various Buffers Surfactants and Solvents on Trypsin Activity – many of these methods are also detrimental to trypsin digestion.

It should also be noted that digestion itself is a means to increased solubility. Most tryptic peptides, due to their smaller size, are more soluble than their parent proteins or corresponding polypeptide chains. Therefore in working to increase protein solubility under any of the conditions which may induce precipitation, we investigated a two-phase approach to maintain protein solubility: modifying the buffer composition to enhance the rate of digestion as well as provide a protein stabilizing agent.

Materials and Methods:

This study employed bovine serum albumin (BSA) as a model protein. Porcine trypsin was purchased from Sigma Aldrich (St. Louis). Temperature stable immobilized trypsin was made by Perfinity Biosciences (West Lafayette, IN). Tris buffered saline (TBS) was used as the default buffer and temperature was employed as the forcing factor in protein precipitation. Different concentrations of BSA were incubated at 70 °C for varying time lengths to incur varying levels of precipitation. All other chemicals used were ACS grade or higher.

Results and Discussion:

BSA in TBS precipitated relatively easily in any concentration above 1.25 mg/mL as seen in Figure 1A. Although this concentration is relatively high for pure protein work, it is low when considering complex matrices, which sometimes contain protein concentrations as high as 70 mg/mL. It should also be noted that as a globular protein, albumin has a relatively high solubility to begin with and many proteins that may be dealt with in a purified situation are less soluble.

First we investigated the effect of digestion on solubility. Knowing that peptides are often more soluble than their parent proteins, we expected the addition of trypsin alone would decrease the amount of precipitation observed. This was borne out in our work, as shown in Figure 1B. The addition of trypsin to the solution, even though it increased the total protein in solution by a small amount, served to dramatically reduce the amount of precipitation observed. However, concentrations of BSA greater than 2.5 mg/mL still showed precipitation after 10 minutes of incubation. This could be in part due to

the denaturation of the trypsin due to the high temperature, limiting the extent to which digestion could be achieved and therefore solubility improved.

Following this we screened a wide variety of additives including organic solvents, chaotropes, surfactants, and detergents. For this screening process we fixed the concentration of BSA at the high end, 12.5mg/mL, and used a buffer that had already been improved for digestion based on the screening performed in our previous application note. We also employed temperature stable immobilized trypsin to prevent the denaturation of the trypsin from impacting our results. Samples were again run at 70 °C and 1400rpm to keep the immobilized trypsin in suspension. The results are shown in Figure 2. These results were then balanced with impacts on digestion as determined previously.

Based on the previous step's results we then employed an optimized digestion buffer which has been designed to stabilize proteins in solution. In using this buffer without trypsin we wanted to investigate it purely based on its ability to increase protein solubility without the aid of digestion seen in the previous step. At this point we found it was possible to keep BSA in solution for over 4 hours at 70 °C, even at concentrations as high as 25 mg/mL as shown in Figure 1C.

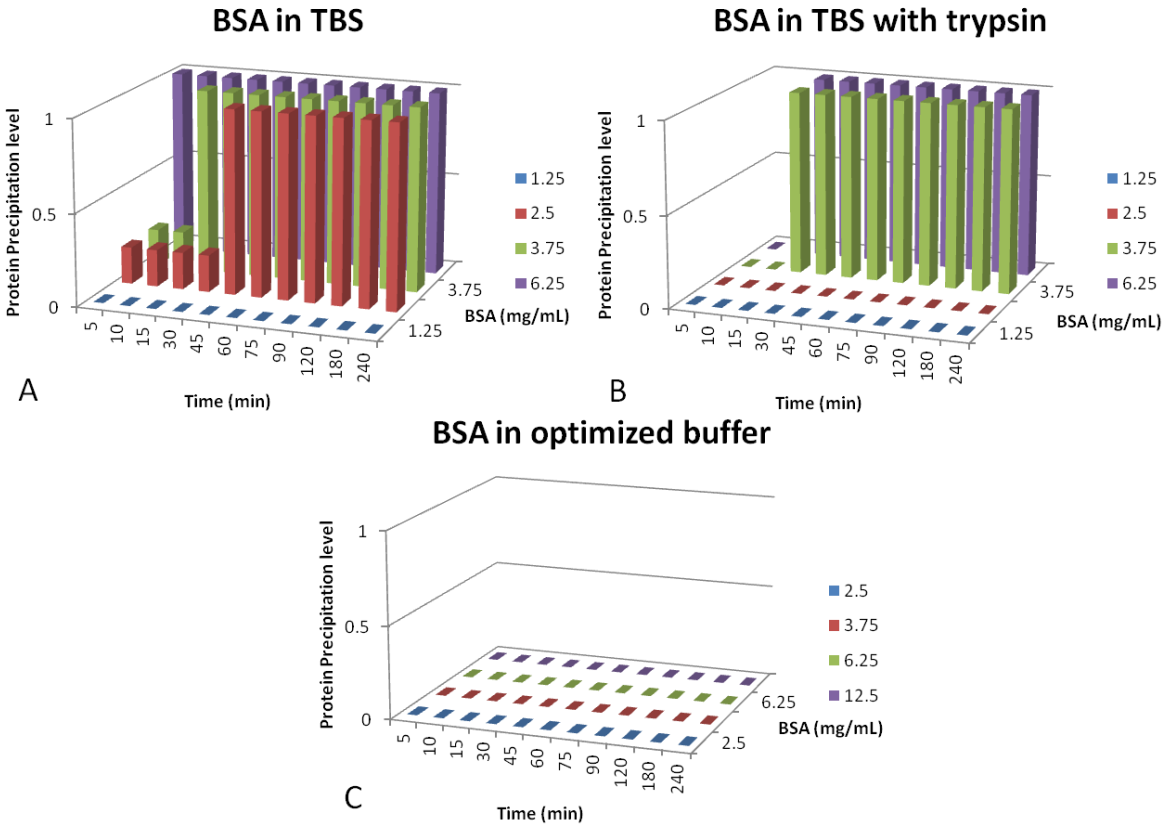

Figure 1 Precipitation of various concentrations of BSA at 70 °C for various lengths of time A: in TBS, B: in TBS with trypsin, and C: in a buffer optimized for solubility.

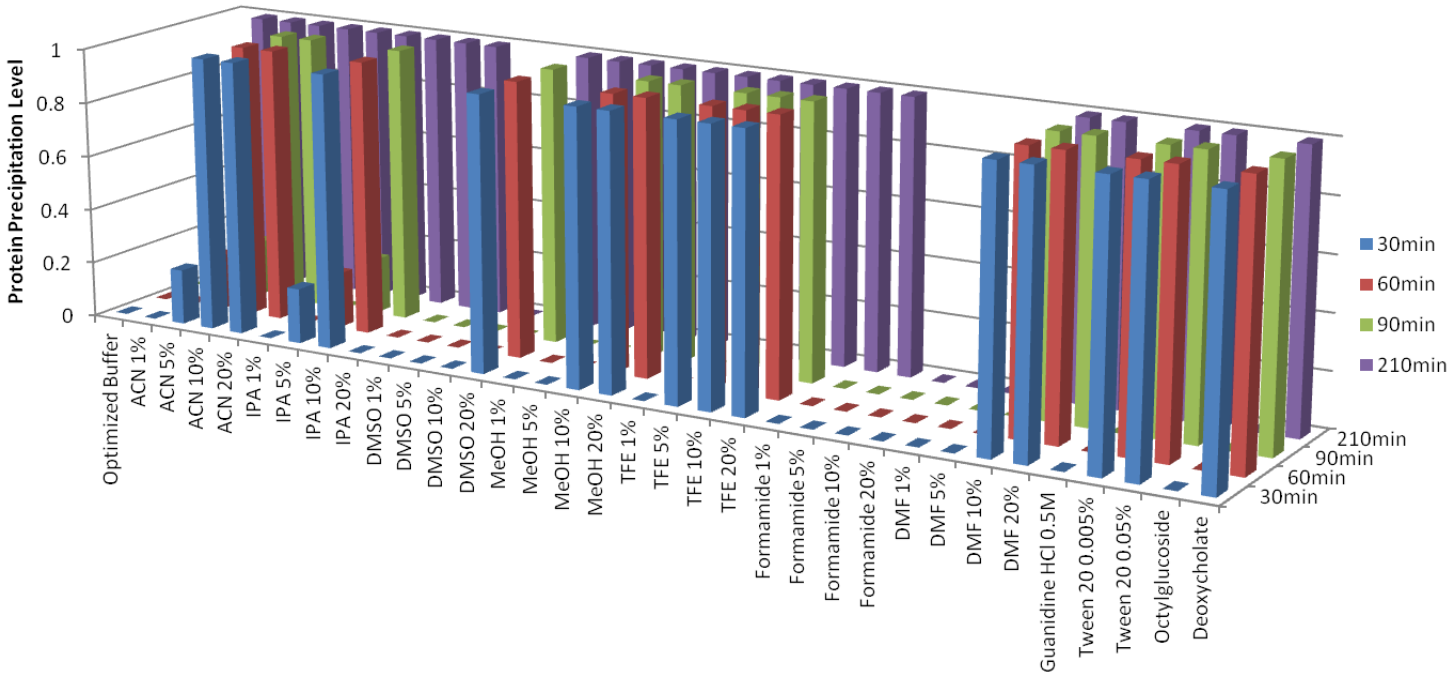

Figure 2 Precipitation of 12.5 mg/mL of BSA at 70 °C for various lengths of time in a buffer optimized for digestion and with various additives, shaken with immobilized trypsin at 1400rpm.

As a final validation of our improved buffer, we combined the digestion buffer optimized for protein stability with one optimized for digestion and employed it on samples of human plasma diluted 4 fold which also contained a thermally stable immobilized trypsin. While this only represents a final protein concentration of 17.5 mg/mL, plasma has many proteins that are far less soluble than albumin. Using this digest buffer we were able to perform digestion of 25% plasma for over 4 hours at 70 °C without any precipitation.

Conclusions:

Understanding protein solubility enables researchers to better make use of it as a tool rather than an impediment to workflows, whether working to achieve protein precipitation for separation purposes or working to avoid precipitation while operating under conditions which favor it. In this investigation we showed that understanding how to prevent protein precipitation makes it possible to perform digestions at high temperatures, even as high as 70°C, enabling the dual advantages of kinetically improving the rate of digestion and enabling simultaneous thermal denaturation of proteins of interest.