In-solution digestion

In-solution digestion

In-solution enzymatic protein digestion is a useful, and sometimes necessary, alternative to in-gel digestion. For samples of low to medium complexity or for samples not amenable to SDS-PAGE in-solution digestion can be used and will provide similar results to in-gel digestion. However, protein folding can protect the amino acid chain from enzymatic cleavage so denaturation is necessary for efficient cleavage. The conundrum with in-solution digestion is finding conditions to denature the sample without denaturing the protease. **Detergents cannot be used in the denaturation process since they will interfere in subsequent MS analysis.** Common denaturants that we use in our laboratory for in-solution digestions include 8 M urea in 10 mM Tris (pH 8.0), 8 M guanidine HCl (pH 8.0 or 1.5[1]) and 6 M urea/2 M thiourea in 10 mM Tris (pH 8.0). Unfortunately trypsin, the most common protease for MS analysis, is not stable in any of these conditions but another enzyme, LysC, is stable. LysC cleaves on the carboxyl side of lysine residues while trypsin targets both lysine and arginine residues. The basic procedure, therefore, is to digest in the high concentration of denaturant first with LysC then dilute the sample to conditions tolerable for trypsin and digest in a second round with that enzyme.

Protocol: In-solution trypsin digestion

Reagents

- 8 M urea in 10 mM Tris (pH 8.0)
- Reduction buffer (10 mM dithiothreitol in water)
- Alkylation buffer (50 mM iodoacetamide in 50 mM NH_4HC0_3)
- LysC ()
- Trypsin, modified sequencing grade (Promega)

Method

1. Spin samples solubilized in 8 M urea, 6 M urea/2 M thiourea or 8 M guanidine HCl in 10 mM Tris (pH 8.0) for 10 min at 10,000 rpm to pellet any insoluble material. The pH of the final solution should be near 8.0 for optimal trypsin digestion. Check with pH strips.
2. Add 1 µL reduction buffer for every 50 µg sample protein and incubate 30 min at room temperature. Only a very rough estimate of protein content is necessary – where sample amount is limited it is better to sacrifice accuracy than waste sample on a protein assay. In this procedure all steps are done at room temperature to reduce unwanted derivatization of amino acid side-chains by the denaturants.
3. Add 1 µL alkylation buffer for every 50 µg sample protein and incubate 20 min at room temperature.
4. Add 1 µg LysC/50 µg sample protein and incubate for 3 h or overnight, at room temperature.
5. Dilute sample with 4 volumes 50 mM NH_4HC0_3.
6. Add 1 µg trypsin/50 µg sample protein and incubate overnight at room temperature.

---

[1] Trypsin & LysC perform optimally at pH 8.0 but we have successfully extracted a sample in 6 M urea/2 M thiourea, pelleted the insoluble material and solubilized this pellet with 8 M guanidine HCl, pH 1.5 and then combined the guanidine and urea to achieve a higher degree of sample solubility than either denaturant alone. As a bonus the Tris buffering the urea is sufficient to maintain the combined solution at or near pH 8.0 when combining equal volumes of the two.