REDUCTION AND ALKYLATION OF CYSTEINES

Introduction

To allow the full proteolysis of a protein it may be necessary to reduce disulphide bonds and then block the free cysteinyl residues to prevent the disulphide bonds reforming. This treatment may help if solubility problems are encountered during proteolysis.

This can be accomplished by reduction of the protein sample followed by either carboxymethylation or pyridethylation.

Method

1. **Solubilise, denature and reduce protein**
    a. Protein sample ideally needs to be >1.5mg/mL. (Concentrate or precipitate if necessary).
    b. Prepare 6M Guanidine HCl in suitable buffer (either 0.1M sodium phosphate pH 7.0 – 7.5 or 0.1M Tris pH 7.5 – 8.0 or 0.1M sodium borate H 8.0 – 9.0)
    c. Add 1 – 10 mg protein to 6M Guanidine HCl solution to give a final volume of 900mL.
    d. Flush sample with nitrogen
    e. Add 2-mercaptoethanol to 20 – 50mM
    f. Incubate at 25°C for at least 2 hours

Alkylate the protein with one of the following methods.

2. **Carboxymethylation**
    a. Add 100mL freshly prepared 0.5M iodoacetic acid (to give a final concentration of 50mM IAA). Caution burns.
    b. Incubate for 3 hours at 25°C. Note pH needs to be >7.0

3. **Or Pyridethylation**
    a. Add 100mL freshly prepared 2% (v/v) 4-vinylpyridine in H_2O (to give a final concentration of 20mM 4-VP)
    b. Incubate in the dark for 3 hours at 25°C

4. **Quench reaction**
    a. Add excess thiol to mop up any unreacted reductant
    b. Isolate protein by one of the following methods.
        i. Dialyse Vs either 0.1M ammonium bicarbonate, buffer of choice or water
        ii. RP HPLC
        iii. Gel filtration chromatography
        iv. Precipitation

Notes

- 4-Vinyl pyridine treatment will result in S-B-(4-pyridyl-ethyl) cysteine which adds 104.058 Da (monoisotopic mass)

- Iodoacetimide treatment will result in carboxymethylcysteine which adds 58.005 Da (monoisotopic mass)

References

Proteolytic enzymes a practical approach. RJ Benyon and JS Bond pp146-147

Advanced methods in protein microsequence analysis. B Wittman-Leibold, J Salnikow, V Erdmann pp245-255