Protocol for reductive dimethylation of surface-exposed lysine residues

Ref: Reductive alkylation of lysine residues to alter crystallisation properties of proteins. ***Ivan Rayment (Methods in Enzymology, Vol. 276, p171-179, 1997)***

The following protocol has resulted in the dimethylation of all surface-exposed lysines in every target tried so far. The protocol results in a homogeneous sample with minimal dilution from crystallisation concentrations, and can be completed in just 2 days. Samples are checked by mass spectroscopy. Crystals have been obtained in a number of cases.

Reagents 1 M borane-dimethylamine complex (in H_2O) (MUST BE FRESH)
1 M formaldehyde (prepared from 16% solution, methanol-free, Polysciences Inc., PA)
1 M glycine, pH 8.6 (with NaOH)
50 mM HEPES, pH 7.5

Chemistry

(i) $R\text{-}NH_2 + CH_2O \text{ ----> } R\text{-}N{=}CH_2 + H_2O \text{ ----> } R\text{-}NH\text{-}CH_3$

(ii) $R\text{-}NH\text{-}CH_3 + CH_2O \text{ ----> } R\text{-}N(CH_3)(CH_2OH) \text{ ----> } R\text{-}N(CH_3)_2$

NB. N-terminal $-NH_2$ group of backbone will also be reductively alkylated.

Protocol

A Buffer exchange of protein using PIERCE protein desalting spin columns

- resuspend resin by inverting
- twist off bottom and loosen cap
- centrifuge @ 1.5 rcf (1500x g), 1 min
- equilibrate column in 50 mM HEPES (no free amino groups, phosphate buffer good too), pH 7.5 by adding 400 µl buffer to column and centrifuging at 1.5 rcf, 1 min.
- rpt 3 times
- add protein sample (30-120 µl, I do 100 µl 10mg/ml sample) to bed of column, making sure not to disturb the resin
- centrifuge at 1.5 rcf, 2 min to elute sample in fresh collection tube

B Reductive dimethylation

Preparation of reagents

- reagents should be made up before use each time.
- a 20-fold molar excess of formaldehyde has been shown to yield essentially complete modification of all exposed lysine residues and free N-termini (note: remember it is the molarity of lysine residues + N-termini in solution which equals the number of lysines + number of free N-termini multiplied by the molarity of your protein solution)

Protocol

- 200 µl 10 mg/ml protein buffer exchanged into 50 mM HEPES, pH 7.5 (or phosphate buffer)

1. Add 20 µl 1 M ABC (borane-dimethylamine complex) per 1 ml of protein solution w/ gentle mixing.
2. Immediately add 40 µl 1 M formaldehyde, again w/ gentle mixing and leave on ice for 2 hrs.
3. Rpt steps 1-2 and leave protein on ice for a further 2 hrs.
4. Add a final aliquot of 10 µl 1 M ABC per 1 ml of protein (initial vol.) and leave the reaction mixture on ice overnight.
5. Reaction quenched by addition of excess amine:
   2-fold molar excess of glycine, pH 8.6 over total added formaldehyde, added to reaction. Incubated on ice for 1 h.
6. Reaction mixture dialysed into 1.5 litres 50 mM HEPES, pH 7.5 using PIERCE Slide-A-Lyzer 0.1-0.5ml dialysis cassettes, 10,000 MW cut-off (or appropriate), 4 h @ 4°C.
7. Cassette transferred into 1.5 litres 50 mM HEPES, pH 7.5 + 2 mM DTT to reverse any modification of cysteine or methionine side chains. Dialysed o/n.
8. Methylated protein retrieved from cassette and analysed by electrospray mass spectroscopy.

Results

Calculating change in molecular weight

-CH_3 grp = 15 Da
- each free amino group is dimethylated since the reaction proceeds rapidly from monomethylated to dimethylated, therefore 15 x 2 = 30 Da added, replacing 2 H atoms, therefore 28 Da added per methylated lysine residue / N-terminus.
- increase in molecular weight should be divisible by 28.