BUSM Mass Spectrometry Resource
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Practical MS of proteins: sample preparation techniques.

1. Sample preparation for MALDI-TOF MS of proteins.

A protein that is at least a major component in a mixture can be analyzed using MALDI-TOF MS without SDS-PAGE.

A. Washing to remove salts.

Sinapinic acid (SA) and α-cyano-4-hydroxycinnaminc acid (CHCA) are soluble in 30-50% organic solutions. Because they are not very soluble in acidic water, dried MALDI spots can be washed with 0.1 % TFA or 1% formic acid in water, a step that removes salts from the crystal surface. This is effective for removal of many buffer salts (tris, guanidine) provided that the concentration of salt is not high enough to inhibit crystal formation. High concentrations of NaCl or phosphate should be avoided. Note that washing does not work for dihydroxybenzoic acid (DHB) because this matrix is soluble in acidic water. The same is true of succinic acid, the most widely used IR MALDI matrix.

B. Droplet recrystallization (1).

This procedure removes detergents or other contaminants that inhibit crystal formation. A layer of matrix (SA or CHCA) is dried on the target and crushed. A droplet of sample diluted in matrix is applied on the crushed matrix and allowed to partially dry. Crystals grow on the matrix surface as the organic solvent evaporates, leaving detergent in the aqueous layer. The droplet is washed with 0.1 % TFA before it dries completely. This requires some artful solvent compositions.

C. Drop dialysis.

A droplet of protein solution is floated on a boat made of dialysis membrane and allowed to sit for 10-30 min. An effective means of removing contaminants that are not tightly bound to the protein.

D. Micro gel filtration.

A small sample of protein is desalted using a microcentrifuge gel filtration cartridge.

E. Micro reversed phase cartridges.

Zip-tips or other commercial pipet tips with reversed phase packing can be used to desalt some proteins, provided that they elute in organic solvent. Microcentrifuge versions of this approach exist.

F. Precipitation

Precipitation methods vary for proteins of different classes, but may work well for desalting or removal of detergents.

2. Sample preparation for MALDI-TOF MS of peptides.

A. General

Techniques are similar to those for proteins. Washing works well for removal of salts with SA and CHCA but not for DHB. For DHB either purify peptides using RP-HPLC or zip tips. Drop dialysis does not work.

B. Sample prep for very hydrophobic/membrane peptides.

Here are four approaches to the problem of MS analysis of membrane proteins:
HPLC of membrane proteins has been conducted using a polystyrene-divinylbenzene column with Solvent A = 60% formic acid and B = 2-propanol with a 5-100% B gradient in 55 min(2). This system was used to produce LC/MS data on the intact proteins. A similar system may work for hydrophobic peptides. On-line LC/MS of cyanogen bromide peptide generated from rhodopsin and bacteriorhodopsin has been accomplished using reversed phase chromatography with a water/2-propanol/TFA solvent system (3). The protein was delipidated by precipitation in 95% ethanol before cyanogen bromide digestion. Rhodopsin was dissolved in 4M urea and then reduced and alkylated(4). Samples were diluted to 2M urea and digested with trypsin (4% by weight, a value higher than typically used). HPLC separation was carried out using a Vydac C4 column with a mobile phase containing octyl-β-glucoside. MALDI-TOF spectra were then acquired on the peptides. Rhodopsin was reduced and alkylated and precipitated using TCA(5). The protein was dissolved in 80% TFA and digested with cyanogen bromide followed by MALDI-TOF MS.

3. Sample preparation for ESI MS of proteins.

Proteins must be free of salts for ESI MS. The method for desalting will depend on the protein. Ethanol precipitation works well for extracellular matrix proteins. Microcentrifuge gel filtration cartridges work well for many soluble proteins. Zip tips work only for well-behaved soluble proteins. Microcon centrifugal dialysis units work well if the protein does not stick to the membrane. Reversed phase desalting cartridges (short columns) work well for the purification of some proteins.

4. Sample preparation for ESI MS of peptides.

Purify peptides for ESI-MS using RP-HPLC or Zip tips.

5. References

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