

Protein sequencing at EPC, BioCentrum-DTU

Detailed information and guidelines

Protein sequencing by Edman degradation

Proteins and peptides with a free α -amino terminal are coupled to phenyl thioisocyanate at pH 9. At acid pH the first amino acid is liberated as an unstable derivative, which rearranges to the stable PTH amino acid (phenylthiohydantoin). The resulting protein or peptide is now one amino acid residue shorter than the original. The procedure is repeated liberating residue no. 2 etc. After each cycle the liberated PTH amino acid is identified and quantified relative to a standard by RP-HPLC and UV-detection. Hereby the sequence is determined residue by residue.

Protein sequencer Applied Biosystems 494

In the sequencer all reagents are kept under argon and the whole series of reactions are carried out in a closed system at argon pressure excluding oxygen. This allows a repetitive yield of a cycle in the Edman degradation of 92-95%. The cleavages occur in a reaction chamber, either a) or b)

a) the sample is adsorbed to a 9 mm glass fiber disc. The fiber is washed and treated with Biobren before the sample is applied in aliquots of 15 μ l.

b) a piece of PVDF membrane containing the sample is fixed in a slit in the chamber.

The maximal stretch of identified residues is normally 30-40. Cystein/Cystin is not detected unless special precautions are taken. Carbohydrate-linked residues are not detected.

Samples for protein sequencing

Sample requirements: Samples must have a free NH_2 -terminus and should be > 90% pure. Samples must not contain amines (e.g. Tris or glycine), large amounts of salt, detergents or residuals of acrylamide. Samples may be in solution or bound to a PVDF-membrane. Coomassie staining is tolerated. Proteins larger than 10,000 Da can be desalted here by centrifugation in Prospin cartridges washing out salt, and binding the protein to a PVDF membrane. In order to avoid blocking the α -amino group especially aldehydes must be avoided, even traces hereof, as well as pyridine, formic acid and formates.

Amounts: The standard level is 10 pmoles, usually allowing determination of up to 20 residues and 20-50 pmol may allow up to 50 residues, depending on the sequence. Higher amounts will only result in a higher background. If enough material is available it is advantageous to determine the accurate amount by amino acid analysis before the sequencing.

Handing in the samples: Freeze dried samples in e.g. Eppendorf tubes or blots may be mailed. Samples in solution should be handed over to Anne Blicher in room 111a/113, building 224.

Each sample or series of samples must be accompanied by a form giving the necessary informations. All available information about known sequences or homologous proteins can be useful for the interpretation. The number of residues to be determined must be stated. Five residues is the minimal number. It is possible to set the instrument to five residues and continue after inspection of the initial results.

Report

The result is presented as a “called sequence” obtained by automatic data analyses and a “user called sequence” obtained by manual data analysis. The report also includes of all chromatograms and yields of the PTH-amino acids at each step. If precise information on the amount of protein is not available, the amount is set to 10 pmol.

Publication

For analyses of samples prepared by the customer and performed according to already described procedures our involvement can be limited to mentioning that the "N-terminal sequencing was performed by Anne Blicher, BioCentrum-DTU by Edman degradation on an Applied Biosystems Procise 494 sequencer according to the suggestions of the manufacturer". If we are involved in method development or other project collaboration it is a cooperation which should result in coauthorship. Even in these cases we must ask for the usual fee, except in cases where the core facility has obtained funding for the analyses in question.

References

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General information on purification and characterisation

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Aguilar, M.-I. Ed. Methods in Molecular Biology, **251**, HPLC of peptides and proteins: Methods and Protocols. Humana press, Totowa NJ. (2004)

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