

Amino Acid Analysis at EPC, BioCentrum-DTU

Detailed information and guidelines

Standard procedures for peptides and proteins

After hydrolysis in 6M HCl the amino acids are separated by ion exchange chromatography and detected after oxidation and derivatization with orto-phthalaldehyd (OPA) (1). Each amino acid is identified and quantified according to a standard mixture containing 1 nmol of the amino acids, which can be determined after acid hydrolysis.

Proteins bound to PVDF-membranes, e.g. blots from Coomassie stained SDS-PAGE, can be hydrolysed as well.

Amino acids have different stabilities towards hydrolysis and different requirements for hydrolysis: **Asparagine** and **glutamine** hydrolysed to aspartic acid and glutamic acid, respectively. **Tryptophan** is degraded by acid hydrolysis. **Serine** and **threonine** are slowly degraded under acid hydrolysis, the losses can be expected to be 10% and 5%, respectively, after 20 hours hydrolysis. The most correct values are obtained by extrapolating to time = 0 from the values of several hydrolysis times (e.g. 24, 48 and 72 hours). **Cysteine** and **cystine** are determined as thiopropionic-cysteine (TPCys) after derivatization with 3,3'- dithiopropionic acid during the hydrolysis (1). **Valine** and **isoleucine** are released slowly. Some bonds with these residues require up to 72-96 hours hydrolysis, but valine and isoleucine are stable under the conditions used for hydrolysis. **Methionine** is sensitive to oxidative contaminations during hydrolysis, but the loss is usually less than 10%. **Glucosamine** can be determined, if desired, but the destruction after 24 hours acid hydrolysis is approx. 85 %.

As a result, the following amino acids can be determined after acid hydrolysis:

Aspartic acid, glutamic acid, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. The content of cysteine and ½-cystine is determined as thiopropionyl-cysteine.

Special analyses

By use of special hydrolysis procedures tryptophan and glucosamine can be protected to some extent towards degradation and determined. Phosphoamino acids can be identified.

Free amino acids, including asparagine and glutamine are determined by reversed phase HPLC

(Waters Accutaq) (2) after precipitation of proteins and peptides.

Samples for amino acid analysis

Sample Requirements: *The sample must not contain chemicals, which before or after hydrolysis are hazardous or contain amines in larger amounts than the highest expected amount of an amino acid.*

In order to protect equipment and columns and the succeeding analyses the samples must not contain lipids, remains of acrylamide, non-hydrolysable polymers, large amounts of amine or ammonia/ammoniumions (the maximal amount of injected contaminating amine, e.g. Tris is approx. 5 nmole). The sample must not contain oxidative components, and it must be free from naturally occurring amino acids (e.g. glycine) from buffers. If the sample is dissolved, the solvent must be volatile. Less than 1 μ mole salt (e.g. 40 μ l 25 mM NaCl) pr. analysis is tolerated.

For each sample or series of samples our data sheet should be filled out with relevant information.

Amounts: The optimal determination is obtained by injection of 1 nmole of every amino acid, corresponding to approx. 2 μ g protein to a single determination (e.g. 100 pmole of a protein with M_r 20,000). As amino acids occur in very different amounts in proteins, it may be necessary to inject different amounts of a given hydrolysate. If the amino acid composition of the protein sample or a homologous protein is known, the information will be useful for estimation of the amount that should be injected.

Optimal amount to hydrolysis: 5 μ g

Smallest amount to hydrolysis : 0.2 μ g

Handing in the sample: Each sample or series of samples must be accompanied by a form giving the necessary informations. 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.

Report

The results are presented as chromatograms and a page with results where the amount for every amino acid is given as nmole in the injected volume and mole-%. The dilution volume is also given. If wanted, the total amount of protein can be calculated. Data can be copied to data discs for further processing in spreadsheets or search in databases.

We do not have the capacity to make a complete calculation of the composition of a protein based on several hydrolysis times and –methods, but we are willing to assist at such calculations.

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 amino acid analyses were performed by Anne Blicher, BioCentrum-DTU. We will provide a suggestion for description of the method used and a reference. 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

- (1) Barkholt, V. & Jensen, A.L.: Amino acid analysis: determination of cysteine plus half-cysteine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. *Analytical Biochem* **177**, 318-322 (1989).
- (2) Wandelen, C.V. & Cohen, S.A. : Using quaternary HPLC-systems for separating 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate-derivatised amino acid mixtures. *J Chromatography* **763**, 11-22 (1997).

General information on purification and characterisation:

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

Kielberg, V. & Rasmussen, L. Ed. *Proteiner – oprensning og karakterisering*. Gyldendal, Copenhagen (2005).

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