Amino acid analyses at the Australian Proteome Analysis Facility (APAF)

Amino acid analysis (AAA) is a technique used in biochemistry and molecular biology to determine the composition and quantity of amino acids in a sample, typically proteins or peptides.

Our amino acid analysis services provide precise and comprehensive identification and quantification of amino acids in various samples, including proteins, peptides, food products, and biological samples. AAA is a versatile and essential tool in many scientific fields, providing valuable insights into the molecular composition of samples and for understanding protein structure, function, and nutritional content.

Applications of Amino Acid Analysis

- **Food Analysis**: Determining the nutritional content and quality of food products
- **Protein Quantitation**: Measuring the amino acid composition of proteins to understand their structure and function
- **Clinical Chemistry**: Diagnosing metabolic disorders by measuring amino acid levels in biological fluids.
- **Extinction Coefficient:** Accurate measurements are essential for calculating extinction coefficients of proteins or to determine the turnover number for an enzyme.

Challenges and Considerations

- **Sample Preparation**: Proper preparation is crucial to avoid contamination and ensure accurate results. We are happy to assist you with advice and assistance, but please note that buffer constituents such as amines, lipids and detergents can interfere with the assay.
- **Sensitivity and Specificity**: Choosing the right detection method is pivotal to balance sensitivity and specificity in a way that is suitable for the sample type.

Method Summary

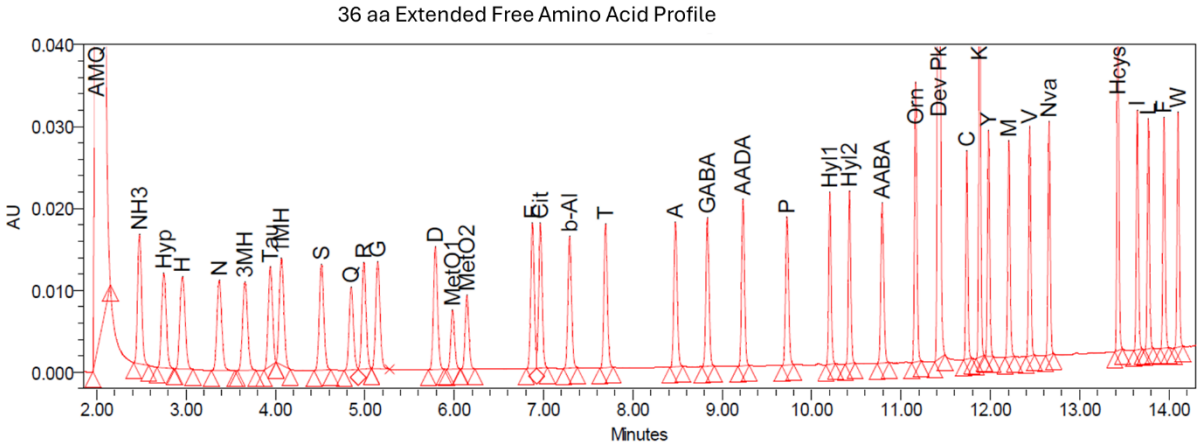
Standard AAA services are ISO 17025-accredited, and generally involve the following processes:

1. **Hydrolysis**: Breaking down the protein into its constituent amino acids. The sample, often a protein or peptide, is hydrolysed under strong acidic or basic conditions.
    a. Hydrolysis is not performed for Free Amino acid analysis, wherein only ‘free’ amino acids that have not been incorporated into peptides/proteins are quantified.
2. **Pre-column Derivatization**: Amino acids are chemically labelled using Waters AccQ•Tag prior to separation to enhance detection sensitivity and selectivity.
3. **Separation**: Amino acids are separated using Ultraperformance liquid chromatography (UPLC).
4. **Detection and Quantification**: Amino acid abundance is detected and quantified against a standard curve.

Amino Acid Analysis Services

Total (i.e., protein-bound and free) amino acid quantification					Free amino acid quantification	
Amino acid profile (liquid hydrolysis)	Amino acid profile with Hydroxyproline & Taurine (liquid hydrolysis)	Tryptophan determination (base hydrolysis)	Cysteine determination (performic acid oxidation)	High sensitivity AAA (purified protein – gas hydrolysis)	Free AAA (no hydrolysis, 20aa)	Additional Amino Acids*
NH3*	NH3*	-	-	-	NH3*	3-Methyl-L-histidine
-	Hydroxyproline (Hyp)	-	-	-	Hydroxyproline (Hyp)*	1-Methyl-L-histidine
Histidine (H)	Histidine (H)	-	-	Histidine (H)	Histidine (H)	Methionine Sulfoxide 1 (MetO1)
-	-	-	-	-	Asparagine (N)	Methionine Sulfoxide 2 (MetO2)
-	Taurine (Tau)	-	-	-	Taurine (Tau)*	Citrulline (Cit)
Serine (S)	Serine (S)	-	-	Serine (S)	Serine (S)	beta-Alanine
-	-	-	-	-	Glutamine (Q)	gamma-Aminobutyric acid (GABA)
Arginine (R)	Arginine (R)	-	-	Arginine (R)	Arginine (R)	alpha-Aminoadipic acid (AADA)
Glycine (G)	Glycine (G)	-	-	Glycine (G)	Glycine (G)	Hydroxylysine 1 (Hyl1)
Aspartic acid (D)	Aspartic acid (D)	-	-	Aspartic acid (D)	Aspartic acid (D)	Hydroxylysine 2 (Hyl2)
Glutamic acid (E)	Glutamic acid (E)	-	-	Glutamic acid (E)	Glutamic acid (E)	alpha-Aminobutyric acid (AABA)
Threonine (T)	Threonine (T)	-	-	Threonine (T)	Threonine (T)	Ornithine (Orn)
Alanine (A)	Alanine (A)	-	-	Alanine (A)	Alanine (A)	Homocystine (Hcys)
Proline (P)	Proline (P)	-	-	Proline (P)	Proline (P)	Theanine
-	-	-	Total Cyste(i)ne (C)	-	Cystine (C)	
Lysine (K)	Lysine (K)	-	-	Lysine (K)	Lysine (K)	* On request
Tyrosine (Y)	Tyrosine (Y)	-	-	Tyrosine (Y)	Tyrosine (Y)	
Methionine (M)	Methionine (M)	-	-	Methionine (M)	Methionine (M)	
Valine (V)	Valine (V)	-	-	Valine (V)	Valine (V)	
Isoleucine (I)	Isoleucine (I)	-	-	Isoleucine (I)	Isoleucine (I)	
Leucine (L)	Leucine (L)	-	-	Leucine (L)	Leucine (L)	
Phenylalanine (F)	Phenylalanine (F)	-	-	Phenylalanine (F)	Phenylalanine (F)	
-	-	Tryptophan (W)	-	-	Tryptophan (W)	

Example Chromatogram



Data reporting

APAF's standard procedure for amino acid analysis is to analyse a client sample in technical duplicate and then to report the averaged results upon a NATA-endorsed report. APAF offers additional replicate alternatives (i.e., triplicate or singlicate), however these must be discussed prior to the commencement of the project and are subject to respective pricing.

Should you require the *results of individual replicates* to be reported, please notify the Technology Manager – Protein Analysis, Dr. David Cantor (david.cantor@mq.edu.au) at the time of quoting or *before* the report has been released. Once the report has been issued, any requests to report individual replicate data will incur an additional reporting charge.

Any modifications to the standard workflow should be identified and discussed with the Technology Manager prior to commencing any project.

Data Formatting

APAF offers two standard output formats for amino acid analysis results, at the discretion of APAF staff:

1. A PDF report, containing project details and up to 6 sample results,
2. A PDF report coversheet, with a Microsoft Excel attachment, for projects with >6 samples.

Should you require small sample number projects to be reported in Microsoft Excel format, please notify APAF staff by listing the requirement on the Service Request Form.

Interpreting your results

Amount (-H_2O; mg/g)

Calculation based on amino acid residue mass in protein. These values express the concentration of amino acids in their protein-bound form (i.e., incorporated into protein/s), and are generally the appropriate value for complex samples/matrices.

Amount (mg/g)

Calculation based on free amino acid molecular weight. These values express the concentration on the assumption that the residues were in their 'free' (i.e., monomeric form) prior to hydrolysis. This value is most appropriate for synthesised amino acids/products.

Amino acid Molar percentage (Mol%)

This value corresponds to the number of specific amino acid residues per 100 residues in a protein. This may be useful for evaluating the data when the protein sequence is known, assisting to corroborate protein/peptide identity.

Sample Submission and Shipment to APAF

For most sample types, we recommend sending 1-10g of material in a sealed container under appropriate temperature conditions. This allows for representative sampling, and repeat analysis, should it be required. Where the available sample amount is lower than this, we recommend discussing this with an APAF team member to determine whether a scaled-down assay is possible/appropriate.

When possible, we recommend sending dry, homogenous samples, particularly for challenging material such as grains, whole plant/animal tissue, etc., to minimise sample heterogeneity, microsampling effects, and to reduce loss from additional processing.

When multiple analyses are requested, we generally recommend sending 5-10g of material for analysis.

When ready to despatch, please direct the parcel to the following address, with attention to the Protein Analysis team:

- Science Stores, Faculty of Science and Engineering, 14 Eastern Road room 186, Macquarie University, NSW, 2109, Australia

Further Enquiries

Please see our website for more information on the Australian Proteome Analysis Facility:

https://www.mq.edu.au/research/research-centres-groups-and-facilities/facilities/macquarie-analytical-and-fabrication-facility/australian-proteome-analysis-facility

Please direct enquiries or requests for further information to info.apaf@mq.edu.au, or to Dr. David Cantor (david.cantor@mq.edu.au).

Thank you,

Protein Analysis Team