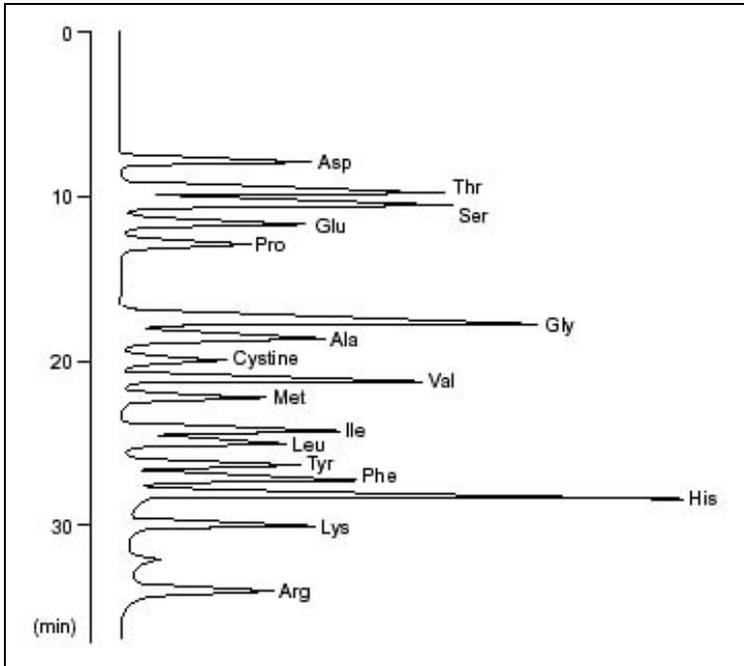


Amino Acid Analysis

By J. Frederick

Amino acid analysis is a technique used to characterize a protein's amino acid content and the concentration of a given sample. We out-source this service through the Keck facility (formally the HHMI Biopolymer – Keck Foundation Biotechnology Resource Foundation) at Yale. This facility is located in the Boyer Center of the Medical School and can be accessed online (<http://info.med.yale.edu/wmkeck/prochem/aaa.htm>).



The Keck website provides detailed information about what happens to your protein during the amino analysis process. In brief, the protein is completely hydrolyzed and then subjected to chromatographic analysis (HPLC) against amino acid standards.

The diagram at the left represents a sample HPLC chromatogram of a hydrolyzed protein. (Adapted from www.shimadzu.com)

The following is taken from <http://keck.med.yale.edu/prochem/procprot.htm>:

“Amino acid analysis is carried out on a Beckman Model 7300 ion-exchange instrument following a 16 hr hydrolysis at 115 degrees C in 100 μ l of 6 N HCl, 0.2% phenol that also contains 2 nmol norleucine. The latter serves as an internal standard to correct for losses that may occur during sample transfers, drying etc. After hydrolysis, the HCl is dried in a Speedvac and the resulting amino acids dissolved in 100 μ l Beckman sample buffer that contains 2 nmol homoserine with the latter acting as a second internal standard to independently monitor transfer of the sample onto the analyzer. The instrument is calibrated with a 2 nmol mixture of amino acids and it is operated via the manufacturer's programs and with the use of their buffers. Data analysis is carried out on an external computer using Perkin Elmer/Nelson data acquisition software.

"During acid hydrolysis asparagine will be converted to aspartic acid and glutamine to glutamic acid. During the HPLC analysis that follows, cysteine co-elutes with proline; and methionine sulfoxide, which is a common oxidation product found in peptides/proteins, co-elutes with aspartic acid. Hence, following normal acid hydrolysis, glutamine and asparagine are not individually quantified and it is possible that the methionine value will be low and (generally to a lesser extent) that the aspartic acid and proline values will be somewhat high. Improved quantitation of cysteine and methionine can be obtained by requesting prior oxidation with performic acid, which converts both methionine and methionine sulfoxide to methionine sulfone and cysteine and cysteine to

cysteic acid. Generally, however, performic acid oxidation destroys tyrosine. Best quantitation of tryptophan is generally obtained by requesting hydrolysis with methanesulfonic acid (MSA) instead of hydrochloric acid. The procedure used in this instance is to carry out the hydrolysis with 20 μ l MSA for 16 hrs at 115C. After hydrolysis, the sample is neutralized with approximately 200 μ l 0.35 M NaOH and 100 μ l (50% of the sample) is then analyzed on the Beckman 7300. Please keep in mind that since we believe the overall extent of hydrolysis with MSA is less than with HCl, we do not recommend MSA hydrolysis for use in quantifying the concentration of protein stock solutions.”

For form submission, and how to fill out the form that must accompany each submission, please see <http://keck.med.yale.edu/yaleforms.htm>.