CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

LCEC of Amino Acids Derivatized with NDA/CN

Purpose

Determination of the amino acids in a protein hydrolyzate using precolumn derivatization with naphthalenedialdehyde/cyanide* (NDA/CN) followed by LCEC (F1).

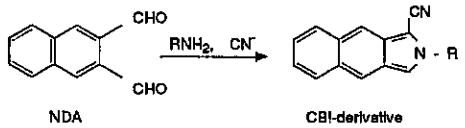

Figure 1. Reaction of NDA/CN with primary amines.

Existing methods

There are several methods that are employed for amino acid analysis. Most of these produce fluorescent derivatives. FMOC, PITC and DANSYL methods suffer from interferences of the parent reagent or hydrolysis products. Ninhydrin lacks the sensitivity of the other methods. Derivatization with orthophthalaldehyde (OPA) 2-mercaptoethanol, is a common approach for the analysis of amino acids using either fluorescence or electrochemical detection. NDA has the advantage over OPA in that the derivatives are stable over a period of hours as opposed to minutes (1).

Conditions

Detector: BAS LC-4B amperometric detector
Electrode: Glassy carbon
Potential: +0.75 V vs. Ag/AgCl
Column: Supelco LC-18 DB (150 X 4.6 mm) 3 µm
Mobile phase: A: 85% (v:v) 0.005 M sodium citrate (pH 7.5), 10% methanol and 5% THF; 0.05 M sodium perchlorate (final conc.)
B: 36% (v:v) 0.005 M sodium citrate (pH 7.5), 63% methanol, 1% THF; 0.05 M sodium perchlorate (final conc.)
Gradient: 0-3 min 10% B
3-20 min 10-100% B
20-30 min 100% B

Sample preparation (BAS P/N CF-1045)

Ten mg elastin was hydrolyzed in vacuo in 1 mL 6 N HCl for 24 h. at 110°C. The HCl was evaporated under nitrogen at room temperature and the residue dissolved in 5 mL of 0.05 M sodium borate. A twenty microliter aliquot of this sample was dissolved in 5 mL borate buffer. One hundred microliters of 0.1 M NaCN and 200 µL of 30 mM NDA were added. The final volume was adjusted to 10 mL with borate buffer and the reaction was allowed to proceed for 30 minutes at room temperature.

Separation of Amino Acid Standards

F2 shows the separation of the 17 common amino acids using the gradient conditions given above. The current response was linear for all amino acids between 8 and 160 picomoles with correlation coefficients of 0.999 or better. The detection limits for lysine and desmosine (an amino acid unique to elastin) were found to be approximately 100 femtomoles under isocratic conditions.

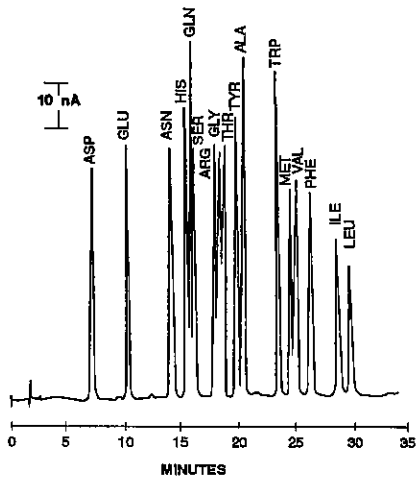

Figure 2. Gradient separation of 17 common amino acids. Each peak corresponds to 160 pmol of the amino acid injected. Chromatographic parameters are as listed above under Conditions.

Detection of Amino Acids in Elastin

F3 shows the detection of amino acids in elastin. The gradient was modified to detect lysine and desmosine. These compounds are multiderivatized, and elute about 15 minutes after the last peak if the original chromatographic conditions are employed. The amino acid composition of elastin as determined by this method agreed well with those reported in the literature.

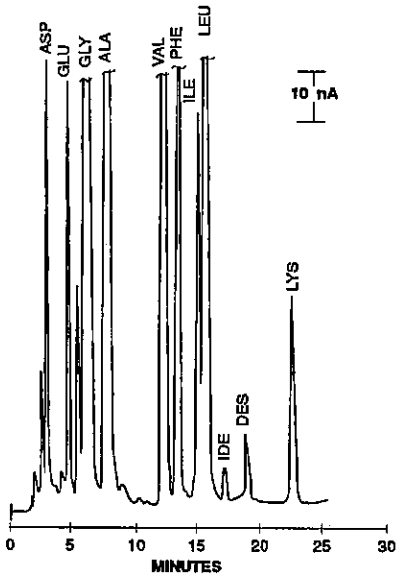

Figure 3. Detection of desmosine and isodesmosine in the elastin hydrolysate using gradient elution LCEC. Mobile phase A was 85% 0.005 M sodium citrate (pH 7.5), 10% methanol, and 5% THF. Mobile phase B was 10% 0.005 M sodium citrate (pH 7.5) and 90% methanol. Both mobile phase A and B were made 0.05 M with respect to sodium perchlorate. Gradient conditions were 50 to 100% B over 30 min; other conditions as in F2. Concentration of elastin, 4.0 µg/mL.

In addition, it has been found that the oxidation potential of the CBI-derivatized amino acids is strongly dependent on the R-group. Multiderivatized compounds, such as lysine, were found to have a lower oxidation potential than monoderivatized amino acids such as alanine. Therefore, voltammetry can be used for the identification of sample components. In reference 2, a dual electrode detector was used to compare the redox potential of the peaks coeluting with lysine and desmosine with that of derivatized standards. The voltammetry was found to be identical.

Notes

The derivatization volumes can be scaled down by a factor of 100. A large volume was used in this case because the sample quantity was not limited.

When determining trace levels of amino acids, it is important to thoroughly clean glassware and to use water that is amine free.

We have found that the addition of NaOH to the sodium borate increases the number of extraneous peaks. Therefore a 0.01-0.05 M sodium borate solution (unadjusted pH about 9.3) is used for all amino acid derivatizations.

It is very important to add the NDA last and to keep the sample protected from light.

References

1. P. de Montigny, J.F. Stobaugh, R.S. Givens, R.G. Carlson, K. Srinivasachar, L.A. Stemson and T. Higuchi, Anal. Chem. 59 (1987) 1096-1101.

2. S.M. Lunte, T. Mohabbat, O.S. Wong, and T. Kuwana, Anal. Biochem. 178 (1989) 202-207.

3. M. D. Oates and J. W. Jorgenson, Anal. Chem, 62 (1990) 577-1580.

* U.S. PATENT # 4837166