Application Note

Detection of Amino Acids Using HPLC with CD Detection

Amino acids are often described as the "building blocks" of the body. Besides building cells and repairing tissue, they form antibodies to combat bacteria and viruses; they are also part of the enzyme & hormonal system; build nucleoproteins (RNA & DNA); carry oxygen throughout the body and participate in muscular activity. The twenty naturally occurring amino acids that comprise proteins are almost all of the L- form. The L (Levorotatory) form is the stereoisomer that rotates plane polarized light to the left. One method to detect amino acids and determine their stereochemistry is with HPLC.

Introduction

An asymmetric carbon atom can be described as having chirality. Chiral molecules have the ability to rotate the plane of polarized light either to the right (dextrorotatory) or to the left (levorotatory). All the amino acids found in proteins exhibit the same absolute steric configuration. Therefore, they are all L-amino acids. D-amino acids are not found in proteins, although they exist in nature and are found in polypeptide antibiotics.

Peptides and polypeptides are polymers of alpha-amino acids. There are 20 α-amino acids that are relevant to the make-up of mammalian proteins. Several other amino acids are found in the body, free or in combined states (i.e. not associated with peptides or proteins). These non-protein associated amino acids perform specialized functions. Several of the amino acids found in proteins also serve functions distinct from the formation of peptides and proteins, e.g., tyrosine in the formation of thyroid hormones or glutamate as a neurotransmitter.

Jasco HPLC System

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Amino acids play a crucial role in the activity of many reactions. In solution, it is the nature of the amino acid R-groups that dictate structure-function relationships of peptides and proteins. The hydrophilic amino acids are generally found on the exterior of proteins as well as at the active centers of enzymatically active proteins. It is the amino acid R-groups that allow enzyme reactions to occur. Equally important is the ability of histidines in hemoglobin to buffer the H+ ions from carbonic acid ionization in red blood cells.

It is this property of hemoglobin that allows it to exchange O2 and CO2 in the tissues or lungs, respectively. The aromatic R-groups in amino acids absorb ultraviolet light with an absorbance maximum in the range of 280nm. The ability of proteins to absorb ultraviolet light is predominantly due to the presence of the tryptophan which strongly absorbs ultraviolet light. This note will describe chiral detection of amino acids using HPLC.

Table 1. The Amino Acids

Amino Acid	Symbol	pK1 (COOH)	pK2 (NH2)
Glycine	Gly - G	2.4	9.8
Alanine	Ala - A	2.4	9.9
Valine	Val - V	2.2	9.7
Leucine	Leu - L	2.3	9.7
Serine	Ser-S	2.2	9.2
Threonine	Thr-T	2.1	9.1
Cysteine	Cys - C	1.9	10.8
Methionine	Met-M	2.1	9.3
Aspartic Acid	Asp - D	2.0	9.9
Asparagine	Asn - N	2.1	8.8
Glutamic Acid	Glu - E	2.1	9.5
Glutamine	Gln - Q	2.2	9.1
Arginine	Arg - R	1.8	9.0
Lysine	Lys - K	2.2	9.2
Histidine	His - H	1.8	9.2
Phenylalanine	Phe - F 2	2.2	9.2
Tyrosine	Tyr - Y	2.2	9.1
Tryptophan	Trp-W	2.4	9.4
Proline	Trp-W	2.0	10.6

Experimental

Several amino acids were examined using a JASCO HPLC system with a CD-2095 circular dichroism detector. The separation was made with a CROWNPAK CR (+) (4.0 mm I.D. x 150 mm/L) column. The eluent was HClO4 with flow rates of 0.4 - 0.8 mL/min. The column temperature varied from 0-25°C with an injection volume of 10 µL (2 mg/mL, 20 µg). Amino acids were also separated following precolumn derivatization with DABS-Cl. For these a CrestPak C18S column was used with a mixture of: A) 8mM Sodium dihydrogenphosphate dihydrate in H_2O with 4% DMF, and B): ACN. Temperature was 40°C, injection volume 10 µl and a flow rate 1 mL/min. The gradient is shown below.

Time (min)	A (%)	B (%)
0	85	15
9	70	30
14	59	41
16	49	51
18	46	54
23	10	90

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Results and Discussion

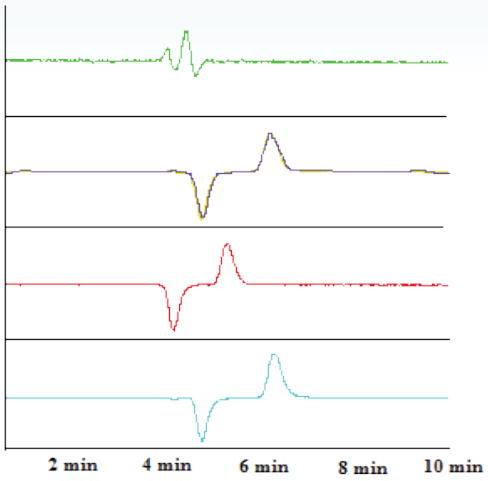

Figure 1. HPLC separation of Pro, Ser, Thr, and Cys amino acids (top to bottom).

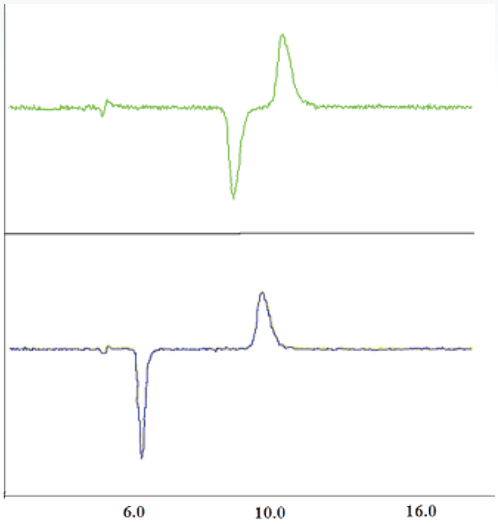

Figure 2. HPLC separation of Lys and Arg.

Figures 1 and 2 show the HPLC separation of proline, serine, threonine, cysteine, lysine and arginine with precolumn derivatization. Each of the compounds evaluated had both a positive and negative peak in the CD spectrum confirming the presence of both enantiomers. In the case of molecules like isoleucine (not shown) which have 2 chiral centers, two separate sets of peaks are found, one set for each chiral center.

The separation of cysteine, proline, histidine, leucine, threonine, and phenanthroline was made following precolumn derivatization with DABS-Cl. In these cases pre-derivatization is required for detection of the isomers because a chiral column was not used.

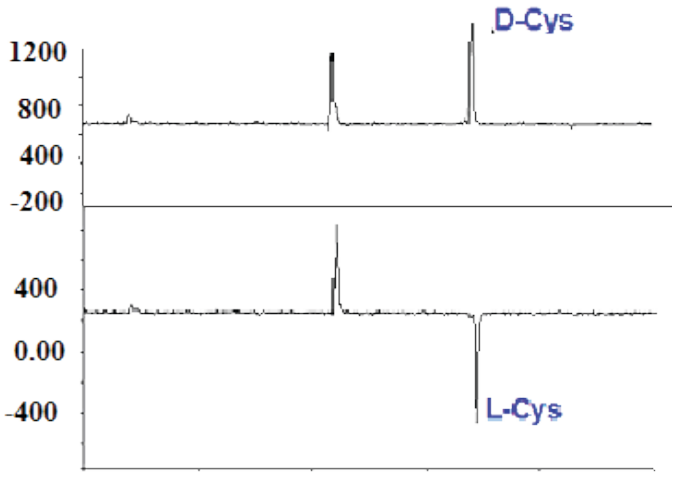

Figure 3. Separation of Cysteine with DABS-CL.

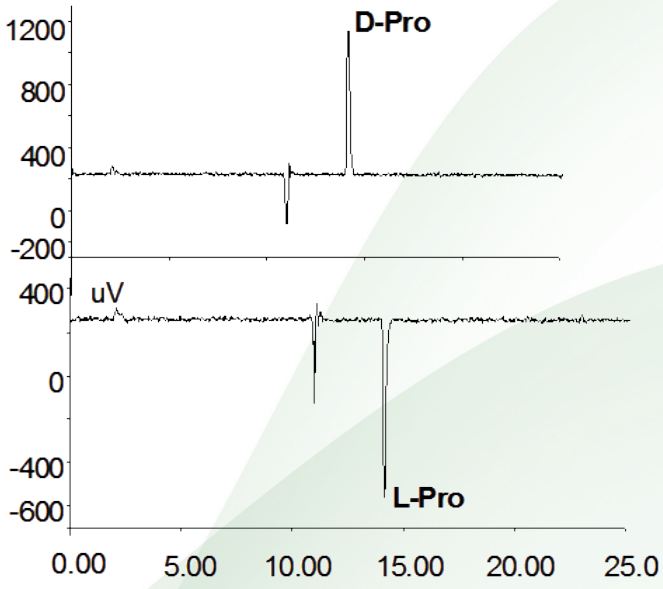

Figure 4. Separation of Proline with DABS-CL

Figures 3 and 4 show the separation of cysteine and proline. Derivatization allows for a more complete separation and sharpens the peaks. Regardless of whether the peaks are separated with a chiral column or with precolumn derivatization, the CD-2095 detector can offer sensitive and selective detection of chiral compounds such as amino acids.

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