application note

Automated Amino Acid Analysis of Protein Hydrolysates

Abstract

The amino acid analysis of three protein hydrolysates, Lysozyme, Chymotrypsinogen A and Pepsin, are described. Derivatisations have been performed on 10 pmol of hydrolysates, and separations conducted on 5 pmol of derivatised samples. The results are in very good agreement with expected values, including those of histidine and tyrosine.

Keywords:
Amino Acid, AMINOMATE, Lysozyme, Chymotrypsinogen A, Pepsin, Protein, Hydrolysate

'...the rapid, sensitive and fully automated analysis of amino acids in protein hydrolysates...'

AMINOMATE, GBC Automated Amino Acid Analyser,1 has been developed for the rapid, sensitive and fully automated analysis of amino acids, especially those in peptide2 and protein hydrolysates. Due to the high sensitivity of the system, with detection limit at 50 fmol, valuable protein hydrolysates as little as 10 pmol is sufficient for analysis. Accurate quantitation of histidine and tyrosine residues, which have proved problematic with existing methods, is also achieved.3

Lysozyme is an enzyme in egg white and human tears which catalyses the hydrolytic cleavage of the bacterial cell wall polysaccharides. It has 130 amino acid residues, with a molecular weight of 14300, but contains only one histidine and three tyrosine residues. The analysis results (Figure 1, Table 1) are in good agreement with the expected values, with accurate determination of both histidine and tyrosine even though they are present at low levels.

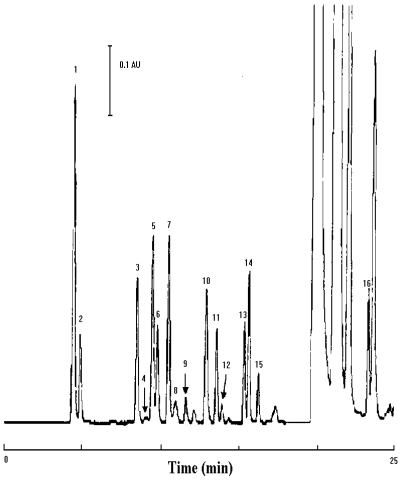

Figure 1 *HPLC Separation of Lysozyme Hydrolysate (5 pmol)*

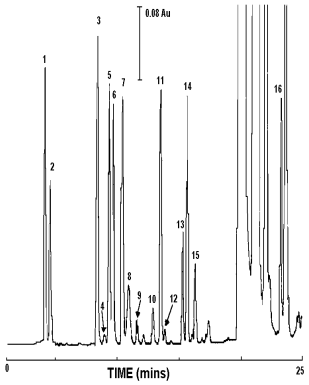

Figure 2 *HPLC Separation of Chymotrysinogen A Hydrolysate*

Chymotrysinogen A is the inactive precursor of chymotrypsin which is a proteolytic enzyme secreted into the small intestine. Chymotrypsinogen A has 244 residues and a molecular weight of 26400. However, it only contains two histidine and four tyrosine residues. Pepsin is an enzyme found in the stomach which is responsible for the hydrolysis of the peptide bonds of aromatic amino acids or ingested proteins. It has 327 residues, with a molecular weight of 34700, but contains only one histidine. The analysis results by AMINOMATE on the hydrolysates of both proteins (Figure 2 & 3, Table 1) are again in good agreement with the expected values.

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Table 1: Amino Acid Composition of Lysozyme, Chymotrysinogen A and Pepsin

No.	Amino acid	Lysozyme		Molar Ratioa Chymotrysinogen A		Pepsin	
1	Asxb	21.4	(21)	23.1	(23)	44.1	(42)
2	Glxc	5.2	(5)	14.4	(15)	26	(26)
3	Ser	9.3	(10)	23.2	(28)	40.7	(44)
4	Zhis	0.8	(1)	2.0	(2)	1.0	(1)
5	Gly	11.8	(12)	21.7	(23)	34.9	(35)
6	Thr	7.0	(7)	21.9	(22)	26.4	(26)
7	Ala	12.6	(13)	22.3	(22)	17.2	(16)
8	Pro	2.1	(2)	8.9	(9)	14.8	(15)
9	Tyr	2.9	(3)	4.0	(4)	13.7	(16)
10	Arg	11.0	(11)	4.3	(4)	2.3	(2)
11	Val	5.6	(6)	2.04	(23)	19.9	(22)
12	Met	1.7	(2)	1.9	(2)	3.6	(4)
13	Ile	5.5	(6)	9.0	(10)	21.9	(26)
14	Leu	8.0	(8)	19.8	(19)	25.8	(26)
15	Phe	3.1	(3)	6.4	(6)	13.8	(14)
16	Lys	5.9	(6)	13.8	(14)	1.3	(1)
17	Trp	nad	(6)	na	(8)	na	(5)
18	Cys	nad	(8)	na	(10)	na	(6)

a: Expected values given parentheses (protein compositions from Swiss protein data bank)
b: Asp + Asn
c: Glu + Gln
d: Not analysed

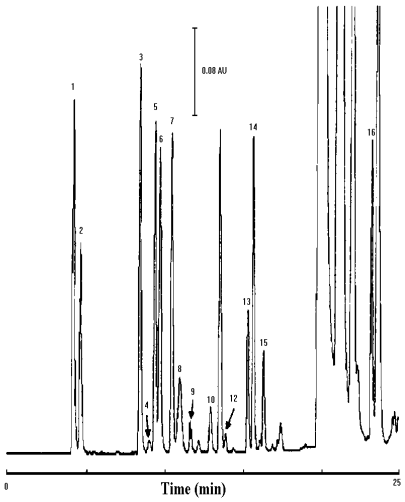


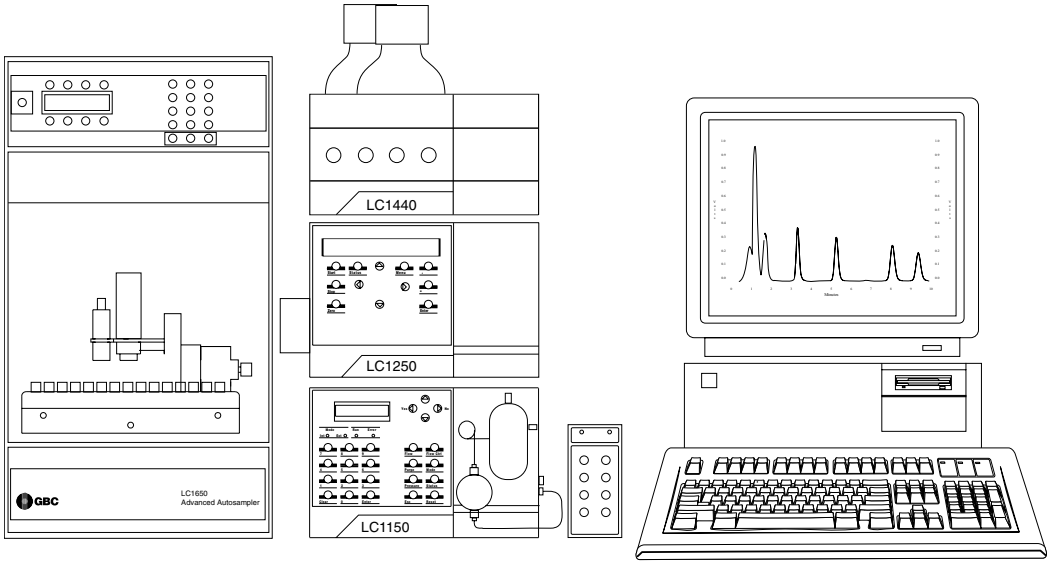
Figure 3 *HPLC Separation of Pepsin Hydrolysate (5 pmol)*

'...the analysis results on the hydrolysates of both proteins are again in good agreement with the expected values...'

GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1250 Fluorescence Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option

Hydrolysis Procedure: see Reference 4.



References

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3. P. Furst, L. Pollack, T.A. Graser, H. Godel and P. Stehle, J. Chromatogr., 499, (1990), 557.
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