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The Analysis of Proteins, Peptides and Amino Acids by Pyrolysis-Gas Chromatography and Mass Spectrometry

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Abstract

A group of seventeen amino acids and ten peptides has been analyzed by a combined pyrolysis gas chromatography-mass spectrometry system.

Identification of a unique pyrolysis product for each amino acid suggests a new method of qualitative analysis for peptides and proteins. Sequence information is probable.

Modern analytical techniques such as gas chromatography and mass spectrometry have provided the chemist with an opportunity to develop more rapid and convenient methods of determining the composition of materials, even when one encounters such complex mixtures as those isolated from natural products. Increasing interest is now current in applying such methods to protein and peptide analysis (1-5). In this study, gas chromatography and rapid scanning mass spectrom-

etry are coupled with pyrolysis for the purpose of providing a means of analysis of the amino acid composition of peptides and proteins.

There are obviously multiple factors influencing the route which a pyrolysis reaction may follow and, therefore, the nature of the reaction products. No attempt has been made to exhaustively examine these factors, although investigation of the principal variables encountered in pyrolysis is recognized as an important area of exploration. The present pyrolysis procedure has been adopted mainly because it gives reproducible results with the gas chromatograph-mass spectrometer system employed.

Experimental

A schematic diagram of the total

analysis system is shown in Figure 1. Pyrolysis products from the sample pyrolyzer are swept onto a gas chromatography column and the separated components are in turn eluted into the ion source of a rapid scanning mass spectrometer. The incorporation of a pyrolysis unit into the combined gas chromatography-mass spectrometry system provides for decomposition of sample, separation of the degradation products, and qualitative identifica-

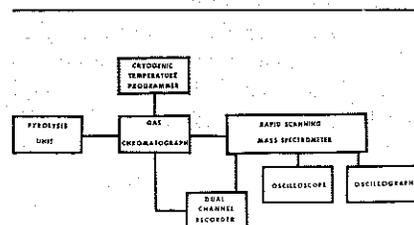


Figure 1. Schematic diagram of analysis system.

tion of these products by means of a single integrated operation.

Programming of the chromatography column temperature at sub-ambient temperatures is an especially important adjunct to the system since the mixtures to be separated contain compounds with a wide range of boiling points. Thus, the gaseous products from the pyrolyzer may be swept onto and condensed upon the cold column with carrier gas. This method of sample introduction provides an effective means of narrow band-on column injection and a chromatogram representing high efficiency and good resolution of the eluted compounds.

The use of a time of flight mass spectrometer, operating with a spectrum scan rate of 10,000 spectra per second, makes it possible to view the instantaneous spectrum on an oscilloscope screen, or with an analog gating system, to display a strip chart recording of the spectrum (in about 5 seconds) on the high speed, direct writing oscillograph.

Moreover, by means of an alternate cycle integrating circuit a tracing of the total ionization current at the spectrometer multiplier is recorded on one channel of a conventional two channel strip chart potentiometer recorder. The mass spectrometer output thus provides a chromatogram as well as a mass spectrum of the components of the mixture. The second channel is used to record the temperature of the chromatography column chamber during a programmed run.

The pyrolyses were conducted with a commercial pyrolysis unit, the Barber-Colman Model 4180, which was readily adaptable to the existing gas chromatography-mass spectrometry system by simply attaching it to the chromatography inlet. When using this system, the contents of the pyrolysis chamber may be swept onto the gas chromatographic column by manipulation of a single multi-position valve.

The pyrolysis products obtained varied widely in boiling point (from less than -180°C to more than $+150^{\circ}\text{C}$) and, therefore, cannot be separated readily under isothermal conditions, regardless of the efficiency demonstrated by the column. Therefore, it was necessary to program the column from a starting temperature of -180°C in order to effectively separate low boiling com-

pounds such as carbon dioxide, methane, and ammonia, all of which are commonly encountered in the pyrolyzed amino acid and peptide samples.

The chromatographic column used was an 8 ft., $\frac{1}{8}$ in. o.d. stainless steel column packed with 5% 1, 2, 3 TRIS (2-Cyanoethoxy) propane on 80/100 mesh untreated firebrick and was programmed from -180°C to $+125^{\circ}\text{C}$ at a rate of 6 deg/min. The temperature program was started concurrently with the introduction of the sample onto the column and continued until the end of the program at 125°C . The TRIS column was especially effective in separating mixtures with multiple functionality; therefore, the individual chromatographic peaks were sufficiently resolved to provide readily interpreted mass spectra.

Results and Discussion

A group of 17 amino acids and 10 peptides has been analyzed by the combined pyrolysis gas chromatography-mass spectrometry system described above.

The complexity of pyrolyzate obtained from the various amino acids ranges from compounds such as tyrosine, for which the product is 98% one compound, through cystine which gives 10-12 major components, and another 12 or more minor components. Despite the occurrence of more than one pyrolysis product for all the amino acids studied, in every case one product was found which was unique or constituted by far the major product among those identified.

The simple mono basic, mono carboxylic amino acids, and the major product from their pyrolysis are listed in Table I. In addition, carbon dioxide, acetonitrile, and propenyl nitrile are found in the pyrolyzate of nearly all the amino acids. Other compounds such as ammonia, methane, and nitrogen are also found to occur frequently. These components cannot provide a means of specific identification. The compounds listed in Table I, however, are unique for each of the amino acids and can thus serve to identify the corresponding amino acid.

The compounds resulting from

Table I

Amino Acid	Unique Pyrolysis Product
Glycine	Acetone
Alanine	Acetaldehyde
β -Alanine	Acetic acid
Valine	2-Methyl propanal
Norvaline	n-Butanal
Leucine	3-Methyl butanal
Isoleucine	2-Methyl butanal
Serine	Pyrazine
Threonine	2-Ethylethyleneimine
Taurine	Thiophene
Methionine	Methyl propyl sulfide
Cystine	Methyl thiophene
Phenylalanine	Benzene
Tyrosine	Toluene
Tryptophan	Ammonia, carbon dioxide
Proline	Pyrrole
Hydroxyproline	N-Methyl pyrrole

the pyrolysis of some trifunctional amino acids are also given in Table I. The presence of an hydroxyl group in serine and threonine or a sulfur containing group in methionine, cystine, and cysteine results in the production of more complex pyrolysis patterns than those produced by the simpler amino acids. The ease of decomposition seems to be increased by introduction of a third function into an otherwise simple aliphatic amino acid. Each of these five compounds pyrolyze under the standard conditions of analysis to give more than 20 components in its pyrolyzate. The majority of these components, however, are present in trace amounts and need not be considered in developing a useful qualitative analysis scheme.

The presence of sulfur dioxide, carbon disulfide, or carbonyl sulfide in the pyrolyzate serves as an indicator for the presence of sulfur-containing amino acids in protein and peptide material.

The major unique pyrolysis prod-

ucts of a group of aromatic and heterocyclic amino acids are also given in Table I. Most of the compounds formed are readily predicted. Tryptophan, however, under the conditions of analysis failed to yield pyrolysis products other than carbon dioxide and ammonia.

The dicarboxylic amino acids do not offer unique pyrolysis products of sufficient quantity to allow for positive identification. However, glutamic acid yields propene; propene spectra are prominent in the analysis of an insulin pyrolyzate; and glutamic acid is prominent among the amino acids in insulin.

A group of glyceryl-peptides was selected for checking out the usefulness of pyrolysis gas chromatography in studying a group of amino acids when they are combined by a peptide linkage to another amino acid. A list of representative glyceryl dipeptides and their corresponding major pyrolysis products is given in Table II. The compounds from the dipeptide pyrolyzates correspond to the major components previously identified from the component amino acids in their uncombined form. Thus, glycine yields acetone in every case, and the other amino acid in the dipeptide may be characterized by its respective unique pyrolysis derivative.

Investigation of the two dipeptide pairs, glycerylalanine:alanylglycine and glycerylleucine:leucylglycine, suggests that arrangement or sequence of an amino acid in the peptide affects the products from pyrolysis of the dipeptide. The dipeptide alanylglycine, for example, with carboxyl terminal glycine, produces acetaldehyde, previously seen to be associated with the pyrolysis of pure alanine. Ammonia is produced in large quantities. The glycine portion of the molecule yields acetone. When alanine is carboxyl terminal, as in glycerylalanine, ammonia is released in barely detectable amounts, and the main component is 2-methyl pyrrole. A simi-

lar situation exists for glycerylleucine and leucylglycine. In glycerylleucine, where leucine is carboxyl terminal, cyclopentane is formed.

Table II

Peptide	Pyrolysis Products
Glyceryl-glycine	Acetone
Glyceryl-valine	Acetone, 2-Methyl propanal
Glyceryl-proline	Acetone, Pyrrole
Glyceryl-methionine	Acetone, Methyl propyl sulfide
Glyceryl-serine	Acetone, Pyrazine
Glyceryl-tryptophane	Acetone, Ammonia
Glyceryl-alanine	Acetone, 2-Methyl pyrrole
Alanyl-glycine	Acetone, Acetaldehyde, Ammonia
Glyceryl-leucine	Acetone, Cyclopentane
Leucyl-glycine	Acetone, Acetic acid

The glyceryl moiety, as usual, gives acetone. On the other hand, in leucyl-glycine, acetic acid is a unique product of pyrolysis for this specific dipeptide sequence. It would appear that, at least in some cases, the pyrolysis products depend on the sequence of the acid in the peptide. If this behavior is more generally observed, pyrolysis procedures may also provide a valuable means of studying end group positions of certain amino acids in the peptide sequence.

As a test of the potentiality of this method of analysis on a rela-

tively large molecule, crystalline bovine insulin was selected for its known structure and variety of amino acid constituents. Table III lists the amino acids in the insulin molecule which were detected, based upon identification of the products in an insulin pyrolyzate. All these identifications were consistent with the known composition of insulin.

Table III

Amino Acid	Insulin Pyrolyzate Product
Glycine	Acetone
Phenyl-alanine	Benzene
Sulfur containing	COS, CS ₂
Proline	Pyrrole
Tyrosine	Toluene
Glutamic acid	Propene

The present work has demonstrated the feasibility of determining the amino acid composition of peptides and proteins by pyrolysis-gas chromatography. A study of the analysis of several typical proteins is now in progress. ■

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