New Techniques in Amino Acid, Peptide, and Protein Analysis

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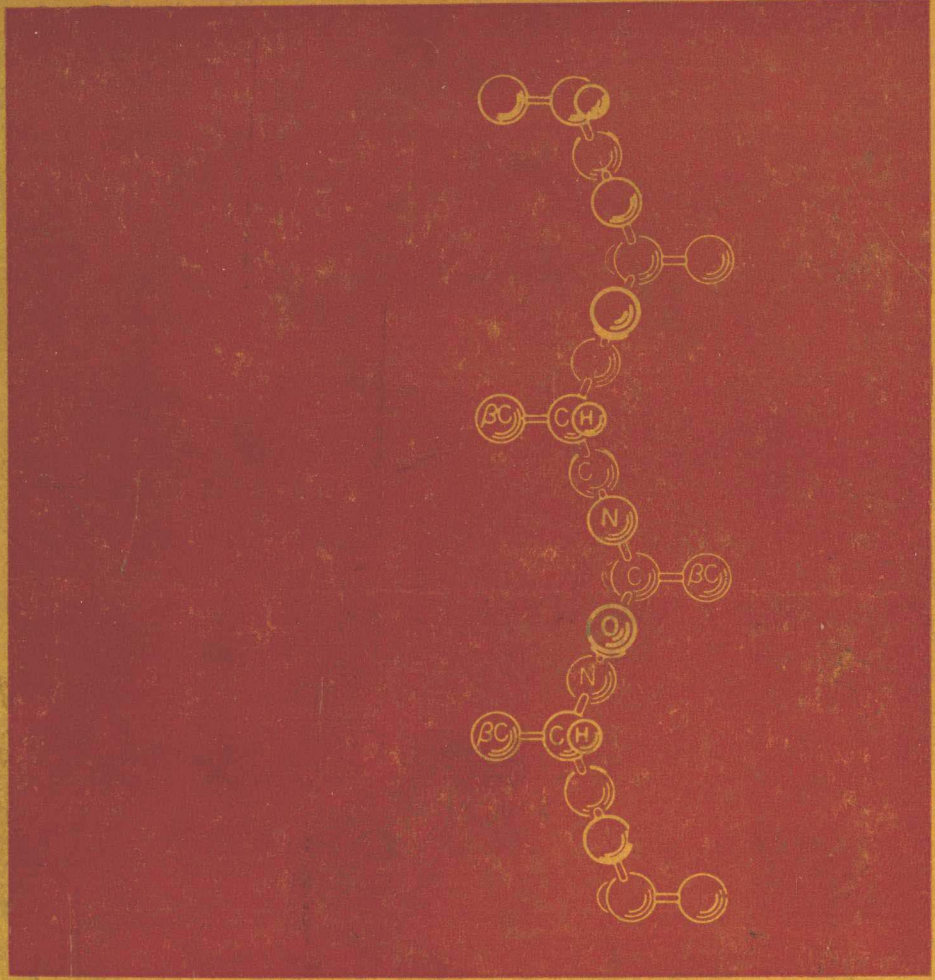

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edited by

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Preface

The exponential increase of scientific activity in the present decades forces the individual researcher to obtain a great part of his information from responsible reviews instead of reading the whole multitude of highly specialized papers. This holds also in the field of amino acid, peptide, and protein analysis. Every few years powerful new techniques are developed which are able to replace the older ones. For instance, automatic ion-exchange chromatography of amino acids according to Stein and Moore, which completely replaced microbiological assays, paper chromatography, and the other quantitative methods used before 1958, has now begun to find competition in gas chromatography. Polypeptide-sequence determination, a laborious task using conventional methods, can now be performed automatically by the Edman sequenator, and the sequence of smaller peptides can be determined most satisfactorily by means of mass spectrometry.

There is insufficient space in a single volume to treat comprehensively all the innovations of recent years. This book presents, instead, selected developments which are believed to be important for anyone working in this large field. All of the chapters have been written by experts who are able to summarize and pass judgment on current work in their specialties. The articles deal with amino acid and peptide analysis by ion-exchange chromatography (J. V. Benson and J. A. Patterson), gas chromatography of amino acids (J. R. Coulter and C. S. Hann), gas chromatography of peptides (B. Kolb), mass spectrometry of peptides (B. C. Das and E. Lederer), molecular-sieve chromatography (S. Hjertén), thin-layer gel filtration (B. G. Johansson), microelectrophoretic determination of proteins in the nanogram range (H. Hydén and P. W. Lange), electrofocusing of proteins (C. W. Wrigley), chemical accessibility and environment of amino acid residues in native proteins (K. Shibata), and methods for investigation of the quaternary structure of proteins (H. Sund).

We are confident that the book will be found useful and that the many efforts involved will be justified.

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G. Pataki

Contents

Chapter 1

Chromatographic Advances in Amino Acid and Peptide Analysis using Spherical Resins and Their Applications in Biochemistry and Medicine

by J. V. Benson, Jr., and J. A. Patterson

The massive work in ion-exchange resin chromatography, which still appears to be in the logarithmic stage of growth, rests firmly on the now classical works of Moore and Stein. An ingenious series of articles[1–13] starting in 1949 precipitated such an abundance of innovations in chromatographic technology that any attempt to cover all of them would far exceed the space limitations of this article. Many excellent reviews[14–25] and monographs[26–30] have been written covering history, theories, methods, and applications.

This chapter, therefore, will be selectively restricted to a discussion of those systems and techniques which, at present, appear to be not only highly satisfactory but also widely applicable. Included in this discussion are the variables which may be manipulated to achieve the results desired for specific analyses. "Playing" with several of these variables simultaneously may produce new and unique results for specific needs.

Finally, specific methodology is detailed to enable the reader to start acquiring data without spending a great deal of time experimenting with methods. While a great number of methods are present in the literature, the only ones included here are those with which we have had extensive experience in our own laboratory and found to be most satisfactory.

1.1 Applications

Ion-exchange column chromatography has been applied to a very diverse and large number of projects to provide basic biochemical

information and to aid in the diagnosis and management of biomedical disorders. The extent of the range of uses can be surmised by a perusal of several of the listed reviews.[14–34]

1.1.1 *Biochemical Applications*

Ion-exchange column chromatography has been applied to three very important areas: (1) qualitative and quantitative analyses of amino acids in peptides and proteins have permitted a useful characterization of molecules. They can be used as a means of detecting some of the specific differences among proteins; (2) the extra-protein amino acid composition of biological fluids not only supplies this basic information but allows one to monitor the changes as influenced by many factors such as environment, physiological state and genetic expression; and (3) the primary structure of proteins has assumed an overwhelming importance in biochemistry today. Many investigators are engaged in determining the amino acid sequence of a great variety of proteins. This will provide the actual chemical structure and permit studies relative to function.

There are many procedures available for accurate quantitative analyses of amino acids and peptides (see Section 1.3). Usually, the proteins or peptides are hydrolyzed to their constituent amino acids by treatment with acid or base, while biological fluids are usually deproteinized.

When studies are made to determine the amino acid sequence, partial hydrolysis using enzymes (such as pepsin, trypsin, chymotrypsin) or chemicals which are quite specific in their cleavage is employed. However, analyses of these hydrolysates provide incomplete information regarding sequence. Additional information can be obtained by C-terminal and N-terminal analyses. By producing different-sized peptides using a variety of enzymatic and chemical techniques, much of the sequence may be deduced from overlapping amino acid residues.

In one of Edman's sequencing procedures[35] the N-terminal amino acid is coupled with phenyl isothiocyanate and cleaved from the peptide with an anhydrous acid as the thiazolinone. The terminal amino acid is identified either by the subtractive method, in which the remaining peptide is sampled, hydrolyzed and analyzed chromatographically, or by converting the thiazolinone to a PTH (phenylthiohydantoin)-derivative which can be identified chromatographically. After cleavage the remaining peptide has a new N-terminal amino acid which can again be coupled and cleaved.

A number of interesting uses of such techniques have been described by Smyth,[36] Benisek,[37] and others.[38–41]