ADVANCES IN PROTEIN CHEMISTRY

EDITED BY

M. L. ANSON
Continental Foods, Hoboken

JOHN T. EDSALL
Harvard Medical School, Boston

VOLUME II

1945

ACADEMIC PRESS INC., PUBLISHERS
NEW YORK, N. Y.

First Printing, 1945
Second Printing, 1947
Third Printing, 1953

Printed in the United States of America

THE MURRAY PRINTING COMPANY
WAKEFIELD, MASSACHUSETTS

ADVANCES IN PROTEIN CHEMISTRY

VOLUME II

CONTRIBUTORS TO VOLUME II

M. L. Anson, *Continental Foods, Hoboken, New Jersey*

M. J. Blish, *Amino Products Division of International Minerals and Chemical Corporation, Rossford, Ohio*

Richard J. Block, *New York Medical College, Flower and Fifth Avenue Hospitals, New York, New York*

Paul R. Cannon, *Department of Pathology, The University of Chicago, Chicago, Illinois*

C. R. Dawson, *Department of Chemistry, Columbia University, New York, New York*

John T. Edsall, *Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts*

I. Fankuchen, *Polytechnic Institute of Brooklyn, Brooklyn, New York*

Sidney W. Fox, *Chemistry Department, Iowa State College, Ames, Iowa*

Dexter French, *Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts*

M. F. Mallette, *Department of Chemistry, Columbia University, New York, New York*

A. J. P. Martin, *Wool Industries Research Association, Torridon, Headingley, Leeds, England*

Karl Meyer, *Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, New York*

Esmond E. Snell, *The University of Texas, Austin, Texas*

R. L. M. Synge, *Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, England*

CONTENTS

The Amino Acid Composition of Food Proteins

By Richard J. Block, *New York Medical College, Flower and Fifth Avenue Hospitals, New York, New York*

The Relationship of Protein Metabolism to Antibody Production and Resistance to Infection

By Paul R. Cannon, *Department of Pathology, The University of Chicago Chicago, Illinois*

Terminal Amino Acids in Peptides and Proteins

By Sidney W. Fox, *Chemistry Department, Iowa State College, Ames, Iowa*

The Copper Proteins

By C. R. Dawson and M. F. Mallette, *Department of Chemistry, Columbia University, New York, New York*

Mucoids and Glycoproteins

By Karl Meyer, *College of Physicians and Surgeons, Columbia University, New York, New York*

The Reactions of Formaldehyde with Amino Acids and Proteins

By Dexter French and John T. Edsall, *Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts*

Wheat Gluten

By M. J. Blish, *Amino Products Division of International Minerals and Chemical Corporation, Rossford, Ohio*

Protein Denaturation and the Properties of Protein Groups

By M. L. Anson, *Continental Foods, Hoboken, New Jersey*

X-Ray Diffraction and Protein Structure

By I. Fankuchen, *Polytechnic Institute of Brooklyn, Brooklyn, New York*

Analytical Chemistry of the Proteins

By A. J. P. MARTIN

Wool Industries Research Association, Torridon, Headingley, Leeds, England

and

R. L. M. SYNGE

Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, England

Contents

1. Introductory

An account of protein analysis is probably best introduced with a discussion of the purpose of the analysis. Work in recent years in the various fields of science grouped around biochemistry has demonstrated both the physicochemical homogeneity and specificity of the proteins and the multitude of their active, specific roles in living organisms. Attention has consequently been focussed on the problem of their detailed structures. In elucidating these, exact knowledge of the nature and number of the amino acid residues composing proteins plays a similar role to knowledge of the nature and number of the component atoms in structural studies

of simpler molecules. The techniques of amino acid analysis are not yet, however, as reliable as those of elementary analysis. We attempt here to review their present condition, and to indicate probable directions of progress. Much improvement in recent years has undoubtedly been stimulated by the desire, variously, to prove or disprove the speculative hypothesis of protein structure of Bergmann and Niemann, insofar as it deals with overall amino acid composition. Present analytical methods are barely equal to this task, even for particular amino acids. There is good reason to hope, however, that in a few years the problems of analysis of protein hydrolyzates or amino acids will have been solved: the emphasis may well shift to the problem of the relation of the composition of the hydrolyzate to that of the protein from which it was derived (cf. paras. 3, 4).

The present review deals only with this problem of determining the nature and number of the component amino acid residues of proteins and related compounds. No attempt is made to deal with 'higher' aspects of protein structure. It should, however, be pointed out that many of the techniques for separating amino acids are also suitable for the much more difficult tasks of separating the peptides resulting from the partial hydrolysis of proteins; some of these techniques have in fact been developed with this as their primary aim. Studies of partial hydrolysis products are likely to be very fruitful for the detailed elucidation of protein structure (cf. 1).

That reliable methods of amino acid analysis should be available is important also for agricultural, clinical, and nutritional work. In these disciplines, accuracy may often profitably be sacrificed in favor of speed and simplicity of manipulation.

In connection with metabolic studies making use of isotopes it is desirable that methods should be available for isolating every amino acid in a high state of purity and from all kinds of biological material. Special methods are also required for checking the purity of 'pure' amino acids.

We attempt here to review advances that have been made in this branch of protein chemistry during the last 15 years. From the qualitative standpoint, Vickery and Schmidt (2), and from the quantitative, Mitchell and Hamilton (3), have given admirable accounts of the position at the beginning of this period.

We hope, by having made the bibliography as comprehensive as possible, to give this review a value separate from any it may have as an expression of our opinion on the most interesting and valuable directions of technical progress. On these topics we have written at length, while others have been dismissed with a briefness which in some cases does them less than justice.

Block and Bolling (3a) have very recently published a reference work bringing together many methods and results of amino acid analysis of

proteins, chiefly from the nutritional standpoint and grouped according to amino acids. Their work is in many ways complementary to the present review, in which we attempt a critical discussion, grouped according to techniques, of the means by which we may hope to arrive at absolute figures for the amino acid residues constituting individual proteins.

2. The Amino Acids Occurring in Nature

For some years there has been increased reason for believing that only few, if any, new amino acid constituents will be discovered in the better known proteins, or are at all widely distributed among living organisms, and we are confirmed in this opinion by the results of 'two-dimensional' partition chromatography (see para. 5.3.4) applied to hydrolyzates of a variety of protein materials. It seems certain, however, that numerous new amino acids will continue to be found that have a limited distribution — particularly in higher plants, fungi, and micro-organisms. In the present section of this review we discuss evidence bearing on this subject subsequent to Vickery and Schmidt's very full review (2) and Dunn's supplementary notes (4), cf. also (5). In the majority of cases, products isolated by procedures not themselves destructive to a postulated precursor may reasonably be regarded as structural components of the intact protein. Doubtful cases, and products derived from altered proteins, are discussed individually below.

Not considering amino acids of abnormal optical form, the only changes required to bring Vickery and Schmidt's 'accepted' list of protein constituents up to date are that threonine should be inserted and hydroxyglutamic acid removed.

Vickery and Schmidt do not mention Leuchs' later work establishing by synthesis that 'naturally occurring' *hydroxyproline* is one of the 2 stereoisomers of γ-hydroxy-*l*-proline (6). The configuration at the γ-C atom of 'natural' hydroxyproline is the only remaining structural prob'em concerning the 'accepted' protein constituents, and recent work (7, 8) suggests that the —OH and —COOH groups may lie *trans* in relation to the pyrrolidine ring.

The presence of a common '*l*-' configuration at the α-C atom has been demonstrated for the amino acids usually found in nature. The varied experimental approaches on which this conclusion is based fall outside the scope of this article (cf. 9).

Citrulline, $H_2N{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$. There seems no doubt that free *l*-citrulline occurs in nature. Citrulline was first described in water-melon press-juice by Wada (10). It is also probable (11, 12) that it is an important intermediary of animal metabolism. However, the citrulline isolated by Wada (13) from a tryptic casein digest may have

arisen by degradation of arginine residues in peptide linkage. There is reason (14) for disbelieving Wada's statement that proline is the main product on treating citrulline with hot mineral acid. The only other evidence that citrulline occurs in proteins seems to be Fearon's (15) color reaction, characteristic of substituted ureas and given by all proteins that have been tested (cf. 16). The possible occurrence of *carbamic acid*, $H_2N{\cdot}COOH$, as a protein constituent deserves serious consideration (17). Peptides of this amino acid would presumably give the Fearon reaction. Citrulline (δ-carbamyl-ornithine) is merely a special case of such a peptide. Model experiments (17, 14) suggest that on acid hydrolysis peptides of carbamic acid do not yield CO_2 or NH_3 stoichiometrically, so the CO_2 evolved in acid hydrolysis of proteins (18) does not set an upper limit to the carbamic acid residues possibly present. The whole problem of the possible occurrence of urea groupings in proteins deserves systematic study.

Wada (19) described *'prolysine,'* $HN{\cdot}CO{\cdot}CH{\cdot}(CH_2)_3{\cdot}CH(NH_2){\cdot}COOH$, with $OC{-}{-}NH$ forming a ring,

as occurring in casein and gelatin hydrolyzates. Nothing further has been published by other workers about this (cf. 20).

Ornithine, $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$. This amino acid, like citrulline, is a known metabolite and is a constituent of ornithuric acid. δ-Monoacetylornithine has been isolated from plant material (21). The presence of ornithine in hydrolyzates of alkali-treated or otherwise altered proteins (cf. 22) can be attributed to the breakdown of arginine residues. The ornithine isolated from acid hydrolyzates of tyrocidine (14) and 'gramicidin S' (23, 109a) may also have originated by such a breakdown during autolysis of the parent bacteria. Failure to detect ornithine in protein hydrolyzates may often have been the consequence of inadequate analytical procedures; however, a recent thorough examination of the products of acid hydrolysis of egg albumin failed to reveal any (16).

Canavanine, $H_2N{\cdot}C(:NH){\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$. Dunn (4) gives references to the isolation, proof of constitution and synthesis of this amino acid, which occurs free in soya-bean meal, etc. (cf. also 24–29).

Octopine, $H_2N{\cdot}C(:NH){\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH(COOH){\cdot}NH{\cdot}CH(CH_3){\cdot}COOH$. Irvin and Wilson (30) provide a bibliography of this compound which occurs free in octopus and scallop muscle. Syntheses (cf. also 31) have shown its structure. Karrer, *et al.* (32, 33), on the basis of enzyme and model experiments, have been unable to determine the optical configuration of the alanine moiety. The arginine moiety is *l*-arginine.

Hydroxylysine. Early reports of the isolation of a base of this character are mentioned by Dunn (4). Subsequently, Van Slyke and colleagues (34–36) have isolated from hydrolyzates of gelatin a base which may have

this constitution, although its carbon skeleton has not yet been identified. Its dissociation constants have been determined, and it has been shown, on treatment with periodate, to yield half its N as NH_3, together with one molecule of formaldehyde. Van Slyke and colleagues suggest that it is either $\alpha\delta$-diamino-ϵ-hydroxy-caproic acid or δ-hydroxylysine. They have determined it quantitatively in hydrolyzates of a number of proteins (37). The quantities present are small, even in the richest known source, gelatin. We have isolated material agreeing in properties (38) from the 'lysine' base-precipitation fraction of gelatin hydrolyzates by solvent extraction as its NN^1-diacetyl-*O*-benzoyl derivative.

If the compound is δ-hydroxylysine, it will be interesting to compare the configuration of the δ-C atom with that of the γ-C atom of hydroxyproline, which is perhaps formed *in vivo* from γ-hydroxyornithine, the next lower homologue of δ-hydroxylysine. In this connection, the possible occurrence (39) of a *hydroxyarginine* in clupein is of interest.

Dunn (4) gives references to the isolation, structural characterization and synthesis of *threonine*, $CH_3{\cdot}CH(OH){\cdot}CH(NH_2){\cdot}COOH$ (cf. also 40–42). Threonine has subsequently been isolated from myosin (43), an *Aspergillus* autolyzate (44) and from human blood-group A substance (45), and must accordingly be added to the list of 'accepted' protein constituents. Threonine has been recognized through its reaction with periodate (para. 5.6.1) as a very widely distributed protein constituent. Higher homologues of threonine were not detected in a number of proteins after a specific search (38).

The periodate reaction has at the same time made it probable that *β-hydroxyglutamic acid* is absent from casein (46, 47) and it is desirable that this amino acid, at least for the present, should be withdrawn from the 'accepted' list (cf. 5). Bailey, *et al.* (48) sum up the present situation, and suggest a possible explanation of some of the reports of its occurrence (cf. also 49).

Dakin (49a) mentions the isolation from a casein hydrolyzate of material that might be a *hydroxyleucine*.

Jacobs and Craig (50) obtained $\beta\beta$-dimethylpyruvic acid or pyruvic acid together with NH_3 by alkaline degradation of various ergot alkaloids, and suggested that these arose by deaminative degradation of unstable α-hydroxyvaline and α-hydroxy-alanine residues respectively. Since pyruvic acid can result from the alkaline degradation of serine (cf. para. 4) it is simpler to postulate *β-hydroxyvaline* and serine as the precursors of these keto acids (cf. 51). Earlier claims (2, 4, 52; cf. 53–55) to have isolated hydroxyvaline from proteins are inadequately supported, and require, like similar claims in respect of *α-aminobutyric acid* (2, 4, 55, 56; cf. 57), *norvaline* (α-aminovaleric acid) (2, 4, 55, 58) and *norleucine* (α-aminocaproic acid)