AMINO ACIDS and PROTEINS

THEORY • METHODS • APPLICATION

Compiled and Edited by

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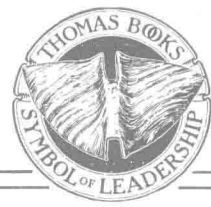

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PREFACE

IMPORTANT GOALS of the researches on proteins are to determine their chemical composition and structure, to develop improved methods of isolation, to establish their purity, to measure their size and shape, to explore their physical and chemical characteristics, to develop precise methods for the analysis of their component units and products, and to evaluate the functions and properties of biologically important proteins, and of protein derivatives.

This book is devoted to the presentation of the methods and results of the main channels of effort by which these goals are being achieved or, at least, approached. It is intended to acquaint students and investigators with the more general methods employed in the study of the proteins and to give a summary of the current knowledge and advances of the subject matter. Topics requiring advanced mathematical treatment have not been included.

This book is a cooperative effort by a group of men well known for their contributions to the special subject matter of the book on which they have written.

May I also point out that the present work stems from the deep and abiding interest in the study of the proteins in the belief that it is a key to an understanding of the phenomenon of life which has been a tradition of the Biochemistry laboratory of our University from its inception. This interest was kindled by the pioneer efforts of such notables as Jaques Loeb and T. Brailsford Robertson. The inspiration received from these men was passed on to and maintained in his pupils by the late Carl L. A. Schmidt, among whom I have the honor to be numbered. The publication of books on proteins from the Biochemistry laboratory is traditional. I hope the present volume is a worthy successor to its predecessors.

Lastly, it gives me great pleasure to acknowledge the valuable assistance of Dr. Theodore Winnick in the editorial work of preparing many of the chapters for publication.

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AMINO ACIDS and PROTEINS

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Chapter I

PROPERTIES OF AMINO ACIDS

E. E. Howe, Ph.D.

I. NOMENCLATURE

THE NOMENCLATURE of the amino acids is at present emerging from a state of confusion. Originally, upon the discovery and isolation of an amino acid, its direction of rotation was determined and the new compound was prefixed d- or l- depending upon whether it was dextro- or levo-rotatory. As the configurational relationship of the amino acids was gradually elucidated it developed that all of the natural acids isolated from proteins, very probably, have the same configuration about the α-carbon atom and that this configuration is identical with that of l(+)-lactic acid. Accordingly, this relationship was expressed by the prefix l- regardless of direction of rotation and the proteinogenic amino acids became known as the l-series.

In order to prevent confusion with the old system, the direction of rotation was indicated by + or − signs in parentheses so that the glutamic acid found in protein hydrolysates, for example, known as d-glutamic acid by the old system became l(+)-glutamic acid by the new. Since the rotation of an amino acid varies in acid and alkaline solution and with temperature, it was necessary before the compound could be properly named to define the conditions under which the rotation was determined. Unfortunately there is some difference of opinion concerning these conditions. Dunn (78) has suggested that the sign of the prefix be determined by the direction of rotation of the free amino acid in water at 25°. On the other hand, since the observed rotation is often more consistent when determined in the presence of an excess of acid or base, Clarke (53) advocated that the sign of rotation of an amino acid be determined in the presence of four or more equivalents of acid.

In 1936 the situation was further complicated when upon the

isolation and clarification of the structure of natural threonine (167) it was designated as d(−)-threonine because of its spatial relationship to d(−)-threose. The spatial arrangement of the groups about the α-carbon atom of this compound is identical with that of the other natural amino acids and it therefore belongs to the l-series.

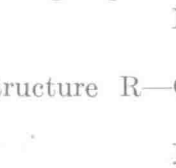
Until recently no group or organization had taken a definite stand and adopted a well defined system of nomenclature with the consequence that both systems are still appearing in the literature.

In the opinion of many, neither system is desirable. If the old system is used the reader must know beforehand the direction of rotation of the amino acid before he can identify it with respect to series or family which, after all, is the only thing he wishes to do. The new system often becomes confused with the old, is unnecessarily long, awkward, and makes no definite provision for the naming of derivatives, i.e., it does not indicate whether they should be given the sign of rotation of their parent substances or should be named according to their own sign of rotation.

Recently, under the leadership of Dr. H. B. Vickery, one of the the editors of the *Journal of Biological Chemistry*, the American Chemical Society's Committee on Nomenclature has approved a set of rules for the clarification of amino acid nomenclature (12, 234). These rules, although necessarily detailed to cover all contingencies, are simple and logical, consisting, in essence, of the use of the small capital letters D and L to indicate the two series or families of the amino acids and their derivatives. This method of nomenclature is in keeping with the system adopted by the American Chemical Society for the carbohydrate series and will be used throughout this volume.

II. CHEMICAL STRUCTURE

To an organic chemist an amino acid is any of a large group of compounds which contains one or more amino groups and one or more acid groups. To the biochemist, however, this term applies only to a very limited number of compounds, all of which have in common an amino group in the α-position to a carboxyl group, i.e., the general structure



In addition, they are all

products of hydrolysis of proteins or are very closely related to these products.

In 1931 Vickery and Schmidt (238) set up certain standards to be met before an amino acid be recognized as a constituent of a protein hydrolysate. These included isolation by some worker other than the discoverer, characterization by suitable derivatives, establishment of structure by synthesis, and if possible, demonstration of utilization by the animal body.

Later Vickery (233) prepared an excellent resume of the status of the various amino acid components of proteins and separated them into four groups as follows: 1) Eighteen amino acids concerning which there is no doubt whatever; 2) Seven amino acids which occupy a special position because of their narrow range of distribution or for other reasons; 3) Five amino acids found in plants that may be constituents of proteins; and 4) Seventeen amino acids for which claims have not been substantiated.

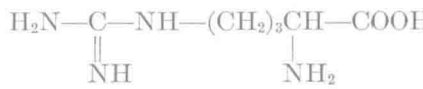
It is beyond the scope of this chapter to consider all of these compounds. It is intended here to discuss only those substances which are of general practical importance to the protein chemist and nutritionist, although some of them have not been established as constituents of protein hydrolysates. They are twenty-eight in number and are listed alphabetically.

1. *Alanine* (α-aminopropionic acid)

In 1850, Strecker (219) in an attempt to synthesize lactic acid treated acetaldehyde ammonia with a mixture of hydrocyanic and hydrochloric acids and obtained instead a crystalline substance which he named alanine. The discovery that this substance is a constituent of most proteins did not come until some years later. In 1875, Schützenberger and Bourgeous (205) isolated from a silk hydrolysate a crystalline substance whose elementary analysis agreed with that of alanine. They characterized it no further and therefore it is Weyl (246) who is usually credited with the demonstration of the presence of alanine in protein hydrolysate. In 1888, he crystallized it from an acid hydrolysate of silk. He reported that it was optically inactive and that its decomposition point was identical with that of synthetic DL-alanine. He also prepared and analyzed the copper salt (247). Fischer and Skita (101) repeated the isolation and obtained an optically active alanine, the structure

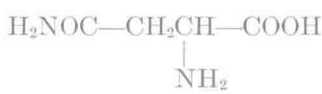
of which they established irrevocably by conversion to lactic acid.

2. *Arginine* (α-amino-δ-guanidovaleric acid)



Schulze and Steiger (212) first isolated arginine in 1886 from etiolated lupine seedlings. It was not until 1895, however, that Hedin (125) obtained it from an acid hydrolysate of a protein. Two years later Schulze and Winterstein (213) established its structure by demonstrating that the products of alkaline treatment were ornithine and urea. This structure was confirmed by Sörensen (217) in 1910, who synthesized the molecule from benzoyl ornithine by condensation with cyanamide and subsequent removal of the benzoyl group by acid hydrolysis. Gulewitsch (120) in 1899 was the first to prepare free crystalline arginine.

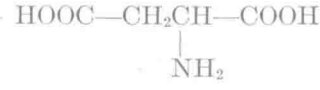
3. *Asparagine* (α-aminosuccinamic acid)



This substance was isolated by Vauquelin and Robiquet (232) from the juice of asparagus in 1806. Although it was long considered to be a constituent of proteins it could not be isolated from acid or basic hydrolysates because of the ease with which it is converted to aspartic acid. Actual proof of its presence in the protein molecule came in 1932 when Damodoran (69) isolated it from an enzymatic hydrolysate of edestin. Its structural relationship to aspartic acid was shown by Piria (184) in 1848 and this structure was confirmed by Piutti (186) who prepared it from the β-monomethyl ester of L-aspartic acid by the action of alcoholic ammonia.

Asparagine, with glutamine, is found in high concentration in etiolated seeds. Asparagine is converted to aspartic acid and ammonia by the enzyme asparaginase.

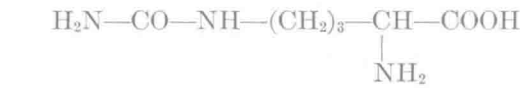
4. *Aspartic acid* (α-aminosuccinic acid)



Although aspartic acid had long since been obtained from asparagine by acid hydrolysis it was first shown to be a constituent

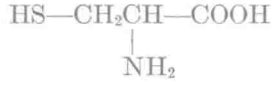
of protein hydrolysates by Ritthausen (194) in 1868. Its structure was established by Piria (184) in 1848. It was first synthesized by Dessaignes (72) who obtained it by the dry distillation of the ammonium salts of maleic, fumaric, and malic acids. Confirmation of structure by synthesis did not come however until 1887 when Piutti (187) prepared the inactive compound by reduction of oximinosuccinic acid diethyl ester. The synthetic compound was shown to be identical with a preparation obtained from optically active aspartic acid by racemization with hydrochloric acid at 180°.

5. *Citrulline* (δ-carbamidonorvaline)



This amino acid was isolated by Koga and Odake (145) from the juice of the watermelon in 1914 and later from a casein hydrolysate by Wada (243). Its importance to the amino acid chemist lies, however, not in its being a constituent of proteins which is not well established but in the fact that it is almost certainly an intermediate in the formation of urea in the animal body (149). Wada (242) not only determined the structure of citrulline by converting it to ornithine by alkaline hydrolysis but also confirmed this structure by synthesis from α-monobenzoylornithine and urethane. Citrulline may be conveniently prepared from arginine by boiling with an equimolecular quantity of alkali in 2.8 *N* solution (108). The amino acid so obtained is optically inactive as was the material isolated by Wada.

6. *Cysteine* (β-mercaptoalanine)

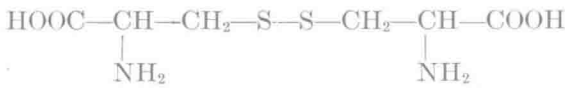


Cysteine was obtained from cystine by reduction with tin in hydrochloric acid by Baumann (19) in 1883. The researches of Neuberg (173) and Friedmann (112) contributed greatly to the elucidation of its structure and in 1904 the suggested structure was proved by Erlenmeyer (86) who synthesized it from benzoylserine. Cysteine in neutral or basic solution is readily oxidized to cystine by the oxygen of the air.

Cysteine has not definitely been shown to be a constituent of proteins although its presence is indicated by the fact that a few

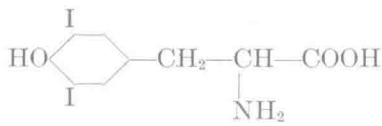
native proteins give a positive nitroprusside test. Mirsky and Anson (169) have presented evidence to show that the sulfhydryl content of proteins does not come from reduction of the disulfide, but exists *per se*. Cysteine is a constituent of the physiologically active tripeptide, glutathione, and in this or other combinations plays a role in oxidation-reduction reactions of the body.

7. *Cystine* (β,β'-dithiobisalanine)



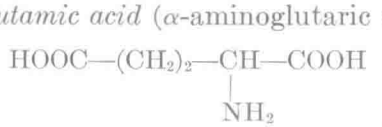
Cystine was the first amino acid to be discovered, having been isolated by Wollaston in 1810 from a urinary calculus. It was not until 1899, however, that Mörner (170) reported its isolation from an acid hydrolysate of horn. Its relationship to cysteine was established in 1883 (19) and its structure was verified by Erlenmeyer (85) who obtained it from benzoylserine without the isolation of the intermediate cysteine. The cystine content of the keratins is very high and, by cross linking, this amino acid is thought to be responsible for the hardness and toughness of these proteins.

8. *Diiodotyrosine* [β-(3,5-iodo-4-hydroxyphenyl) alanine]



In 1896 Drechsel (75) isolated diiodotyrosine from a species of coral, *Gorgonia Calvolinci*, and called it iodogorgoic acid. Since the determination of structure and synthesis of this compound by Wheeler and Jamieson (248) in 1905 the two terms have been used interchangeably. Diiodotyrosine is easily converted to tyrosine by warming with hydriodic acid. Apart from its presence in certain marine animals it occurs only in the thyroid gland protein and is of importance as a possible precursor of thyroxine.

9. *Glutamic acid* (α-aminoglutaric acid)

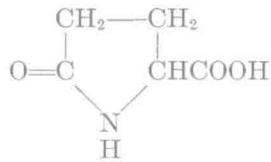


Ritthausen (193) was the first to isolate glutamic acid having obtained it in 1866 from an acid hydrolysate of wheat gluten.

Later he obtained it from other proteins, prepared its barium, copper, and silver salts, and established its empirical formula (194). Dittmar (73) reduced the product obtained by the action of nitrous acid on glutamic acid and Markownikoff (166) proved that this reduction product was identical with glutaric acid. Wolff (256) in 1890 synthesized glutamic acid from levulinic acid, deaminated it with nitrous acid and proved the hydroxy acid he obtained to be identical with that derived from the natural product.

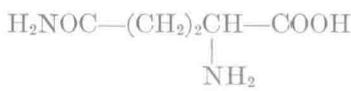
Glutamic acid occurs widely in nature and in especially high concentration in cereal proteins. It is of considerable economic importance because its monosodium salt is used widely as a condiment.

In solution over a wide pH range glutamic acid is converted by boiling to optically active pyrrolidonecarboxylic acid (pyroglutamic acid) (253). At pH 4 equilibrium is reached when about 98% of the glutamic acid has been lactamized.



Solid L-glutamic acid upon heating to 160°, melts and is converted to L-pyrrolidonecarboxylic acid; while increasing the temperature to 190° racemizes the product (7, 14).

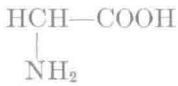
10. *Glutamine* (α-aminoglutaramic acid)



Glutamine (13), like asparagine, was isolated and characterized long before its presence in the protein molecule was established. It was obtained from beet juice in 1883 (209) and while it was long agreed that most of the glutamic acid found in proteins existed as glutamine positive proof was not forthcoming until 1932 when Damodoran, Jaaback and Chibnall (70) isolated it from an enzymic digest of gliadin. The structure of glutamine was clarified in 1900 by Schiff (202) and it was converted to glutamic acid by Micko (168) in 1908 by hydrolysis with barium hydroxide. Its synthesis from L-glutamic acid was accomplished by Bergmann and his collaborators (30) by application of their ingenious carbobenzoxy synthesis.

Glutamine upon heating in solution to 100° at pH 6.5 is rapidly converted to the ammonium salt of L-pyrrolidonecarboxylic acid (235). Upon treatment with nitrous acid in acetic acid it yields nitrogen equivalent to 180% of its amino nitrogen (13), a unique property in that amides including asparagine react with nitrous acid only in the presence of strong mineral acids.

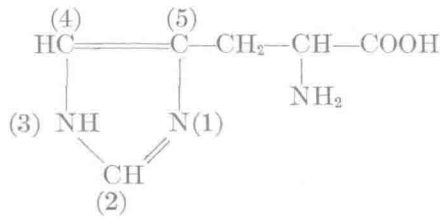
11. *Glycine* (aminoacetic acid)



Glycine, the simplest of protein constituents, is the only amino acid which contains no asymmetric carbon atom. It was isolated by Braconnot (37) in 1820 from a sulfuric acid hydrolysate of gelatin. It was probably the first amino acid to be obtained from a protein hydrolysate in a pure state. Cahours (44) in 1857 established the structure of the molecule and a year later he synthesized it from chloroacetic acid and ammonia.

Glycine, apart from its role as a constituent of most proteins, is of great interest as one of the precursors of creatine (33) which plays an important part in muscle contraction.

12. *Histidine* (α-amino-β-5-imidazolepropionic acid)



This amino acid was discovered in 1896 by two investigators independently. Kossel (148) obtained it from protamines while Hedin (126) isolated it from the acid hydrolysates of other proteins. Pauly (181) established that histidine contained an imidazole (glyoxaline) group and that the molecule through this group coupled with diazotized sulfanilic acid to yield a highly chromogenic compound. Its constitution was determined mainly by the work of Frankel (111), Pauly (181), and Knoop and Windaus (144). In 1911 Pyman (191)synthesized it from citric acid and resolved the racemic compound into its optically active isomers. Histidine, serine and threonine alone of the free amino acids give the biuret test.