

BIOCHEMISTRY
of
THE AMINO ACIDS

by **ALTON MEISTER**

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PREFACE

The amino acids have been the subject of considerable study by biochemists in recent years. The literature of this field is voluminous and provides ample evidence of the striking advances which have been made since the isolation of the first amino acid one hundred and fifty years ago. In this volume, the author has attempted to present a comprehensive picture of the present status of the biochemical interrelationships and transformations of the naturally occurring amino acids.

The subject matter of this volume is organized in five major sections. The first chapter is devoted to a consideration of the amino acids which occur in nature and the forms in which these have been found. Attention is given to the general properties of amino acids, including stereochemical considerations, which have profound biological significance. The second chapter is concerned with observations resulting from nutritional studies. The third and fourth chapters summarize the present state of knowledge of the metabolism of the amino acids, including their synthesis and degradation, and the relationships among the amino acids and other metabolites. The final chapter deals with abnormalities of amino acid metabolism associated with disease states; this discussion is intended to supplement the presentation of "normal" metabolism in earlier chapters.

Our knowledge of amino acid biochemistry appears, in general, to have developed in several phases. The first of these was the isolation and characterization of amino acids which occur in nature. Subsequently, nutritional studies on experimental animals and microorganisms were performed. These investigations, which began with the observation that proteins were necessary dietary components for animals, were facilitated by amino acid analyses of proteins and improved techniques of amino acid isolation. Such studies led to the discovery of new amino acids and established that certain amino acids could be synthesized by the organism while others could not. They also provided important clues to metabolic interrelationships. The application of isotope procedures ushered in a new phase of study, which resulted in recognition of the dynamic state of the body proteins and in the elucidation of many individual biochemical reactions. The cellular catalytic components responsible for some of these reactions are now known. Genetic, isotopic, and chemical procedures have uncovered mechanisms involved in the synthesis and metabolism of amino acids by microorganisms. It is noteworthy that intermediary metabolism of the

amino acids is often (though not always) similar in widely different species.

Obviously these "phases" have overlapped and contributed to each other. Thus, although the 22 amino acids which commonly occur in proteins were identified by 1935, more than 80 natural amino acids (at least half of which were discovered in the last ten years) are now known. Chromatographic methods and new synthetic organic techniques have greatly contributed to recent progress. Nutritional approaches continue to serve significantly in genetic and metabolic studies and in work on amino acid antagonists. Notable is the recent extension of nutritional studies to man and to human cells grown in tissue culture.

In a book such as this, it is necessary to exclude from consideration, sometimes arbitrarily, some areas which overlap with and relate to the basic subject matter. Accordingly the chemical and physical properties of amino acids have received only superficial treatment. The most closely related area not extensively covered here is that of the proteins. This subject has been masterfully presented in the recently published four-volume work, *The Proteins*, edited by H. Neurath and K. Bailey. While much information concerning proteins is available, there is no definitive knowledge of the mechanisms of protein synthesis. It is probable that the events leading to the synthesis of proteins represent some of the most important reactions of the amino acids. This problem must await a new phase of investigation.

This book owes its origin to a review on amino acids written by the author in 1951, while he was a member of the National Institutes of Health. The contributions of many of his colleagues there through valuable and stimulating discussions over a period of ten years have been of inestimable value. The author is particularly indebted to his former chief, Dr. Jesse P. Greenstein, for his guidance and encouragement in those years.

The cogent and constructive criticisms of the entire manuscript offered by Dr. Sidney Weinhouse are gratefully acknowledged. The author also wishes to thank his colleagues in the Department of Biochemistry of Tufts University School of Medicine, especially Dr. Kivie Moldave, Dr. Richard Peabody, and Dr. A. N. Radhakrishnan, and also Dr. Carl G. Baker and Dr. Sidney Udenfriend of the National Institutes of Health for their valuable suggestions and for help in editing the manuscript. Special acknowledgment is due the author's wife, Leonora Garten Meister, for her tireless efforts in editing the manuscript, reading the proofs, and compiling the subject index.

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CHAPTER I

THE NATURAL AMINO ACIDS

A. GENERAL CONSIDERATIONS

An amino acid may be defined as a chemical compound which possesses amino and acid groups. Most of the natural amino acids are described by



the general structure, $\text{R}-\text{C}-\text{COOH}$, in which the amino group is in the



α -position to a carboxyl group. Exceptions to this generalization include amino acids which possess more than one carboxyl or amino group, several possessing imino groups, those which have other types of acid groups, and several with amino groups not attached to the α -carbon atom. The majority of the amino acids possess at least one asymmetric carbon atom, and may therefore exist in at least two optically active forms. Although most of the natural amino acids are of the L-configuration, a number of D-amino acids also occur in nature.

The nomenclature of the amino acids may be considered from two points of view. First, it is customary for biochemists to use a series of trivial names for the amino acids. Such terminology represents a useful simplification; as in any special area of scientific work, a knowledge of the accepted professional jargon is essential. Secondly, it is usually necessary and desirable to indicate the optical configuration of an amino acid, where known, by means of the small capital letters D or L. If the configuration is not known, an amino acid may be designated *d* or *l*, depending upon the direction of its optical rotation in water. Some confusion has attended the designation of optical configuration and rotation. This subject will be considered in greater detail in Section G.

This chapter will be devoted to a discussion of the amino acids which have been found in nature. Asparagine and cystine, the first two amino acids to be recognized, were discovered in 1806 and 1810, respectively. Since this time, the existence of more than 80 amino acids has come to light. The "discovery" curve (Fig. 1) shows a dramatic rise in the recognition of new amino acids in the last decade. On the other hand, the curve describing the recognition of the major amino acid constituents of protein

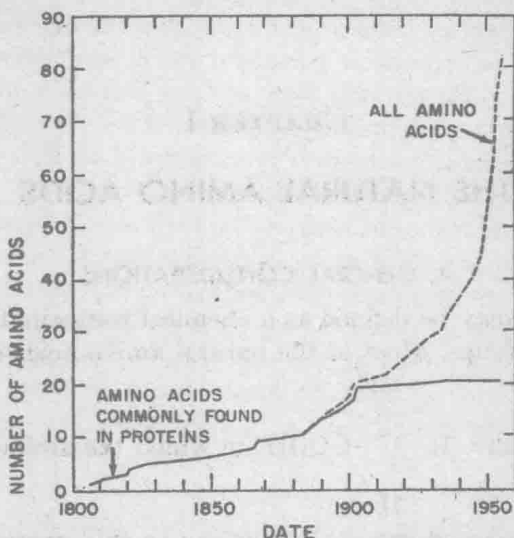


Fig. 1. Discovery of the natural amino acids.

appears to have reached a plateau, since no new amino acids in this category have been found since the discovery of threonine in 1935.

The amino acids which are frequently found in protein hydrolyzates will be considered in the next section. Those which occur occasionally in protein, and those which are found in other molecules or in the free state will be discussed under separate headings (Sections D, E, F).

B. THE COMMON AMINO ACIDS OF PROTEINS

"On examining the products of plants more carefully than had been done previously, modern chemists have distinguished a great number of compounds unknown to previous workers; but for a long time, I believe, a compound has not been found in plants which is as unique and as interesting as that of which we are now to speak. . . . in a certain quantity of asparagus juice, concentrated by evaporation, I observed a rather large number of crystals, among which two appeared to me to belong to new substances: since they had a different shape, transparency, and flavor, it was easy for me to separate them".—VAUQUELIN (1806).

Introduction

There are 22 amino acids which may be said to occur frequently in protein hydrolyzates. The discovery of these amino acids represents an important chapter in the development of modern biochemistry; many of the details of these stimulating and basic investigations have been recorded in the review of Vickery and Schmidt (1). These authors suggested that an amino acid be "accepted" as present in a protein hydrolyzate only if it had been isolated by at least two independent workers, and if its constitution

had been established by comparison with the synthetic product. These criteria continue to be important guides for the evaluation of reported amino acid discoveries. Evidence that they are not infallible, however, is the inclusion by Vickery and Schmidt themselves of β -hydroxyglutamic acid (see p. 69) in the "accepted" category. Norleucine is another amino acid which temporarily achieved "accepted" status.

The amino acids discussed below have been repeatedly isolated from protein hydrolyzates, and their structures have been conclusively established. Except for β -hydroxyglutamic acid and the iodine-containing amino acids, all of the amino acids listed by Vickery and Schmidt in 1931, plus asparagine, glutamine, cysteine, and threonine, have been included. It should be stated that the term "commonly found" as applied here may be somewhat arbitrary, or at least open to some interpretation. For example, it has been known for some time that 3,5-diiodotyrosine and thyroxine are present in thyroglobulin. There is also excellent evidence for the occurrence in certain proteins of δ -hydroxylysine. These, and other amino acids which have been isolated less frequently from protein hydrolyzates, will be considered in Section D.

The amino acids considered in this section are listed in alphabetical order; they may be classified as follows:

ALIPHATIC:

MONOAMINOMONOCARBOXYLIC:

Glycine
Alanine
Isoleucine
Leucine
Valine

HYDROXYMONOAMINOMONOCARBOXYLIC:

Serine
Threonine

MONOAMINODICARBOXYLIC:

Aspartic acid
Glutamic acid

MONOAMINODICARBOXYLIC- ω -AMIDES:

Asparagine
Glutamine

DIAMINOMONOCARBOXYLIC:

Arginine
Lysine

SULFUR-CONTAINING:

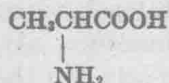
Cysteine and Cystine
Methionine

AROMATIC:

Phenylalanine
Tyrosine

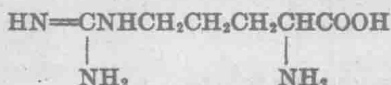
HETEROCYCLIC:

Tryptophan
Histidine
Proline
Hydroxyproline

L-ALANINE (α -AMINOPROPIONIC ACID)

Alanine is one of the amino acids which was prepared by chemical synthesis prior to its recognition as a natural substance. In 1850, Strecker, in an attempt to prepare lactic acid, treated acetaldehyde-ammonia with hydrocyanic and hydrochloric acids; alanine was obtained in crystalline form and converted to lactic acid by reaction with nitrous acid (2). The Strecker reaction leads to the formation of an aminonitrile, which gives the corresponding amino acid on hydrolysis; the procedure has proved useful for the preparation of a number of other amino acids from the appropriate aldehydes. The name *alanine*, first employed by Strecker, contains the first syllable of the word aldehyde.

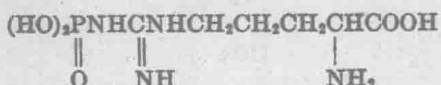
Weyl, 38 years after Strecker's synthesis of alanine, isolated alanine from an acid hydrolyzate of silk (3), which is particularly rich in this amino acid. Later, Fischer and Skita obtained L-alanine from silk and established the structure and configuration by conversion to lactic acid (4).

L-ARGININE (α -AMINO- δ -GUANIDINOVALERIC ACID)

Schulze and Steiger isolated arginine from etiolated lupine seedlings in 1886 (5). In 1895, Hedin reported the isolation of the silver nitrate salt of arginine from hydrolyzates of horn (6). Subsequently, Kossel and Gross found that arginine was a major constituent of the basic proteins of fish sperm (7). The structure of arginine was established by alkaline hydrolysis to ornithine and urea (8) and by synthesis from benzoylornithine (9).

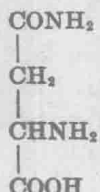
Arginine may be cleaved to ornithine during acid hydrolysis of proteins, thus accounting for the occasional presence of ornithine in protein hydrolyzates. Conversion of arginine to citrulline by alkaline hydrolysis has also been described (10). Arginine gives a red color when treated with 1-naphthol and sodium hypochlorite or sodium hypobromite, a reaction first described by Sakaguchi (11). Arginine may be precipitated from solution in the form of the mono- or diflavinate (7); such precipitation has been used for the isolation of arginine from protein hydrolyzates.

In addition to its occurrence in proteins, and in the free state, arginine occurs as phosphoarginine

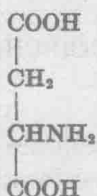


in invertebrate muscle, where it appears to perform a function analogous to that of phosphocreatine in higher forms (12, 13). Arginine also occurs in octopine (see p. 44) and argininosuccinic acid (see p. 284). A chemically related compound, canavanine, has been isolated from jack bean meal (see p. 36).

L-ASPARAGINE (α -AMINOSUCCINAMIC ACID)

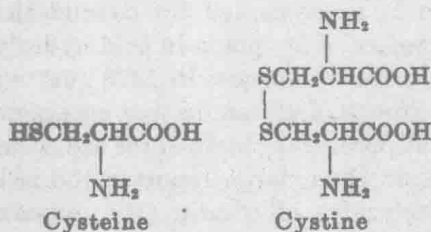


Asparagine was the first amino acid to be isolated from nature. Vauquelin and Robiquet, in 1806, isolated asparagine from asparagus juice (14). Acid hydrolysis of protein is accompanied by deamidation of asparagine to aspartic acid. The presence of ammonia in acid hydrolyzates of protein led Hlasiwetz and Habermann to suggest in 1873 that such ammonia might arise from the amide groups of glutamine and asparagine (15). However, it was not until 1932 that proof was obtained for the occurrence of asparagine in proteins; in this year Damodaran reported the isolation of asparagine from enzymatic hydrolyzates of edestin (16). Asparagine is widely distributed, and accumulates in considerable concentrations in certain higher plants. It also occurs in the free state in animal tissues. Although hydrolyzed during acid hydrolysis of protein, the amide group of asparagine is relatively stable as compared to that of glutamine. Asparagine and certain other β -aspartyl derivatives may give a brown color with ninhydrin rather than the characteristic purple ninhydrin reaction observed with most amino acids. It has been reported that the brown ninhydrin reaction of asparagine is not accompanied by release of carbon dioxide (17). Infrared absorption (18) and x-ray diffraction studies (19, 20) are consistent with the occurrence of intramolecular interaction between the amide and carboxyl groups of asparagine; such interaction may be related to its anomalous behavior with ninhydrin. The suggestion that asparagine may exist in a hydrated cyclic imide form (17) has been disproved by synthesis of α -aminosuccinimide and the demonstration that this compound and asparagine are not identical (21).

L-ASPARTIC ACID (α -AMINOSUCCINIC ACID)

Aspartic acid was first known as a product of hydrolysis of asparagine. Ritthausen later isolated aspartic acid from a protein hydrolyzate (22). Piria obtained malic acid from aspartic acid by reaction with nitrous acid (23), and later workers succeeded in synthesizing aspartic acid (24, 25).

N-Acetyl-L-aspartic acid has been found in extracts of cat brain, where it occurs in concentrations of about 100 mg. per 100 g. of tissue. It is also present in rat brain, and to a smaller extent (1 to 3 mg. per 100 g.) in cat liver, kidney, muscle, and urine (26).

L-CYSTEINE (α -AMINO- β -MERCAPTOPROPIONIC ACID) AND L-CYSTINE (β,β' -DITHIODI(α -AMINOPROPIONIC ACID))

Cystine was isolated from a urinary calculus by Wollaston in 1810 (27). In 1899, Mörner accomplished its isolation from hydrolyzates of horn (28). Cystine is a major constituent of keratins and is present also in many other proteins. Although there is good evidence for the occurrence of cysteine in proteins, acid hydrolyzates of protein usually contain only cystine, the oxidation product of cysteine. In the presence of large quantities of tryptophan, cysteine may be formed during acid hydrolysis of protein (29). On the other hand, both cysteine and cystine are destroyed by treatment with alkali.

The presence of cysteine in certain proteins is indicated by the observation that these proteins give a positive color (red) reaction when treated with nitroprusside. Cysteine is readily oxidized to cystine in neutral or alkaline solution, especially in the presence of metal ions. The reduction of cystine to cysteine by treatment with tin and hydrochloric acid was re-