

McGILVERY

# BIOCHEMISTRY

## A FUNCTIONAL APPROACH

# **BIOCHEMISTRY**

## **A FUNCTIONAL APPROACH**

Third Edition

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# **PREFACE TO THE THIRD EDITION**

This book is intended for those who wish to understand living organisms, especially those who strive to define and ameliorate human ills. Biochemistry has become an essential tool for these purposes. It would be almost impossible for a student to survey on his own the massive body of existing knowledge, constantly augmented by a remarkable torrent of brilliant discoveries that shows no signs of diminishing. The purpose of the book, then, is to organize our knowledge into something that can be comprehended in a relatively short time and still convey a reasonably complete picture of the chemical structure and function of man. Readability without sacrifice of coverage has been a prime goal; I still agree with Ernest Hooton that you ought to treat science seriously, but you don't have to act as if you are in church. An important device in gaining that goal is to keep attention constantly focused on function, showing how individual chemical structures and reactions contribute to maintenance of the whole being, and how deviations in these chemical entities produce disease.

Keeping the whole subject within bounds has involved some sacrifices. The subject is paramount, and the author's interests are secondary. Much of our present understanding of biochemistry is sound; it will still be valid for students a century hence, whereas much of the experimentation used to develop that understanding will shortly be obsolete. It is that basic knowledge of body function, rather than the experiments by which it was developed, that is covered in this book. These experiments have been exciting stuff, and it is painful to acknowledge that they rapidly acquire the musty stigma of historical detail to most students. However, some methodologies are so widely used or have such important implications that they warrant general familiarity; these are covered.

I hope the book serves its purpose well, but it clearly would have had much less chance of doing so without the intensive collaboration of Gerald Goldstein. Skilled internist by profession, perpetual student at heart, he has the most important qualification for a great teacher: the desire to share his joy upon learning new things. He contributed to the draft of every chapter, and every page has had the benefit of his repeated critical review. However, he is not to be blamed for any defects; I always had the last word. I had additional support from Joyce Hamlin, who reviewed Chapters 4 through 7, and Gary Balian, who reviewed Chapter 9.

I am grateful to those who gave me permission to use published materials in this edition; these are individually acknowledged where they appear. Peter Agre, Robert J. Fletterick, Jane S. Richardson, and Michael J. Stock went out of their way to supply drawings or prints for inclusion in the book. Struther Arnott clarified the vagaries of DNA structure, and Michael Thorn offered his helpful analysis of renal acid-base compensation. Bernard L. Trumpower attempted my conversion to the new faith on mitochondrial electron transport. In addition, Tom Maniatis and Roscoe Brady clarified particular points. Many colleagues and students throughout the

world have continued my own education through comments on the previous editions and copies of manuscripts and publications.

Roberta Kangilaski again acted as editor of the book. Once more, her enthusiasm, skill, and conscientious monitoring made her a true colleague. The book also had the benefit of the copy editing of Edna Dick, design by Lorraine Kilmer, and the skilled competence of the production departments at the W.B. Saunders Company and York Graphic Services under the patient direction of Frank Polizzano.

Finally, my biggest debt continues to be to my wife, Alice, diplomatic counselor and smoother of the path.

R.W. MCGILVERY  
*Charlottesville, Virginia*

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# CHAPTER 1

## INTRODUCTION

Organisms are marvelously complex assemblages of chemical compounds constantly involved in intertwining arrays of reactions, and one of the great delights in the study of biochemistry is the realization that it is possible to organize this complexity into comprehensible patterns. Much is to be learned, but one sees many large truths about all living things along with patches of finely detailed knowledge.

Biochemistry is essential to an understanding of living beings. Knowledge of biochemistry, like knowledge of mathematics, has become a routine tool for students of many disciplines, who in turn often make significant additions to that knowledge.

However, as with other branches of learning, there are ill-defined boundaries to biochemistry beyond which its value as a device for description and explanation diminishes, even though its principles are determinative. We do not discuss the vagaries of the London gold market in terms of the biochemistry of the participants any more than we would discuss that biochemistry in terms of the physics of the constituent atoms upon which the chemistry rests. As we go from molecule to cell to tissue to organ to individual to society, we must introduce at each stage a more simplified system of statistics to handle the larger domain, statistics that we label as anatomy, physiology, psychology, economics, history, music, and so on. Even so, the basic biochemistry sometimes shows through in previously unsuspected ways. "Chemistry" has long been blamed for the human's chronic rut, but newer psychochemistry and its byproduct drug culture have exposed the extent to which distinctively human activity can depend upon the concentration of single chemical compounds.

To illustrate the boundaries of application of biochemistry, consider the type behavior of the surgeon and the internist. The surgeon manipulates what he can see, and he thinks of his procedures in terms of anatomy and physiology, even in cases in which the ultimate effectiveness of his efforts is gauged in biochemical terms. On the other hand, the stock in trade of the internist largely consists of chemicals. He injects them or asks the patient to swallow them in order to alter the biochemistry of the patient or of some foreign organism, and biochemical language is often the natural medium for discussing what is happening.

Of course, we are making a very simplistic distinction between the specialties. The modern surgeon includes sophisticated biochemical thinking among his tools, and the internist always has probed and poked, and in modern times called in the radiologist, in order to visualize structure.

What kind of prospectus can we offer about biochemistry as a useful medium for describing the living? To begin, biochemistry describes the origin of form. The chemical constituents and the forces developed between them determine in describable ways the microscopic anatomy—the nature of cells and their constituent organelles. Biochemical principles are the tools of choice for that purpose. Biochemistry also describes the forces involved in the association of cells, its language merging into the language of anatomy as more complex levels of organization are considered.

Biochemistry describes heredity. The information on the nature and behavior of an organism that is passed from generation to generation and the mode of its transmission are molecular in nature. The complex summation of that information constitutes genetics, but the details are biochemistry.

Biochemistry describes much more. It is a social nicety to greet an acquaintance after several years of separation with the words, "You haven't changed a bit." We secretly note the coarsened skin, the deepened lines, the graying hair, but in a quantitative physical sense this conventional courtesy is frequently not far from the truth. Given a primitive environment during his absence, this one man, using the simplest of tools, could have diverted a stream, cut down hectares of forest, and in other ways changed his environment so drastically that even the most unobservant would know of his existence. Yet at the end of all this, we might well be hard put to measure more than a small change in the physical dimensions or chemical composition of the man himself.

All of this is well known, even trite, but many of the phenomena that biochemistry can address are contained in the small tale. Motion is a molecular phenomenon resulting from the cyclical formation and cleavage of chemical bonds. The energy for that motion is derived from reactions of compounds that enter the organism in a constant stream, with the products excreted into the environment. The entire process of energy generation and utilization is described by biochemistry.

The major point of the tale is the ability of the organism to maintain its character over long periods of time while acting as a chemical machine to change the environment, and this is also an important part of our story. We shall see how chemical reactions are used to constantly rebuild nearly all parts of the body; the molecular structure and the reactions proceeding within it are subject to continual review of need—a kind of zero-base budgeting, which enables adaptation to changing circumstances.

Finally, we see that the machinery does wear out. The species has built within it limiting devices that say, "Enough. Let the next generation take over." Most attempts at explaining aging are made in biochemical terms, but we know too little to say how effective this approach ultimately will be.

# AMINO ACIDS AND PEPTIDES

### THE NATURE OF AMINO ACIDS

#### *Properties Conveyed by Side Chains*

Glycine Has No Side Chain

Hydrophobic Bulk

$\pi$ -Bond Interaction

Ionized Side Chains

Binding of Metallic Cations

Hydrogen Bonding

#### *Amino Acids as Acids and Bases*

Acidic and Basic Side Chains

Histidine is Different

pH and Bond Formation

Isoelectric Point

Ionization and Solubility

#### *Stereoisomerism of the Amino Acids*

Dating by Racemization

The Fischer Convention

R and S Nomenclature

### PEPTIDES

#### *Nomenclature*

#### *Functions of Peptides*

#### *Chemistry of Peptides*

The Peptide Bond

Electrophoresis

*Blood Plasma Proteins*

Centrifugation

Much of the chemistry of living organisms concerns five major classes of compounds: carbohydrates, lipids, minerals, nucleic acids, and proteins. We shall begin our study with an exploration of the nature and formation of proteins because they are the compounds that define most of the properties we ascribe to life. They determine our metabolism, form our tissues, give us motion, transport compounds, and protect us from deleterious invasion. Even the heredity of an organism is nothing more than an expression of its ability to make various kinds of proteins at different rates.

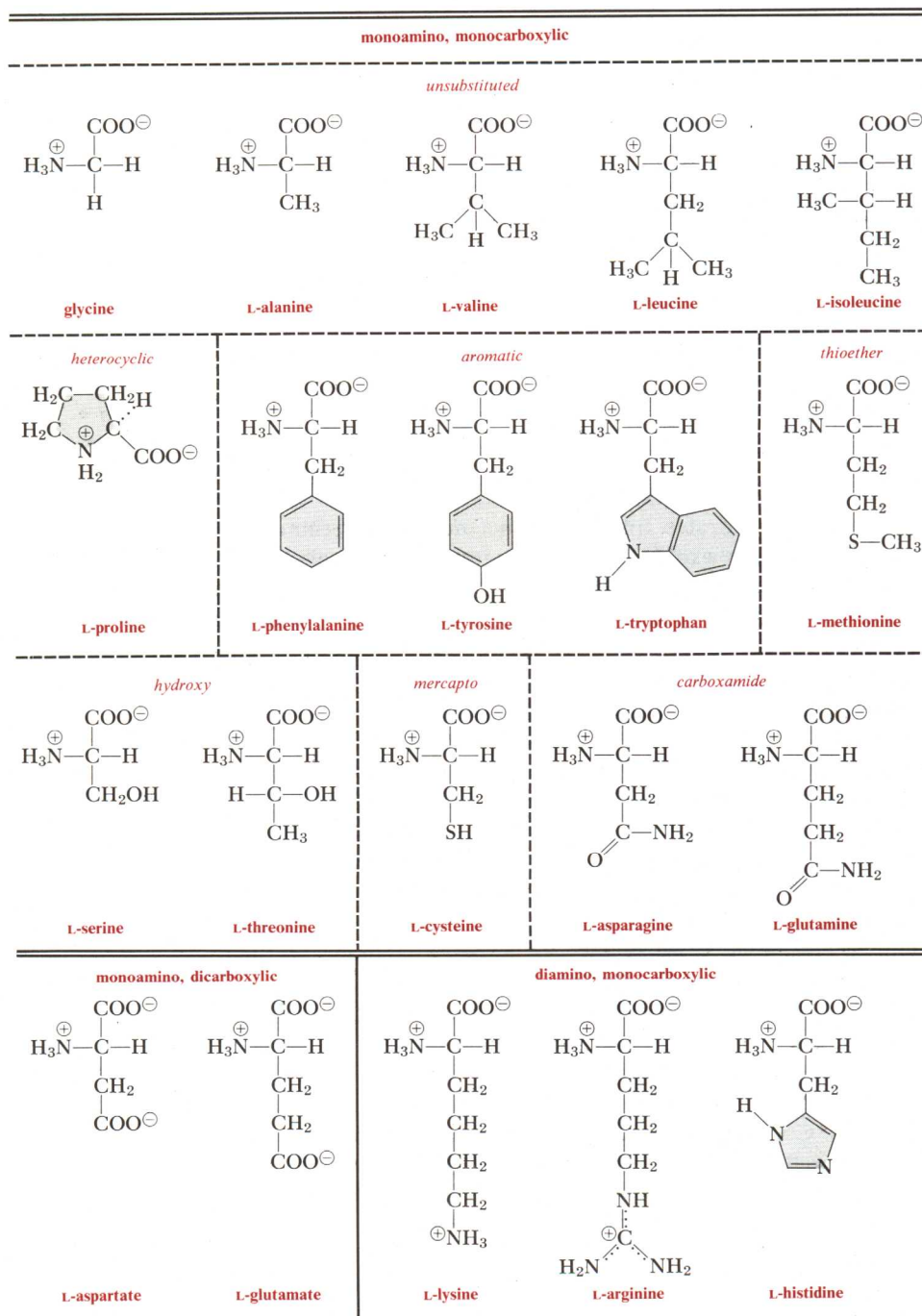
What are proteins? In operational terms they are structures for placing reactive chemical groups in particular three-dimensional patterns and for controlling access to these groups. Looking at proteins in this way, we have three tasks. We must understand how proteins are made; that is, how their various three-dimensional patterns or conformations are created. We must understand the constitution of these patterns—the chemical groups that are available and how they can be distributed in space. Finally, we must describe how these patterns perform biological functions. Only then can we properly appreciate these functions and their response to disease.

In chemical terms, a protein is a polymer of  $\alpha$ -amino acids, that is, 2-amino carboxylic acids. We are familiar with man-made polymers; the ingenuity of the organic chemist has made available a selection with a wide range of properties, but this selection is trivial compared to the diversity possible with polymers of the natural amino acids. An adult human contains thousands upon thousands of different proteins. Consider for the moment only three of these myriads: collagen, myosin, and hemoglobin, each of which is discussed in more detail later.

Collagen is a long rod that associates into stiff fibers used to strengthen bone, cartilage, skin, and so on by resisting deformation. Myosin is also a long rod, but it ends in a two-headed bundle. It is deliberately constructed to change shape because a bending of the molecule is responsible for the contraction of muscles. Hemoglobin, on the other hand, is a compact globular protein built to wrap around iron com-

plexes that will bind oxygen and to be very soluble in water so that it can be packed into circulating cells.

Here we see examples of proteins used for structure, mechanical motion, and transport. How can polymers constructed on the same basic pattern—a linear combination of  $\alpha$ -amino acids—perform such diverse functions? The answer lies in the diversity of the amino acids themselves. Let us first examine the nature of these building blocks.



## THE NATURE OF AMINO ACIDS

Proteins are constructed from 20 different amino acids. Tissues contain substantial amounts of each of these, sometimes as much as several millimoles per kilogram. This pool of free amino acids must be present if proteins are to be made, but amino acids also serve other important purposes. Some are used as chemical messengers to transmit impulses between nerves; others are actively metabolized to form products with important physiological functions. Derangement of amino acid metabolism frequently has severe consequences.

The amino acids have this general structure:



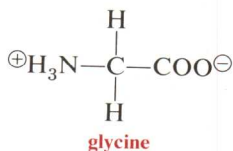
The molecule is shown in two ways, the first indicating every bond, and the second using a common kind of shorthand notation in which substituent hydrogen atoms are lumped together without indicating bonds, and individual C=O bonds also are not drawn.

Amino acids draw their properties from a hydrogen atom and three substituent groups on C-2, the  $\alpha$ -carbon atom. It is the nature of the R group that gives character to an individual amino acid, and these groups, usually called the **side chains**, are all-important in determining the properties of proteins.

### Properties Conveyed by Side Chains

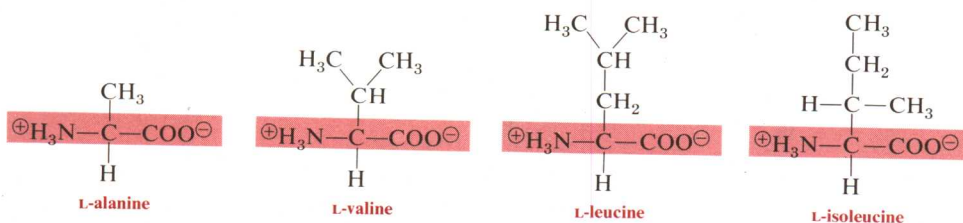
The amino acids are commonly classified according to the character of their side chains, as is shown on the opposite page. Let us survey the nature and function of these side chains in general terms now and consider them in more detail as we encounter their specific effects in subsequent chapters. The chemical functions in the structures can be recognized without the necessity of rote memorization.

**Glycine has no side chain:**

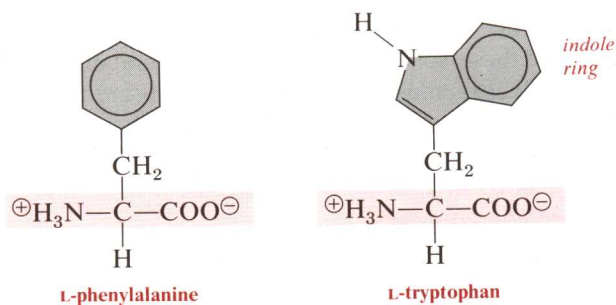


Therefore it occupies the least space of all of the amino acids. This is an important property in itself; the polymer chain can be packed tightly where glycine occurs.

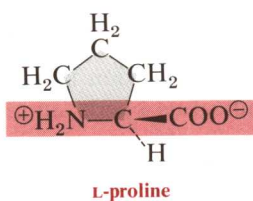
**Hydrophobic Bulk.** Many of the amino acids are built to take up space without interacting with water. They are especially useful in shaping the interior of protein molecules. Side chains that serve in this way include alkyl hydrocarbon groups:



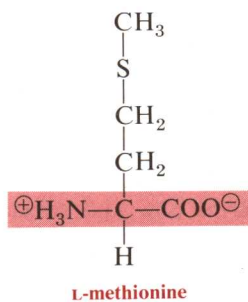
aromatic rings:



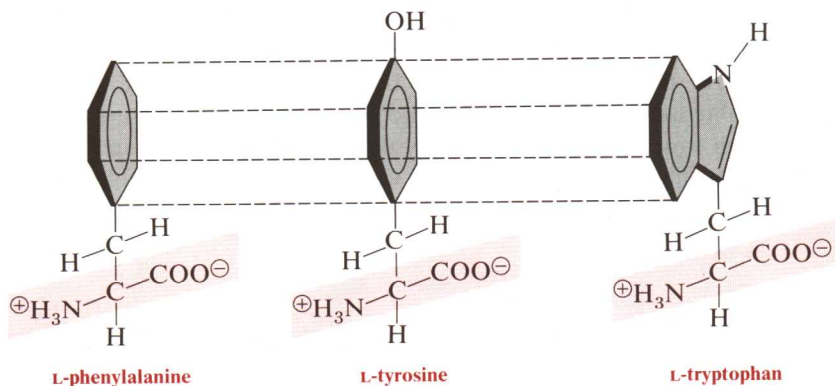
a heterocyclic ring, in which the side chain is also attached to the ammonium group on C-2:



and a thioether:



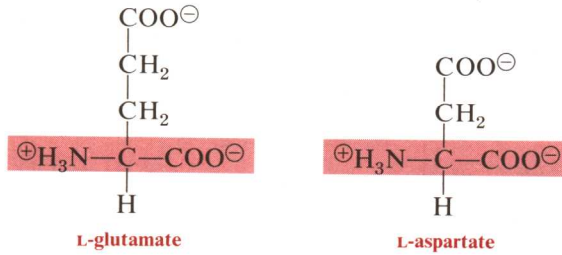
**$\pi$ -Bond Interaction.** When aromatic rings are stacked side by side, the  $\pi$ -electrons of the rings interact to form weak bonds. Some amino acids have aromatic rings that bond in this way with each other or with other flat resonant structures.



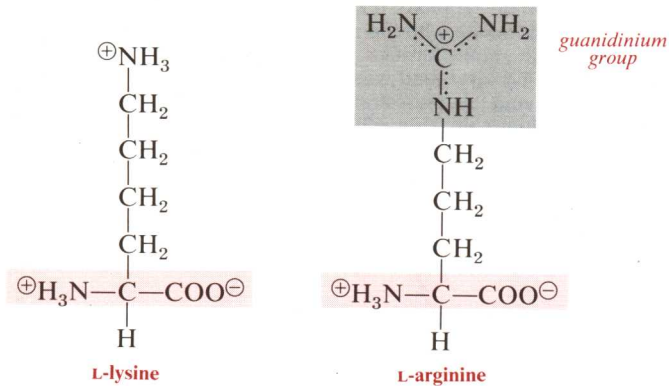
(The dashed lines are intended to convey the existence of interaction between the rings.)



**Ionized Side Chains.** Some amino acids have ionized groups in their side chains that cause strong affinity for water where they occur in proteins. These include amino acids with carboxylate groups:

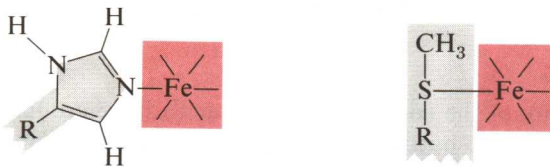


and amino acids with positively charged groups containing nitrogen atoms:

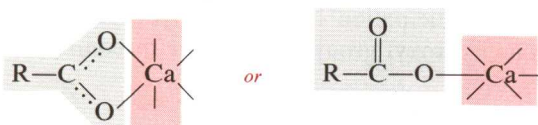


The positively and negatively charged side chains can form bonds through electrostatic interaction.

**Binding of Metallic Cations.** Atoms with unshared electrons are sometimes used to bind metals, or groups containing metals. For example, histidine occurs in hemoglobin at positions at which its side chain can bind an iron atom, and the thioether group of methionine has a similar purpose in other proteins:

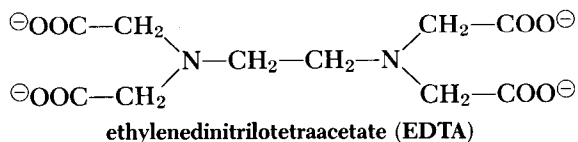


The carboxylate group of side chains in aspartate or glutamate is sometimes used to bind zinc or calcium:

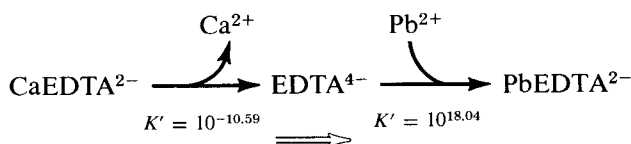




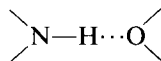
Synthetic amino acids have been developed to bond metallic ions with the carboxylate and uncharged amino groups. One that is widely used both as a reagent and as a drug is ethylenedinitrilotetraacetate, or EDTA (also known as ethylenediaminetetraacetate):



EDTA has a high affinity for metal cations with two or more positive charges. It is used to treat lead poisoning because the lead chelate is soluble and can be excreted. It is necessary to give EDTA in excess in order for it to compete with the many reactive groups in the body that also have a high affinity for lead. If the tetrasodium salt were given, any excess remaining after lead was bound would then bind calcium and remove it. To avoid this undesirable event, EDTA is administered as the disodium calcium salt. This will still remove lead, since EDTA has a higher affinity for lead than it does for calcium, which will be displaced:



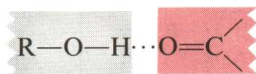
**Hydrogen Bonding.** The hydrogen bond is one of the most important in forming the structure of proteins. It is created when a proton is shared between two atoms containing unpaired electrons, such as O, N, or S:



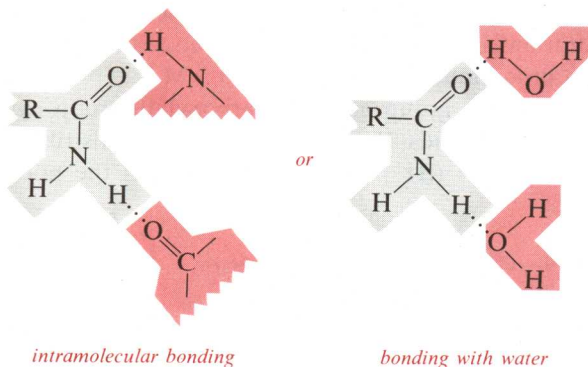
Not only do many of the constituent amino acids have groups in their side chains containing such atoms, but they are also present in the  $\alpha$ -amino and carboxylate groups through which the amino acids are polymerized, and all of these groups, in either side chain or backbone, may form hydrogen bonds.

Amino acid side chains containing N and O are frequently exposed at the surface of proteins where they can interact with water, not only because of their greater polarity but also because they form hydrogen bonds with water. This tendency is often counteracted by participation in internal hydrogen bonding. For example, burial of the alcoholic hydroxyl group of serine or threonine and the phenolic hy-

droxyl group of tyrosine is often facilitated by hydrogen bonding, sometimes to an adjacent carbonyl group:



Even the strongly polar side chains, nearly always in contact with water, are sometimes buried through formation of hydrogen bonds. These include not only those with charged groups mentioned earlier, but also those of asparagine and glutamine, which are amide derivatives of aspartate and glutamate:

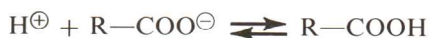


### Amino Acids as Acids and Bases

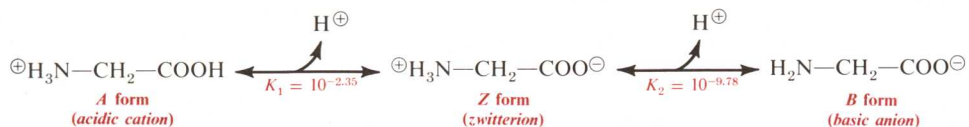
Amino acids can both donate and accept protons; they are therefore said to be **amphoteric**. Every amino acid in neutral solution can behave as an acid because it contains at least one charged ammonium group from which a proton can dissociate:



Similarly, it behaves as a base because it contains at least one charged carboxylate group that can accept a proton:



Consider the simplest amino acid, glycine. It can equilibrate with  $\text{H}^+$  in two ways:



The form shown in the middle is a **zwitterion**, meaning hermaphrodite ion, because it has equal numbers of positive ammonium groups and negative carboxylate groups, although its net charge is zero. It behaves as a base because the carboxylate groups will combine with increasing concentrations of  $\text{H}^+$  to form uncharged COOH groups. The remaining ammonium group then gives the molecule a net positive charge (cationic form). On the other hand, the zwitterion can behave as an acid because the ammonium group will lose  $\text{H}^+$  when the concentration of  $\text{H}^+$  is lowered, leaving an uncharged amino group. The molecule then has a net negative charge from the remaining carboxylate group (anionic form).