Understanding Bioanalytical Chemistry

Principles and applications

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School of Biomedical Sciences
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Preface

Telling first year life and health science students they have to study chemistry as part of their degree programme is often met with disillusionment or despair. To many the very word chemistry conjures up images of blackboards filled with mind-numbing facts and formulae, seemingly irrelevant to their chosen career paths. This textbook is our response to the very many students who plead with their tutors to 'please teach us what we need to know'. Rather than the simplistic interpretation of this statement as an indirect way of asking tutors to 'please tell us what's on the exam paper' we would see this as a more meaningful and reasonable request.

In recent years we have completely overhauled the way in which we teach bioanalytical chemistry. Taking a 'back to the drawing board' approach, we embraced the challenge of carefully considering the key aspects of chemistry every life and health scientist really needs to know. Our goal was to produce a stand-alone first year undergraduate module comprising a discrete series of lectures and practical classes, using relevant real-life examples to illustrate chemical principles and applications in action. This represented a radical departure from the former module in approach and content, and was extremely well received by students, with a marked improvement in student feedback and academic performance.

On reflection we are at a loss as to why it is tradition for life and health science students not to be introduced to the bioanalytical tools of their trade from the outset of their course. To us this is like teaching students the principles of computer science without actually introducing them to a computer and what it can do. With this in mind, we purposely chose to take an applied approach to chemistry, with an introduction to relevant methods and technologies up front, in order to familiarize students with these tools before they encounter and study them in more detail later in their courses.

Our message to students: To argue that life and health scientists don't need chemistry is like arguing that the world is flat. That is, as much as you might be convinced that it is the case, it does not mean that you are correct. Whether we like it or not, the fact is chemistry lies at the heart of the vast majority of scientific disciplines. Given this, it is pretty much impossible to expect that you will really grasp the fundamentals of core disciplines such as physiology, pathophysiology and pharmacology or be prepared for the diverse range of careers in the life and health sciences without at least a basic knowledge of core chemical principles and applications. This book is designed to complement delivery of first year chemistry, focusing on bioanalytical techniques and their real world applications.

Our message to tutors: We know, we've been there; despite all your best efforts, enthusing life and health science students to study (never mind enjoy) chemistry is like trying to encourage a physicist to build a time machine. The task has not been made any easier by the stereotypical stodginess of chemistry, the expansive nature of the subject, or the encyclopaedic nature of the average chemistry textbook. To compound the problem, few academics in life and health science departments either choose or wish to teach chemistry. Often considered the 'poisoned chalice' and the fate of many an unsuspecting fresh-faced newcomer, effective teaching and learning of first year chemistry represents a considerable challenge.

We hope that you will find this book a useful approach to the subject of bioanalytical chemistry and that it will help raise awareness of the vast scope and topics encompassed in what is a rapidly expanding and advancing field. Moreover, we hope that studying the content of this book will provide a fundamental introduction to the tools adopted by life and health scientists in the evolving and exciting new age of 'omics', with the promise of personalized medicine and novel approaches to the screening, diagnosis, treatment, cure and prevention of disease.

1 Introduction to biomolecules

Bioanalytical chemistry relies on the identification and characterization of *particles* and *compounds*, particularly those involved with life and health processes. Living matter comprises certain key *elements*, and in mammals the most abundant of these, representing around 97% of dry weight of humans, are: carbon (C), nitrogen (N), oxygen (O), hydrogen (H), calcium (Ca), phosphorus (P) and sulfur (S). However, other elements such as sodium (Na), potassium (K), magnesium (Mg) and chlorine (Cl), although less abundant, nevertheless play a very significant role in organ function. In addition, miniscule amounts of so-called *trace elements*, including iron (Fe), play vital roles, regulating biochemical pathways and biological function. By definition, *biomolecules* are naturally occurring chemical compounds found in living organisms that are constructed from various combinations of key chemical elements. Not surprisingly there are fundamental similarities in the way organisms use such biomolecules to perform diverse tasks such as propagating the species and genetic information, and maintaining energy production and utilization. From this it is evident that much can be learned about the functionality of life processes in higher mammals through the study of micro-organisms and single cells. Indeed, the study of yeast and bacteria allowed genetic mapping before the Human Genome Project. This chapter provides an introduction to significant biomolecules of importance in the life and health sciences, covering their major properties and basic characteristics.

Learning Objectives

- To be aware of important chemical and physical characteristics of biomolecules and their components.

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- To recognize different classifications of biomolecules.
- To understand and be able to demonstrate knowledge of key features and characteristics of major biomolecules.
- To identify and relate structure–function relationships of biomolecules.
- To illustrate and exemplify the impact of biomolecules in nature and science.

1.1 Overview of chemical and physical attributes of biomolecules

Atoms and elements

Chemical elements are constructed from *atoms*, which are small particles or units that retain the chemical properties of that particular element. Atoms comprise a number of different *sub-atomic particles*, primarily *electrons*, *protons* and *neutrons*. The *nucleus* of an atom contains positively charged protons and uncharged neutrons, and a cloud of negatively charged electrons surrounds this region. Electrons are particularly interesting as they allow atoms to interact (in bonding), and elements to become ions (through loss or gain of electrons). Further topics in atomic theory relevant to bioanalysis will be discussed throughout this book, and an overview of *atomic bonding* is given below.

Bonding

The physical processes underlying attractive interactions between atoms, elements and molecules are termed *chemical bonding*. Strong chemical bonds are associated with the sharing or transfer of electrons between bonding atoms, and such bonds hold biomolecules together. *Bond strength* depends on certain factors, and so-called *covalent bonds* and *ionic bonds* are generally categorized as ‘strong bonds’, while *hydrogen bonds* and *van der Waal’s forces of attraction* within molecules are examples of ‘weak bonds’. These terms are, however, quite subjective, as the strongest ‘weak bonds’ may well be stronger than the weakest ‘strong bonds’. Chemical bonds also help dictate the structure of matter. In essence, covalent bonding (electron sharing) relies on the fact that opposite forces attract, and negatively charged electrons *orbiting* one atomic nucleus may be attracted to the positively charged nucleus of a neighbouring atom. Ionic bonding involves *electrostatic attraction* between two neighbouring atoms, where one positively charged nucleus ‘forces’ the other to become negatively charged (through electron transfer) and, as opposites attract, they bond. Historically, bonding was first considered in the twelfth century, and in the eighteenth century English all-round scientist, Isaac Newton, proposed that a ‘force’ attached atoms. All bonds can be explained by quantum theory (in very large textbooks), encompassing the *octet rule* (where eight is the magic number when so-called *valence electrons* combine), the *valence shell electron pair repulsion theory* (where valence electrons repel each other in such a way as to determine geometrical shape), *valence bond theory* (including orbital hybridization and resonance) and *molecular orbital theory* (as electrons are found in discrete orbitals, the position of an electron will dictate whether or not, and how, it will participate in bonding). When considering bonding, some important terms are *bond length* (separation distance where molecule is most stable), *bond energy* (energy dependent on separation distance), *non-bonding electrons* (valence electrons that do not participate in bonding), *electronegativity* (measure of attraction of bound electrons in polar bonds, where the greater the difference in electronegativity, the more polar the bond). *Electron-dot structures* or *Lewis structures* (named after American chemist Gilbert N. Lewis) are helpful ways of conceptualizing simple atomic bonding involving electrons on outer valence shells (see Figure 1.1).

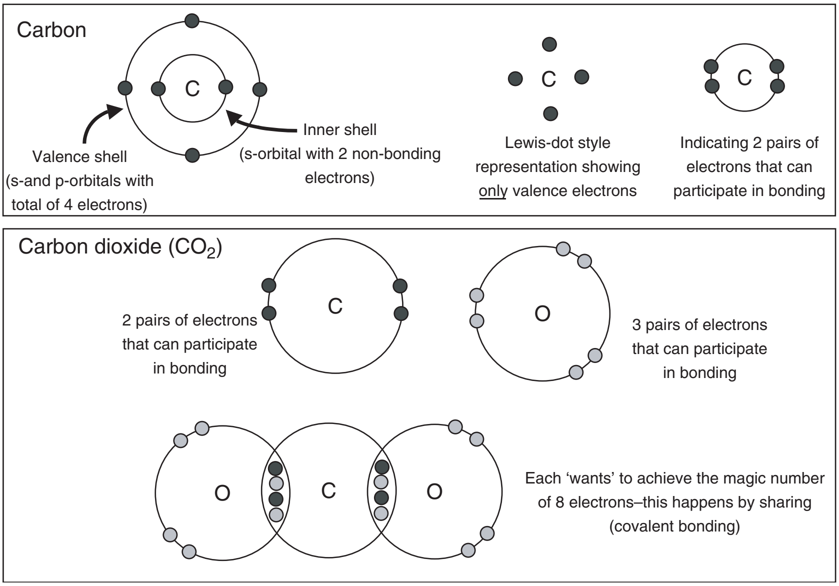


Figure 1.1 **Lewis structures illustrating covalent bonding in carbon dioxide.**

Phases of matter

Matter is loosely defined as anything having mass and taking up space, and is the basic building block of everything. There are three basic *phases of matter*, namely *gas*, *liquid* and *solid*, with different physical and chemical properties. Matter is maintained in these phases by pressure and temperature, and as conditions change matter can change from one phase to another, for example, solid ice converts to liquid water with rise in temperature. These changes are referred to as *phase transitions* inherently requiring energy, following the *Laws of Thermodynamics*. When referring to matter, the word *states* is sometimes used interchangeably with that of *phases*, which can cause confusion as, for example, gases may be in different thermodynamic states but the same state of matter. This has led to a decrease in the popularity of the traditional term *state of matter*. While the general term *thermodynamics* refers to the effects of heat, pressure and volume on physical systems, chemical thermodynamics studies the relationship of heat to chemical reactions or physical state following the basic Laws of Thermodynamics. Importantly, as energy can neither be created nor destroyed, but rather exchanged or emitted (for example as heat) or stored (for example in chemical bonds), this helps define the physical state of matter.

Physical and chemical properties

Matter comprising biomolecules has distinct physical and chemical properties, which can be measured or observed. However, it is important to note that physical properties are distinct from chemical properties. Whereas physical properties can be directly observed without the need for a change in the chemical composition, the study of chemical properties actually requires a change in chemical composition, which results from so-called chemical reactions. Chemical reactions encompass processes that involve the rearrangement, removal, replacement or addition of atoms to produce a new substance(s). Properties of matter may be dependent (*extensive*) or independent (*intensive*) on the quantity of a substance, for example mass and volume are extensive properties of a substance.

Studying physical and chemical properties of biomolecules

A diverse range of bioanalytical techniques have been used to study the basic composition and characteristics of biomolecules. Typically these techniques focus on measures of distinct physical and/or chemical attributes, to identify and determine the presence of different biomolecules in biological samples. This has been important from a diagnostic and scientific standpoint, and some of the major technologies are described in this book. Examples of physical and chemical properties and primary methods used to study that particular property are as follows:

Physical properties: Charge (see ion-exchange chromatography; Chapter 7); Density (see centrifugation; Chapter 6); Mass (see mass spectrometry; Chapter 9); and Shape (see spectroscopy; Chapter 5).

Chemical properties: Bonding (see spectroscopy and electrophoresis; Chapters 5 and 8); Solubility (see precipitation and chromatography; Chapters 6 and 7); Structure (see spectroscopy; Chapter 5).

1.2 Classification of biomolecules

It is important to note that whilst biomolecules are also referred to by more generic terms such as molecules, chemical compounds, substances, and the like, not all molecules, chemical compounds and substances are actually biomolecules. As noted earlier, the term *biomolecule* is used exclusively to describe naturally occurring chemical compounds found in living organisms, virtually all of which contain carbon. The study of carbon-containing molecules is a specific discipline within chemistry called *organic chemistry*. Organic chemistry involves the study of attributes and reactions of chemical compounds that primarily consist of carbon and hydrogen, but may also contain other chemical elements. Importantly, the field of organic chemistry emerged with the misconception by nineteenth century chemists that all organic molecules were related to life processes and that a ‘vital force’ was necessary to make such molecules. This archaic way of thinking was blown out of the water when organic molecules such as soaps (Michel Chevreul, 1816) and urea (Friedrich Wöhler, 1828) were created in the laboratory without this magical ‘vital force’. However, despite being one of the greatest thinkers in the field of chemistry, the German chemist Wöhler was pretty smart not to make too much out of his work, even though it obviously obliterated the vital force concept and the doctrine of vitalism. So from this it is important to remember that not all organic molecules are biomolecules.

Life processes also depend on *inorganic molecules*, and a classic example includes the so-called ‘transition metals’, key to the function of many molecules (e.g. enzymes). As such, when considering biomolecules it is imperative to understand fundamental features of transition metals and their interaction with biomolecules. Indeed, transition metal chemistry is an effective means of learning basic aspects of inorganic chemistry, its interface with organic chemistry, and how these two fields of study impact on health and disease, and a whole chapter of this book is devoted to this important subject (Chapter 3). There are very many ways of classifying molecules and biomolecules, which often causes some confusion. The simplest division of biomolecules is on the basis of their size, that is, small (*micromolecules*) or large (*macromolecules*). However, while the umbrella term *macromolecule* is widely used, smaller molecules are most often referred to by their actual names (e.g. amino acid) or the more popular term *small molecule*. Yet even the subjective term *macromolecule* and its use are very confused. Historically, this term was coined in the early 1900s by the German chemist Hermann Staudinger, who in 1953 was awarded a Nobel Prize in Chemistry for his work on the characterization of polymers. Given this, the word macromolecule is often used interchangeably with the word *polymer* (or polymer molecule). For the purposes of this book the authors will use the following three categories to classify biomolecules:

Small molecules: The term *small molecule* refers to a diverse range of substances including: lipids and derivatives; vitamins; hormones and neurotransmitters; and carbohydrates.

Monomers: The term *monomer* refers to compounds which act as building blocks to construct larger molecules called *polymers* and includes: amino acids; nucleotides; and monosaccharides.

Polymers: Constructed of repeating linked structural units or monomers, polymers (derived from the Greek words *polys* meaning many and *meros* meaning parts) include: peptides/oligopeptides/polypetides/proteins; nucleic acids; and oligosaccharides/polysaccharides.

1.3 Features and characteristics of major biomolecules

Differences in the properties of biomolecules are dictated by their components, design and construction, giving the inherent key features and characteristics of each biomolecule that enable its specific function(s). There are a number of classes of more abundant biomolecules that participate in life processes and are the subject of study by bioanalytical chemists using a plethora of fundamental and state-of-the-art technologies in order to increase knowledge and understanding at the forefront of life and health sciences. Before considering important biomolecules it is first necessary to examine their key components and construction.

Building biomolecules

Biomolecules primarily consist of carbon (C) and hydrogen (H) as well as oxygen (O), nitrogen (N), phosphorus (P) and sulfur (S), but also have other chemical components (including trace elements such as iron). For now, focus will be placed on the core components carbon, hydrogen, and oxygen, and simple combinations (see also Table 1.1).

Carbon: The basis of the chemistry of all life centres on carbon and carbon-containing biomolecules, and it is the same carbon that comprises coal and diamonds that forms the basis of amino acids and other biomolecules. In other words, carbon is carbon is carbon, irrespective of the product material, which may be hard (diamond) or soft (graphite). Carbon is a versatile constituent with a great affinity for bonding other atoms through *single bonds* or *multiple bonds*, adding to complexity and forming around 10 million different compounds (Figure 1.2). As chemical elements very rarely convert into other elements, the amount of carbon on Earth remains almost totally constant, and thus life processes that use carbon must obtain it somewhere and get rid of it somehow. The flow of carbon in the environment is termed the *carbon cycle*, and the most simple relevant example lies in the fact that plants utilize (or recycle) the gas carbon dioxide (CO_2), in a process called *carbon respiration*, to grow and develop. These plants may then be consumed by humans and with digestion and other processes there is the ultimate generation of CO_2, some of which is exhaled and available again for plants to take up, and so the cycle continues. Being crude, in essence humans and other animals act as vehicles for carbon cycling, being *d*esigned for life in the womb, *d*evouring food and fluids, *d*eveloping, *d*efecating, *d*ying and *d*ecaying, the ‘6 D’s of life’.

Hydrogen: This is the most abundant (and lightest) chemical element, which naturally forms a highly flammable, odourless and colourless diatomic gas (H_2). The Swiss scientist Paracelsus, who pioneered the use of chemicals and minerals in medical practice, is the first credited with making hydrogen gas by mixing metals with strong acids. At the time Paracelsus didn’t know this gas was a new chemical element, an intuition attributed to British scientist Henry Cavendish, who described hydrogen gas in 1766 as ‘inflammable air’, later named by French nobleman and aspiring scientist, Antoine-Laurent Lavoisier, who co-discovered, recognized and named hydrogen (and oxygen), and invented the first *Periodic Table*.

Gaseous hydrogen can be burned (producing by-product water) and thus historically was used as a fuel. For obvious safety reasons helium (He), rather than

Table 1.1 **Examples of simple combinations of carbon, hydrogen and oxygen**

Compound	Chemical formula	Notes
Acetaldehyde	CH_3CHO (MeCHO)	Flammable liquid, fruity smell, found in ripe fruit, and metabolic product of plant metabolism. Chemical associated with the 'hangover' following overindulgence in alcohol.
Acetic acid	CH_3COOH	Hygroscopic liquid, gives vinegar its characteristic taste and smell. Corrosive weak acid.
Acetylene (ethyne)	C_2H_2	Gas containing C to C triple bond. Unsaturated chemical compound which can volatilize carbon in radiocarbon dating.
Benzyl acetate	$C_6H_5CH_2OCOCH_3$	Solid, sweet smelling ester, found naturally in many flowers (e.g. jasmine). Used in perfumes, cosmetics and flavourings.
Carbon dioxide	CO_2	Colourless, odourless and potentially toxic gas which can also exist in solid state (dry ice). Important component of the carbon cycle, a 'greenhouse gas', and contributes to the 'carbon footprint'.
Carbon monoxide	CO	Colourless, odourless and extremely toxic gas, produced by incomplete combustion of carbon-containing compounds (e.g. in internal combustion engines–exhaust fumes).
Ethanol	C_2H_5OH	Flammable, colourless, slightly toxic liquid, found in alcoholic beverages.
Methane	CH_4	Simplest alkane. Gaseous and principal component of natural gas. When burned in O_2 produces CO_2 and H_2O.
Water	H_2O	Normally odourless, colourless and tasteless liquid, but can also exist in solid (ice) or gas (water vapour) states. Non-inert common universal solvent.

hydrogen, was the gas of choice for floatation of Zeppelin airships. Indeed, the now famous Zeppelin airship 'The Hindenburg' was to be filled with He, but because of a US military embargo, the Germans modified the design of the airship to use flammable H_2 gas; an accident waiting to happen, and the rest is history.

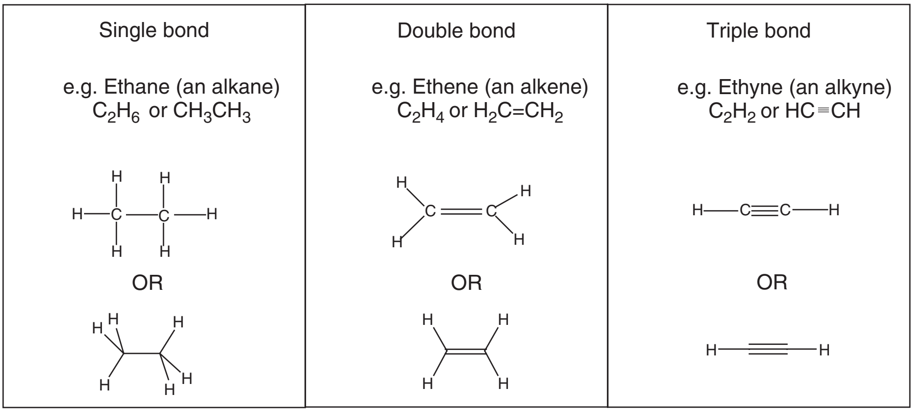


Figure 1.2 Illustration of carbon single, double and triple bonds.

In terms of biomolecules, hydrogen atoms usually outnumber both carbon and oxygen atoms.

Oxygen: As Lavoisier first generated oxygen from acidic reactions, he falsely believed that it was a component of all acids, deriving the name from the Greek words *oxys* (acid) and *genēs* (forming). Oxygen is usually bonded covalently or ionically to other elements such as carbon and hydrogen, and dioxygen gas (O_2) is a major component of air. Plants produce O_2 during the process of photosynthesis, and all species relying on *aerobic respiration* inherently depend on it for survival. Oxygen also forms a triatomic form (O_3) called *ozone* in the upper layers of the Earth's atmosphere, famously shielding us from UV radiation emitted from the

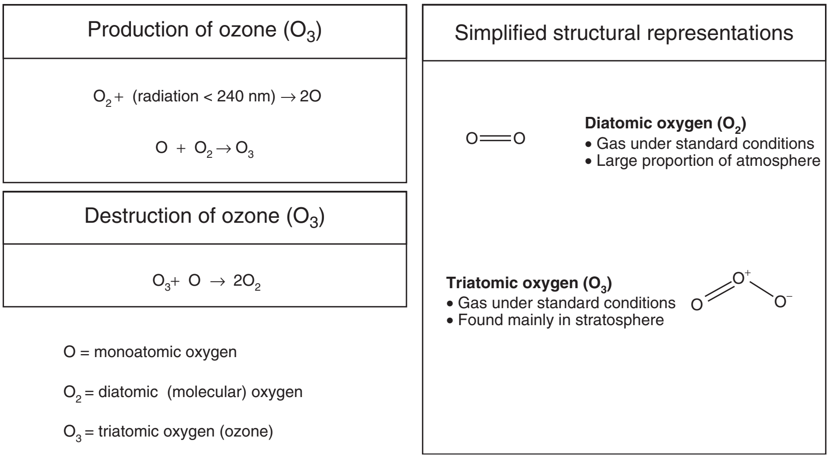


Figure 1.3 Chemical reactions involved in the production and destruction of ozone.

Sun (Figure 1.3). From a physiological and biochemical perspective, oxygen is both friend and foe; without it vital metabolic processes stop (friend) but exposure to oxygen in the form of certain oxygen-containing species (e.g. free radicals such as singlet oxygen) can be harmful (foe), and in extreme cases toxic, to body tissues, by exerting damaging actions on biomolecules regulating cellular and functional integrity.

Constructing complex biomolecules

As indicated above, C, H, O and other elements (such as N or P) can bind in a range of combinations to make simple compounds such as those given in Table 1.1. However, the same elements can also bind together to form much more complex structural and functional compounds (or biomolecules) which play vital roles in physiological processes. Major classes of these complex biomolecules are outlined in the boxes below.

Nucleotides

- Nucleotides consist of three components: a heterocyclic nitrogenous base, a sugar, and one or more phosphate groups.
- Nitrogenous bases of nucleotides are derivatives of either purine (adenine, A; or guanine, G) or pyrimidine (cytosine, C; thymine, T; or uracil, U) (see Figure 1.4).
- Nucleotides may be termed *ribonucleotides* (where component sugar is ribose) or *deoxyribonucleotides* (where component sugar is 2-deoxyribose).
- The bases bind to the sugar through glycosidic linkages.
- Also, one or more phosphate groups can bind to either the third carbon (C3) of the sugar of the nucleotide (so-called 3’ end) or the fifth carbon (C5) of the sugar (so-called 5’ end).
- Nucleotides are structural units of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and cofactors such as coenzyme A (CoA), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), with important roles in energy transfer, metabolism and intracellular signalling.

- Notably, polynucleotides are acidic at physiological pH due to the phosphate group (PO_4^-); this negatively charged anion is important for bioanalysis.

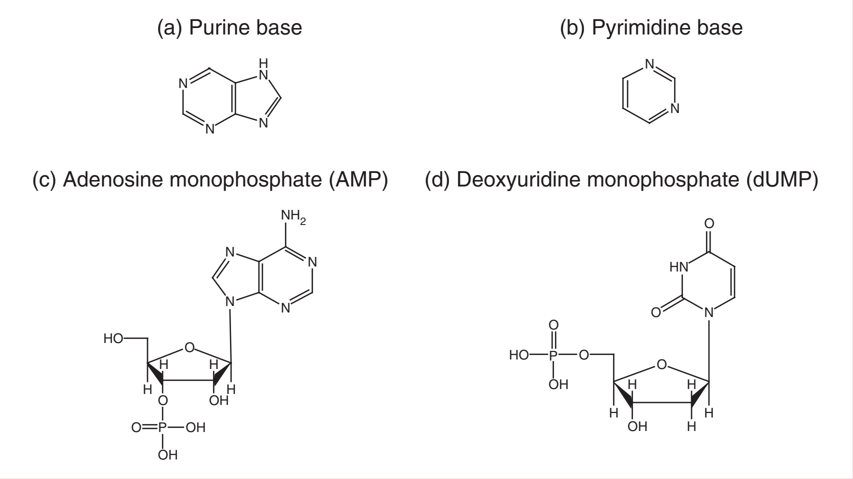


Figure 1.4 Diagrammatic representations of (a) a purine base, (b) a pyrimidine base, (c) a ribonucleotide, adenosine monophosphate (AMP) and (d) a deoxyribonucleotide, deoxyuridine monophosphate (dUMP).

Nucleic acids (e.g. RNA and DNA)

- Nucleic acids are polymers constructed from nucleotides (monomers) and found in cell nuclei.
- RNA comprises ribonucleotides while DNA contains deoxyribonucleotides.
- RNA can comprise the bases adenine (A), cytosine (C), guanine (G), and uracil (U).
- DNA can comprise the bases adenine (A), cytosine (C), guanine (G), and thymine (T).
- A nucleotide comprising a nucleic acid joins with another nucleotide through a so-called phosphodiester bond.
- Polymers of nucleic acids typically have different properties from individual units (nucleic acid monomers).
- There are also structural differences; RNA is usually single-stranded (alpha helix) while DNA is usually double-stranded (double helix). (Figure 1.5)

- DNA can replicate by separation of the two strands of the helix, which act as a template for synthesis of complementary strands.

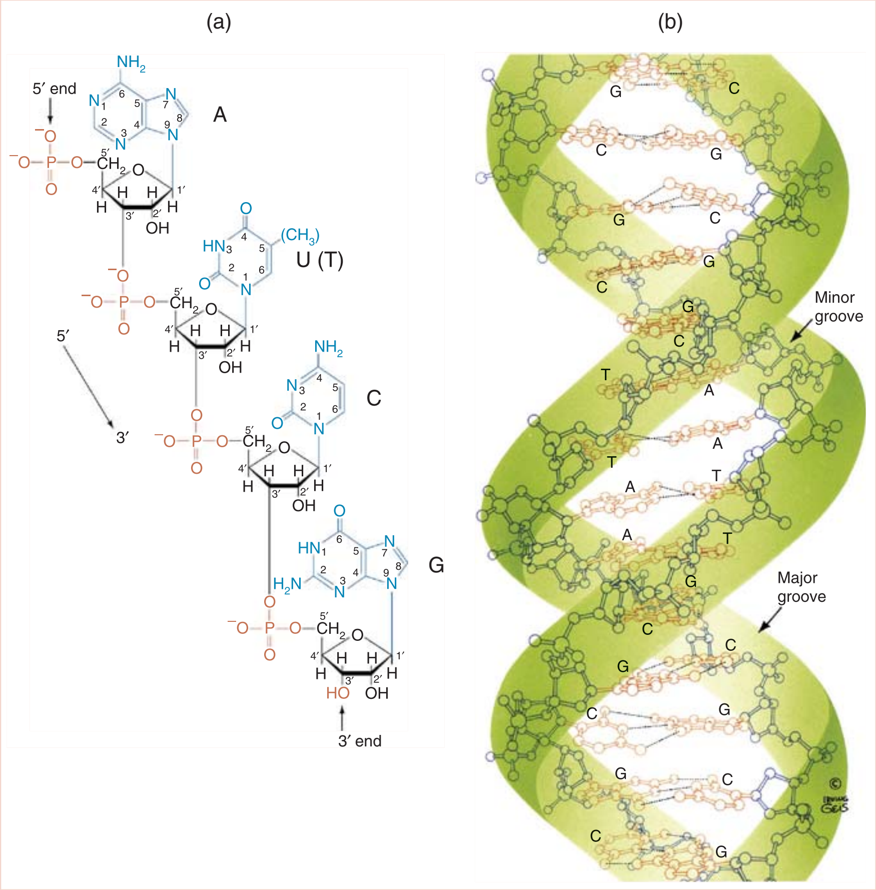


Figure 1.5 Diagrammatic representation of (a) a nucleic acid and (b) double helix structure of DNA. Illustrations, Irving Geiss. Rights owned by Howard Hughes Medical Institute. Reproduction by permission only.

Amino acids

- Molecules that contain a central carbon atom (alpha-carbon) attached to a carboxyl group (COOH), an amine group (NH_2), hydrogen atom (H), and a side chain (R group). (Figure 1.6)

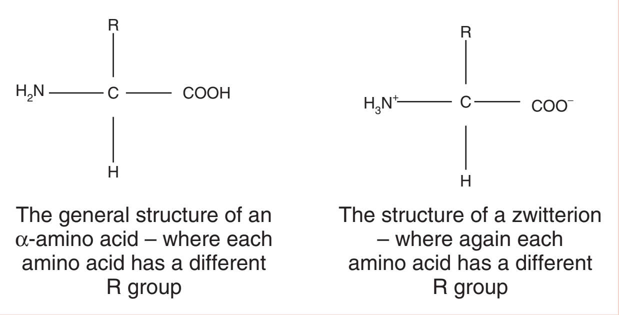


Figure 1.6 General representative chemical structure of an amino acid.

- The R group essentially defines the structure and function of an amino acid; these can generally be classified under three main groups: non-polar, uncharged polar, or charged polar amino acids.
- Amino acids (exceptions include Gly and Cys) are so-called chiral molecules (four different groups attached to alpha-carbon), which means they can exist as two different optical isomers called D (e.g. D-Ala) or more abundant L (e.g. L-Ala).
- There are 20 standard proteinogenic amino acids, of which 10 are essential amino acids that cannot be synthesized in the body so must be derived from the diet.
- Essential amino acids include: Iso, Leu, Lys, Met, Phe, Thr, Trp, Val, Arg and His, where the last two, Arg and His, are only actually essential under certain conditions.
- Amino (NH_2) and carboxylic acid (COOH) groups of the amino acid can readily ionize (to NH_3^+ and COO^-) at certain pHs to form an acid or base.
- The pH at which an amino acid is not in its ionized form (i.e. bears no electric charge) is known as its *isoelectric point*.
- When amino acids contain both positive and negative charges and are electrically neutral they fulfil the criteria of being a zwitterion (dipolar ion), which are highly water-soluble.
- Amino acids can be polymerized to form chains through condensation reactions, joining together by so-called peptide bonds, and they are often referred to as the building blocks of peptides and proteins.

Table 1.2 Classification of essential amino acids

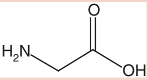
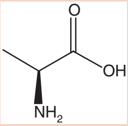
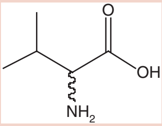
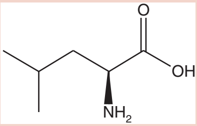
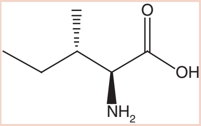
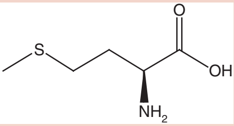
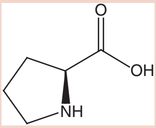
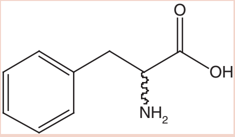
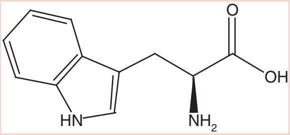
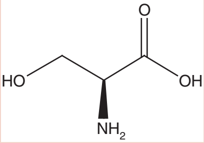
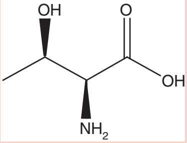
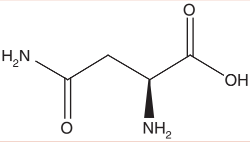
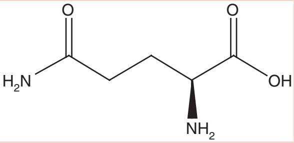
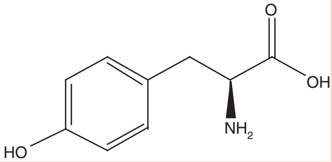
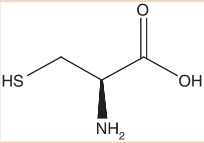
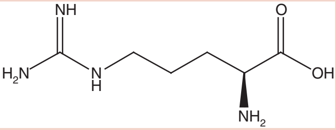
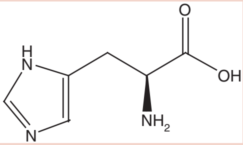
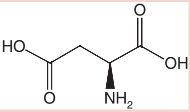
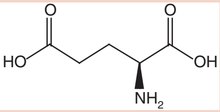
Classification	Name	3-letter code	1-letter code	Structure
Non-polar	Glycine	Gly	G	
	Alanine	Ala	A	
	Valine	Val	V	
	Leucine	Leu	L	
	Isoleucine	Ile	I	
	Methionine	Met	M	
	Proline	Pro	P	
	Phenylalanine	Phe	F	

Table 1.2 (*continued*)

Classification	Name	3-letter code	1-letter code	Structure
	Tryptophan	Trp	W	
Uncharged polar	Serine	Ser	S	
	Threonine	Thr	T	
	Asparagine	Asn	N	
	Glutamine	Gln	Q	
	Tyrosine	Tyr	Y	
	Cysteine	Cys	C	

(*continued overleaf*)

Table 1.2 (*continued*)

Classification	Name	3-letter code	1-letter code	Structure
Charged polar	Lysine	Lys	K	
	Arginine	Arg	R	
	Histidine	His	H	
	Aspartic acid	Asp	D	
	Glutamic acid	Glu	E	

Peptides and proteins

- Each peptide or protein is constructed as a string or chain of amino acids, creating huge numbers of variants, analogous to how letters of the alphabet make words.
- Importantly, peptides and proteins are assembled from amino acids on the basis of genetic coding (gene-nucleotide sequence), or can be synthesized in the laboratory.

- Peptides and proteins have individual and unique amino acid sequences (residues), giving them unique structural conformations and biological activity.
- A peptide is a short molecule formed by amino acids linked through amide (peptide) bonds. (Figure 1.7)

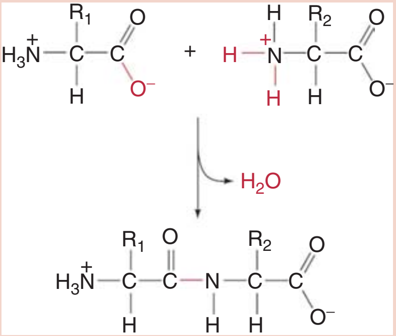


Figure 1.7 Formation of a peptide (amide) bond. From Voet, Voet & Pratt Fundamentals of Biochemistry, 2nd edn; © 2006 Voet, Voet & Pratt; reprinted with permission of John Wiley & Sons, Inc.

- When two amino acids join they form a dipeptide, three a tripeptide and so forth.
- Oligopeptides usually comprise between 3 and 10 amino acids and peptides with more than 10 are often referred to as *polypeptides*.
- Some peptides are called *peptide hormones* and as the name suggests these peptides have hormonal (endocrine) function.
- Proteins comprise one or more polypeptides and while peptides are short, polypeptide proteins are long.
- There are different ways of classifying proteins and one of the most important is on the basis of their structure.
- Biochemically there are four major classifications of protein structure: primary (amino acid sequence), secondary (local spatial arrangement), tertiary (overall 3D structure) and quaternary (protein complex structure). (Figure 1.8)

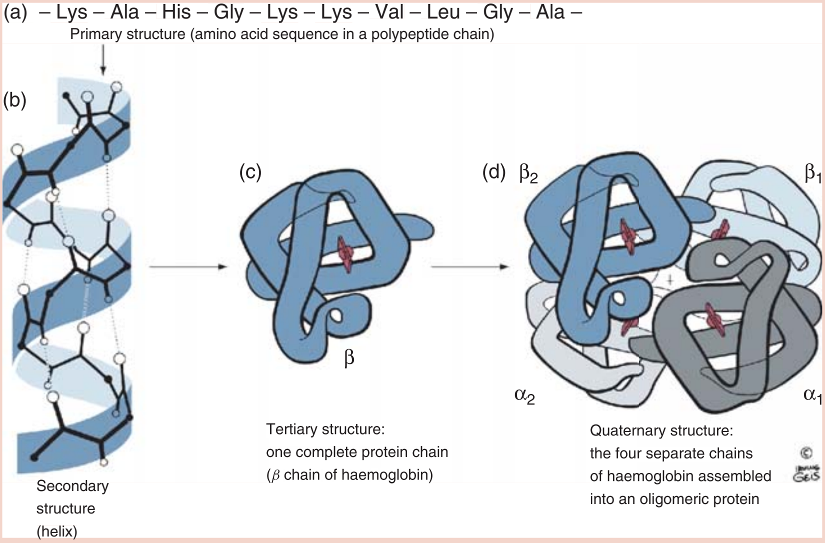


Figure 1.8 Diagrammatic representations of secondary, tertiary and quaternary protein structures. From Voet, Voet & Pratt Fundamentals of Biochemistry, 2nd edn; © 2006 Voet, Voet & Pratt; reprinted with permission of John Wiley & Sons, Inc.

Carbohydrates

- Simple, neutral biomolecules composed of C, H and O, often referred to as *saccharides*.
- All carbohydrates have an aldehyde (aldose) or ketone (ketose) functional group (containing C=O) and a hydroxyl group (–OH).
- Importantly, as carbohydrates contain aldehyde or ketone groups they undergo the same reactions as individual aldehyde or ketone molecules (e.g. oxidation and reduction reactions).
- Classification is based on the number of structural sugar units (and aldehyde or ketone group) in the chain, where 1 unit makes a monosaccharide (e.g. glucose), 2 units are disaccharides (e.g. lactose), 3–10 units are oligosaccharides (e.g. raffinose) and greater than 10 sugar units are polysaccharides (e.g. starch). (Figure 1.9)
- Sugar units are joined together through oxygen atoms, forming a so-called glycosidic bond.