

*Physical Biochemistry*

*Applications to Biochemistry  
and Molecular Biology*

*David Freifelder*

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*Applications to Biochemistry and Molecular Biology*

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# *Preface*

Modern biochemistry and molecular biology are concerned with the functions of biological systems. A century ago, the only means of study was by direct observation of such systems at work. Today, much more sophisticated and detailed observations can be made through electron microscopy and the specialized microscopic techniques that have been developed in the past forty years.

Scientists realized in the late nineteenth century that something was to be gained by studying the chemistry of cells. For decades thereafter, biochemists relied on the chemical methods available to them, and, indeed, great advances in understanding were achieved. Probably the most significant improvement in chemical technique was the development of the use of radioisotopic tracers; this vastly increased the sensitivity of detection and the number of kinds of biological molecules that could be identified. As it became necessary to have more sensitive methods for separating the various components of a biochemical reaction, chromatography and electrophoresis became routine procedures.

When the attention of physicists and physical chemists was directed toward biology (perhaps because of the ability of living cells to create local order even though the laws of physics state that there is a tendency toward disorder in the universe), the techniques of physics and physical chemistry—hydrodynamics, spectroscopy, scattering, and diffraction—entered the biological field.

A significant advance in biochemistry was the recognition that biological systems contain not only the small molecules with which organic chemistry is concerned, but also giant molecules, the *macromolecules*, whose molecular weights we now know can be at least 100 billion times the mass of a hydrogen atom. The importance of macromolecules to biological systems lies in the specificity that they confer both on biological reactions and in forming structural units. It is probably fair to say that in the past twenty years the greatest effort in biochemistry and molecular biology has been to characterize and understand macromolecules and their interactions with one another. This has required sophisticated methods both for separation and purification and for detailed observation of small parts of the molecules. Hence, the greater part of this book deals with techniques for the characterization of macromolecules with which to find answers to the following questions.

1. What is the precise structure at the atomic level of a macromolecule or an aggregate of macromolecules?
2. What properties of a macromolecule determine its structure and what forces participate in stabilizing it?
3. If a macromolecule binds other molecules (either small molecules or other macromolecules), what is their structure, what is the number of binding sites, and what are the physical constants (e.g., dissociation constants) for the binding?
4. Where is a particular macromolecule located within a cell or a small unit like a virus?

It should be noticed that the greatest concern is with the determination of physical parameters and structure. This derives from the (correct) belief that these characteristics determine biological function. For example, it has been said that a protein with a specialized function (e.g., an enzyme) can be thought of as an active site consisting of a few amino acids held together by  $\alpha$  helices,  $\beta$  sheets,  $\beta$  turns, and random coils so that the structure reflects the requirements of the protein for its activity.

At present, the precise structure (at the atomic level) of a macromolecule can be determined only by x-ray diffraction analysis, and no technique has had a greater impact on the study of macromolecules than this one. (Consider, for example, the impact on genetics of the determination of the structure of DNA.) By knowing a few precise structures not only can we establish rules for determining

structure, but also we have reference molecules to study using other techniques, thus explaining how to interpret the data so obtained. X-ray diffraction, unfortunately, is a very difficult and complex technique, and often years are required to determine the structure of a protein or a macromolecular complex. Traditionally, books of this sort contain general descriptions of the theory of x-ray diffraction. However, this book does not for two reasons: (1) it is very unlikely that any but a few readers will ever use the technique or even have an occasion to attempt to unravel an x-ray diffraction pattern, and (2) it is virtually impossible, in less than an entire book, to explain the principles and analytical methods so that they can be thoroughly understood. However, throughout the book, reference is made to information gained by such analysis.

Students wishing to understand modern biological thinking can find excellent texts that give biological facts and theories as we know them today. However, well-read students who attempt to read current scientific literature (i.e., journals and review articles) quickly discover that a great deal of technical information is needed. To obtain this information, they can turn to textbooks of biophysical chemistry and find excellent theoretical analyses of the available methods, together with detailed mathematical derivations describing the physics underlying the methods. Alternatively, there are manuals available that describe procedures for the use of various instruments and techniques.

In many years of teaching physical biochemistry, I have come to realize that a student who has labored through mathematical derivations rarely has achieved sufficient understanding of the techniques to read, understand, and judge the work presented in current scientific literature. Furthermore, many students lack the mathematical sophistication required for obtaining any information at all from this standard approach. For these reasons, I teach physical biochemistry in a way that completely avoids mathematical derivations. In presenting an equation, I make clear what assumptions have been made in its derivation and what conditions must be satisfied before the equation is usable. Techniques are described in detail, but mostly with words, and many examples are given as a teaching device. I feel that the approach has been successful, and this book is designed in accord with it. Derivations are left to those instructors who feel that they are necessary to round out understanding. The single aim of this book is to enable the student to read and understand the current literature.

The book is directed at advanced undergraduates or beginning graduate students who have a general knowledge of chemistry and physics. For a few of the techniques presented (especially in the sections on spectroscopic methods), a more extensive background in physics is required, and the student may have to draw on the instructor's knowledge of such techniques for a thorough grasp of them. In the course of writing this book, I had the help of many active researchers as reviewers and was pleased to discover that the book is informative to such people, filling them in on techniques developed since graduation.

I would like to thank the following people whose aid was invaluable in achieving correctness and clarity: Elliot Androphy, Dan Alterman, Carol Orr, and Jon Tumen, four undergraduates at Brandeis University, and Robert Suva, a Brandeis graduate student, who combed the manuscript for ambiguities and inaccuracies; Andrew Braun, who read the entire manuscript in search of flaws in presentation; Richard Mandel, without whom I could not have written the chapters on spectroscopy; Alfred Redfield, Helen Van Vunakis, Lawrence Levine, Robert Baldwin, Bruno Zimm, Ross Feldberg, Sherwin Lehrer, Inga Mahler, and Serge Timasheff, who read the specialized chapters; Phil Hanawalt, Paul Schimmel, and Peter Von Hippel, who reviewed the manuscript submitted for publication. Further thanks go to all of the people who gave me data and allowed the use of their illustrations and photographs. I also want to thank Mildred Kravitz and Barbara Nagy, who typed thousands of pages to bring the manuscript into its final form. My final debt is to my wife, Dorothy, who made use of her extraordinary skills as proofreader, scientist, editor, and logician, requiring perfection of me and of this book as she read the page proofs.

*February 1976*

DAVID FREIFELDER

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# Characterization of Macromolecules

For roughly half a century the aim of biochemistry has been to assemble a complete catalog of the chemical *reactions* occurring in living cells. The motivation for this great effort was the belief that a significant number of the biological properties of cells could be understood in terms of the reactions in which covalent bonds are formed or broken. Indeed, from the great collection of biological reactions that has been obtained, we now understand in some detail how energy is generated by chemical degradation, how biological molecules are interconverted, and how giant molecules—the *macromolecules*—are assembled from amino acids, nucleotides, sugars, and lipids.

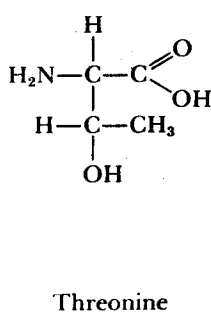
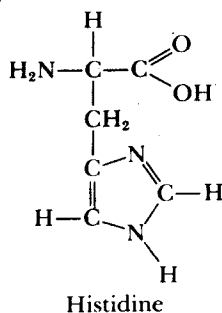
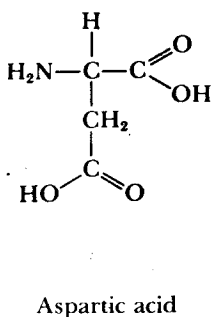
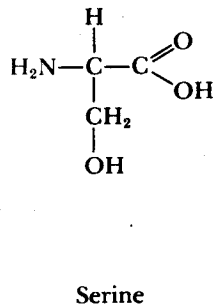
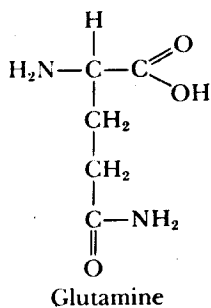
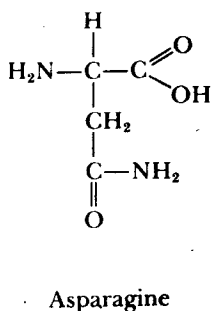
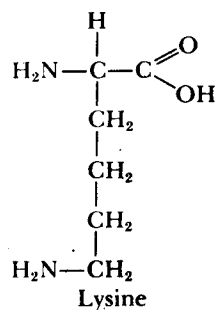
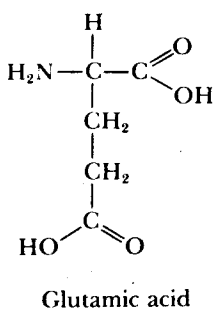
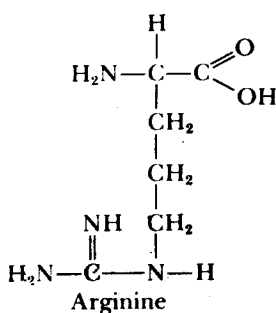
In the past thirty years, it has become apparent that the physical *interactions* between molecules—that is, those that do not form or break covalent bonds—are at least as important as the chemical *reactions*. For example, the *regulation* of chemical reactions (i.e., the degree to which they are allowed to occur) is accomplished both by physical changes in the structure of macromolecules and by variation in the availability of active sites on macromolecules resulting from the noncovalent binding of both small and large molecules. Furthermore, the large macromolecular aggregates found either in cells or in organisms (i.e., membranes, cell walls, chromosomes, tendons, hair, etc.) derive many of their special properties from noncovalent physical interactions. Therefore, chemical reactions are only half the story; clearly, to understand a complex biological system, knowledge of the physical properties of the constituent molecules is essential. The attainment of this knowledge is the goal of *physical biochemistry*. The application of the information so obtained to biological systems is the foundation of the modern discipline called *molecular biology*. A large part

of this book describes methods for characterizing macromolecules. Because the language describing macromolecules is generally unfamiliar to the student of biochemistry, the terminology and concepts used in considering the properties and shapes of macromolecules and the transitions between various forms will be explained first.

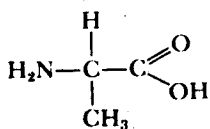
FIGURE 1-1

The amino acids and their chemical structures.

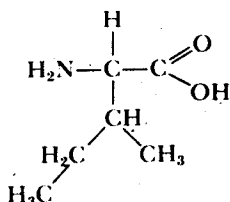
*Polar amino acids (tend to be on protein surface)*



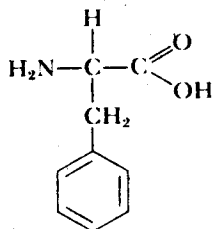
*Nonpolar amino acids (tend to be internal)*



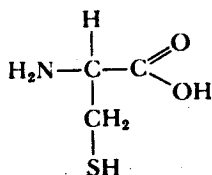
Alanine



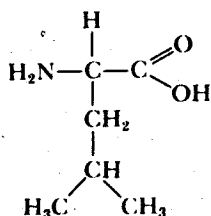
Isoleucine



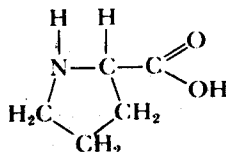
Phenylalanine



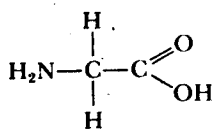
Cysteine



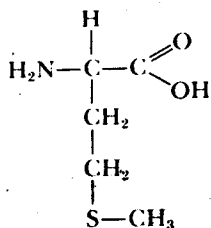
Leucine



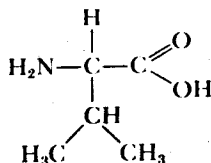
Proline



Glycine

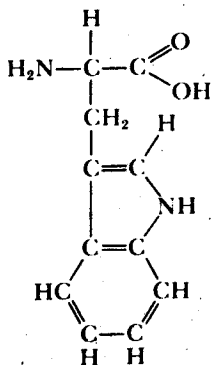
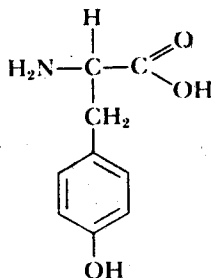


Methionine



Valine

*Amino acids equally frequently internal and external*

Tryptophan  
(nonpolar)Tyrosine  
(polar)

## POLYPEPTIDES AND POLYNUCLEOTIDE CHAINS

The components of proteins and polypeptides are the *amino acids*. The chemical structures of the common amino acids are shown in Figure 1-1 (on pages 2 and 3), in which the amino acids are grouped to indicate their usual locations in proteins. *Polar* amino acids carry charged groups that interact significantly with water (i.e., they are *solvated*). They are also called the *hydrophilic* amino acids. Because of this strong interaction with water, polar amino acids tend to be on the surfaces of proteins, thereby maximizing contact with water. Many polar amino acids carrying opposite charges (e.g., on the negative hydroxyl and positive amino groups) tend to interact with one another to form hydrogen (H) bonds (Figure 1-2) and are therefore often in close proximity. The *nonpolar* amino acids are not charged nor easily solvated by water, and therefore tend to be internal, thus minimizing contact with water. They are also called *hydrophobic* amino acids. The sulfhydryl (SH) group of the amino acid cysteine can combine with the SH of another cysteine to form a *disulfide bridge* ( $-S-S-$ ).

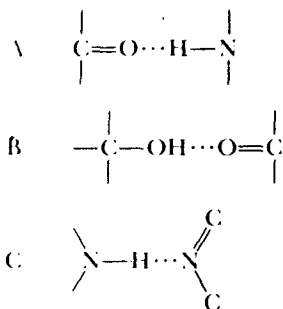


FIGURE 1-2

Structures of three types of hydrogen bonds (indicated by three dots): (A) the type found in proteins and nucleic acids; (B) a weak bond found in proteins; (C) the type found in DNA.

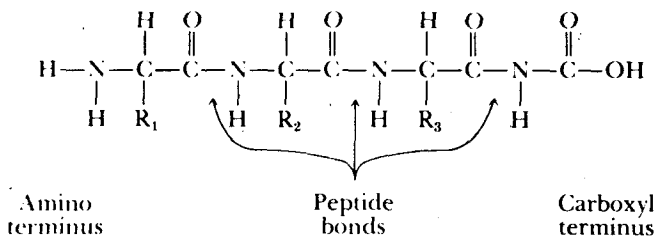


FIGURE 1-3

Structure of a polypeptide chain, showing amino and carboxyl termini, peptide bonds, and the locations of the side chains ( $\text{R}_1$ ,  $\text{R}_2$ , and  $\text{R}_3$ ).

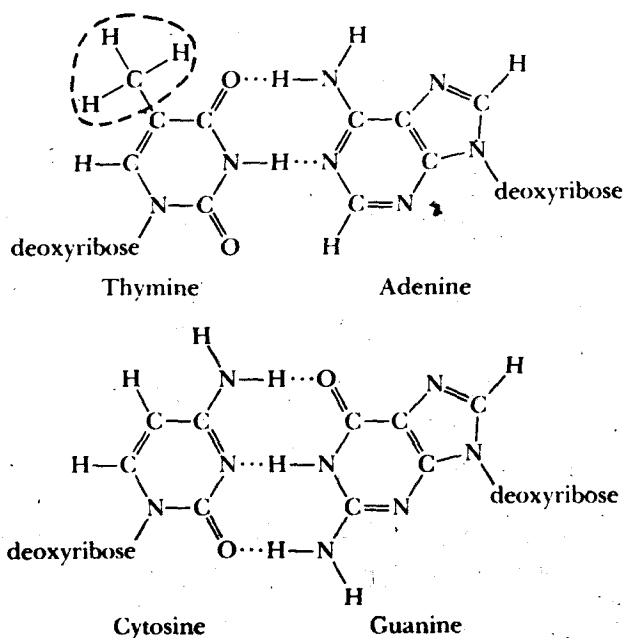


FIGURE 1-4

The two common base pairs of DNA. If the encircled methyl group were replaced by a hydrogen, the result would be uracil.

Amino acids polymerize by forming a covalent bond, called a *peptide bond*, between the carboxyl group of one and the amino group of another. The resulting structure of a polypeptide or protein is shown in Figure 1-3, in which  $R_1$  (like  $R_2$  and  $R_3$ ) represents the *side chain*, or distinguishing group, of an amino acid.

The components of nucleic acids are the bases depicted in Figure 1-4. These bases consist of relatively hydrophobic rings to which are attached charged groups that interact by means of hydrogen bonding to form the base pairs indicated. A base is covalently coupled with a sugar (deoxyribose, for DNA, or ribose, for RNA) to which a phosphate group is attached; the structures thus formed are polymerized by means of phosphodiester bonds to form a nucleic acid, as shown in Figure 1-5. When hydrogen bonds form between bases, the polar part of each base becomes less accessible to water; because the ring part is fairly hydrophobic—and considerably so compared with the sugar and the highly charged phosphate group—in a nucleic acid in which there is hydrogen bonding, the bases will be situated in a way that tends to minimize contact with water. This line of reasoning gives a rationale for the fact that, in a totally hydrogen-bonded nucleic acid such as DNA, the base pairs are internal and surrounded by the hydrophilic sugar-phosphate chains.

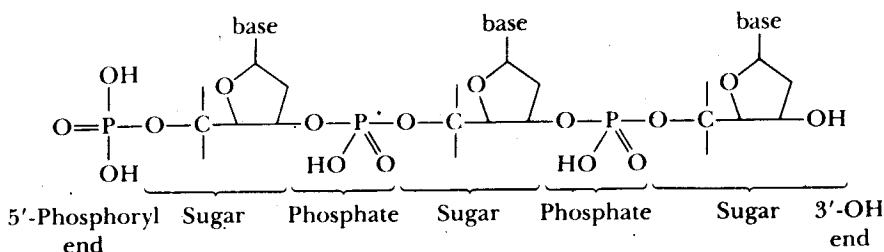


FIGURE 1-5

Structure of a single polynucleotide chain. The sugar (ribose or deoxyribose) and phosphate moieties alternate, a phosphate always connecting the 3'- and 5'-carbon atoms.

## POLYMER STRUCTURES

The *primary structure* of a polymer consisting of different monomer types refers to the monomer sequence—for example, the amino acid sequence of a protein and the base sequence of a polynucleotide. Such sequences can be determined by chemical analysis, using many of the separation procedures described in Chapters 8 and 9. The actual chemical methods, however, will not be discussed in this book.

Because of the interactions between various amino acid side chains and between nucleic acid bases and because of the relative degree of interaction of different molecules with water, biological polymers are rarely fully extended linear chains; instead, they fold to form complex three-dimensional structures. The orientation of each monomer unit with respect to another is called the *secondary structure*. By convention, secondary structure usually (but not always) refers to the *configuration* or *conformation* of the backbone—that is, the polypeptide and the sugar-phosphate chains. The shapes commonly encountered are helices, coils, sheets, and rods, and combinations thereof.

The relative orientation of the side chains (amino acids or nucleic acid bases) is usually called the *tertiary structure*. Many biological polymers interact with one another to form complex structures such as multisubunit proteins, viruses, membranes, filaments, and so forth. This is sometimes called the *quaternary structure*. However, the use of the terms tertiary and quaternary structure is sometimes ambiguous.

The peptide bond is *planar* (Figure 1-6), which puts several constraints on the possible types of secondary structures of proteins. On the other hand, all bonds involving the  $\alpha$ -carbon are flexible and allow a wide variety of possible structures.

The phosphodiester bonds of nucleic acids are also flexible (Figure 1-7). However, because the bases consist of planar, strongly hydrophobic ring

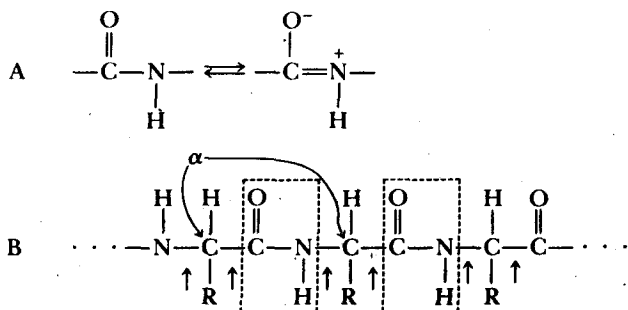


FIGURE 1-6

A. Tautomeric structure of peptide bond, showing the rigidity conferred by partial double-bond characters. This is why the peptide bond is planar. B. Part of a polypeptide chain: arrows point to bonds about which there is free rotation; the rigid peptide units are inside the boxes.

systems surrounded by only a few charged groups, they tend to stack one above the other (see Chapter 16), thereby minimizing contact with water. This tends to increase the rigidity of the structure, even in a single-stranded polynucleotide.

A linear polymer that has free rotation about all bonds in the chain and has no interaction of side groups is called a *random coil* (Figure 1-8). It does not have a unique three-dimensional structure or size because it is continually being distorted by Brownian motion. Its size can be described by an average value—the average *radius of gyration*:

$$R_G = \sqrt{\frac{\sum R_i^2}{N}}$$

in which  $N$  is the number of segments (or monomers) and  $R_i$  is the average distance of a segment,  $i$ , from the center of mass. It turns out that  $R_G$  is actually proportional to  $\sqrt{N}$ . It is also a measure of the average size of a hypothetical sphere occupied by the coil. A protein in which all hydrogen

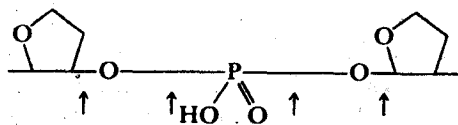


FIGURE 1-7

A phosphodiester bond; the points of possible rotation are indicated by the arrows.



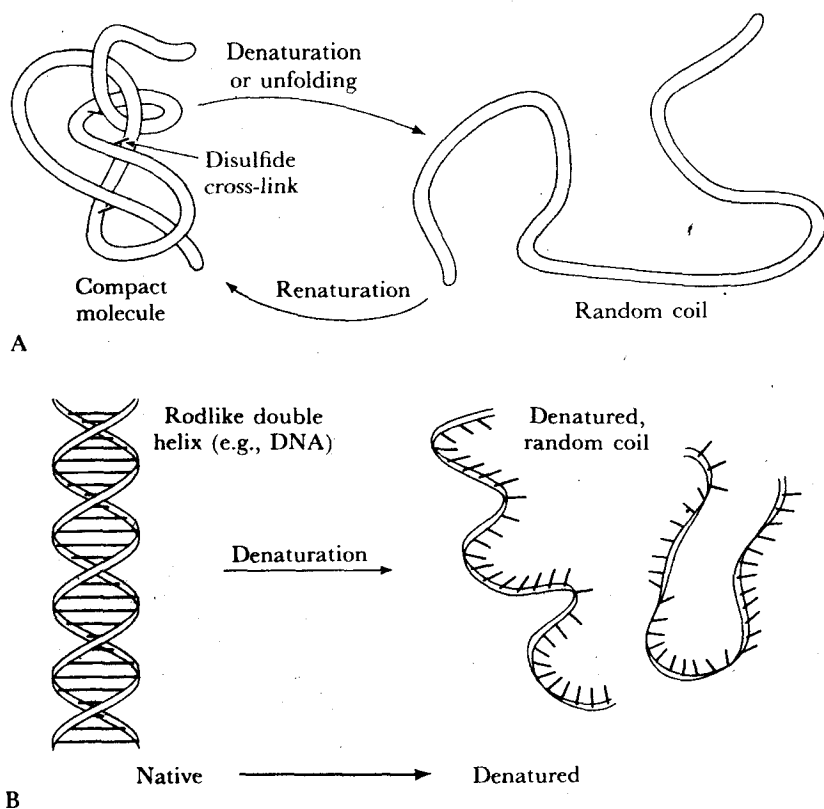


FIGURE 1-8

Several forms of macromolecules and how they are interconverted.

bonds are broken but a *few* disulfide bonds remain—which reduces the radius of gyration—is sometimes called a *near-random coil*.

Most biopolymers have substantial *side-chain interactions*, which tend to reduce the size of the molecules to less than that of a random coil. These molecules are *globular* and are said to be *compact* (Figure 1-8).

Many polymers have a *helical structure* (Figure 1-9). For proteins this means that the planar bonds are rotated about a particular point such that the rotation from plane to plane is constant. Such a structure can fit into a cylinder. A simple analogy is the structure that would be obtained if a stack of playing cards were pinned together with a straight pin at one corner but each card were rotated at a fixed angle with respect to the one below. In many proteins, rotation occurs about the  $\alpha$ -carbon atom of the amino acid (Figure 1-6) and the structure is stabilized by bonds between carboxyl and amino groups. This particular helix is called an