The Institute of Biology's
Studies in Biology no. 42

The Structure and Function of Enzymes

Second Edition

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General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology has sponsored this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

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Readers' comments will be welcomed by the Education Officer of the Institute.

1979

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Preface

The study of biochemistry is changing. Like the subject itself it is in a state of dynamic equilibrium and is very responsive to internal and external stimuli. This is certainly true of those who work in the area referred to as enzymology. Some have become more chemical in their approach and attempt, often successfully, to synthesize compounds that mimic the action of enzymes. Others take up the challenge issued by the problems of biology. Our more detailed knowledge of the structure of enzymes, particularly the existence of subunits and their interaction, has facilitated the explanations of the control of metabolism and hence the control of the cell itself. This booklet attempts not only to explain enzymes in strict chemical and physical terms but also to show how recent work has provided more satisfactory explanations of the precision of specific responses of biological systems. It is hoped that it may provide the stimulus for others to take up the challenge.

Manchester, 1978

C. H. W.

Contents

1 Enzymes as Chemical Catalysts

1.1 The need for catalysis

A common feature of the myriad chemical reactions that take place in a living cell is that these reactions occur at a far faster rate than similar reactions occurring in a non-living environment. An outstanding example of this is the contraction of a muscle where tremendous energy is used and must be made available in a very short period of time. To account for this phenomenon of the increase in reaction rate it is necessary to postulate the existence of catalysts. Catalysts find widespread use in many industrial processes and their properties have been studied extensively. In the specific case of catalysts of biological origin the term 'enzyme' is used, meaning 'in yeast' (Greek, *en* – in, *zyme* – yeast), and showing historically that much of our knowledge of the properties of enzymes was derived from studies on yeasts and other microorganisms. Thus enzymes may be defined as molecules of biological origin which increase the rate of specific reactions, although not affecting the final position of the equilibrium established, and which may be recovered from the reaction mixture at the end of the reaction.

Since earliest recorded time man has made use of the properties of enzymes in the production of alcohol and in cheese-making. At first this use must have been purely accidental and ill-controlled, but with the passing of time it is probable that early man began to realize the influence of warmth and of the other constituents on these processes called fermentation and took unwittingly the first steps in biochemistry. Slow progress ensued and it was not until the late eighteenth and early nineteenth century that more detailed investigations of the processes of fermentation were undertaken. Among the major contributions of this period were those of Schwann, who recognized that yeast was a plant capable of converting sugar to alcohol and carbon dioxide, and of Pasteur, who studied the influence of oxygen on these processes and analysed the end product of many different fermentations. Unfortunately further progress at this time was impeded by a widespread belief in vitalism, which held that a vital force was necessary for the synthesis of organic compounds and that this vital force was present only in living organisms. Finally, Buchner's demonstration of fermentation of sugar by a yeast extract from which all living yeast had been removed paved the way for the detailed examination of the chemical and physical properties of the enzymes.

In the early twentieth century the individual enzymes involved in fermentation were isolated and the intermediate compounds formed in

the production of alcohol from sugar were found. The last forty years have seen tremendous advances in our knowledge of both the pathways of metabolism and the enzymes responsible. These advances have been made possible by the development of modern technology and by the rejection of the concepts of vitalism. The belief that all the processes that occur within a living cell are capable of interpretation by the laws of physical science has been rewarded by the rapid advances that have been made; a belief in vitalism inhibits experimentation.

It is constructive to consider for a given reaction the mechanism of the uncatalysed reaction and to compare this with what is known about the mechanism of the catalysed reaction. In this way we shall be able to realize the true nature of catalysis and its control.

1.2 Ribonucleic acid and its hydrolysis by acid and alkali

Ribonucleic acid, commonly abbreviated as RNA, is a macromolecule of biological importance in the growth of cells, since, amongst its many functions is that of carrying the genetic information contained in the nucleus to the cytoplasm where it is translated into specific proteins. The essential features of a segment of the RNA molecule are shown in Fig. 1–1 and the main features of the structures summarized.

When RNA is treated under fairly stringent conditions with acid or alkali, the molecule is completely hydrolysed yielding mixtures containing molar proportions of phosphate, ribose and heterocyclic bases. However, when RNA is hydrolysed under less drastic conditions, for example 0.1M sodium hydroxide at room temperature, various larger fragments can be identified. It is interesting to consider in detail the mechanism of action of alkali on RNA. The first stage in this mechanism is nucleophilic attack by OH^- ions (as shown by the arrows in Fig. 1–2) and the first product formed is cyclic 2′,3′-phosphate (so called because the 2 and 3 positions of the ribose ring are esterified). The cyclic phosphate is only an intermediate in this reaction and is attacked by further OH^- ions cleaving the C—O—P bonds with equal facility and hence producing a mixture of 2′- and 3′- phosphates. From this it can be seen that, under conditions of mild alkaline hydrolysis, the breakdown of RNA is brought about in several steps in a partially controlled manner.

1.3 Ribonuclease and its mechanism of action

It is a fundamental property of living systems that they are in a state of dynamic equilibrium, i.e. that the molecules which make up the chemical skeleton of the cell are constantly being synthesized and degraded. Ribonucleic acid is no exception and there are present in all cells enzymes, ribonucleases, which hydrolyse this molecule. The chemical structure and physical properties of these enzymes have been investigated extensively

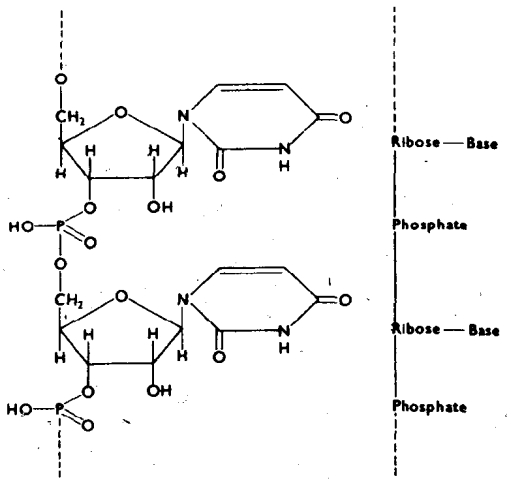


Fig. 1–1 A segment of the ribonucleic acid molecule (RNA) showing the nature of the bonding and also a summary of the relative positions of the component moieties. In this example only one type of base, uracil, is shown although in the normal RNA molecule three other bases, adenine, guanine and cytosine also occur.

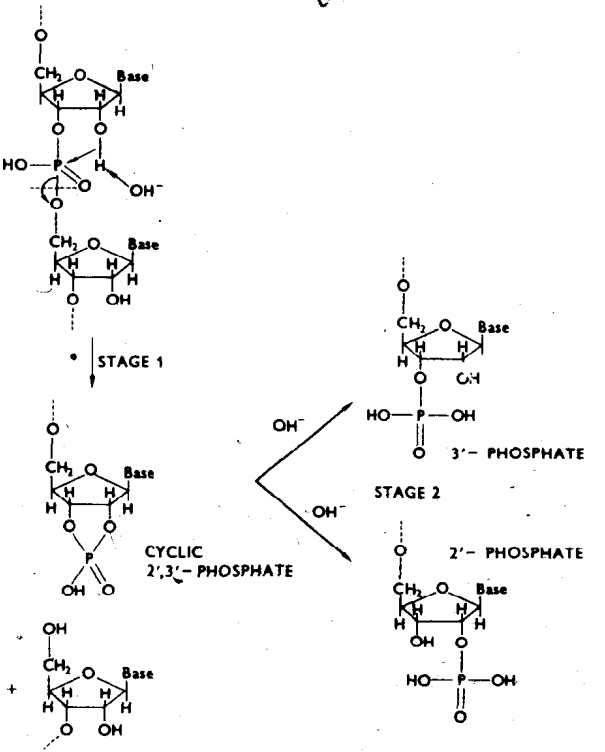


Fig. 1–2 The mechanism of mild alkaline hydrolysis of RNA.

and from these studies have emerged various theories of the mechanisms of the reactions catalysed.

The ribonuclease of bovine pancreas has been shown to hydrolyse the RNA in two stages, in a manner analogous to the action of alkali. The first stage is the production of the cyclic phosphate and this is followed by the breakdown to the 3′-phosphate. Lest it be thought that this can be simply explained by the presence of OH^- ions, it is pertinent to note that the enzyme acts in buffer of approximately neutral pH where the OH^- ion concentration is extremely small.

A possible mode of action of this enzyme is shown in Fig. 1–3. There are at least two ionizable groups in the enzyme which are involved in the catalytic activity and which form part of what is termed the 'active centre' of the enzyme. In the first stage of the reaction, group A serves as a proton acceptor paralleling the basic character of the OH^- ion while group B acts as a proton donor, i.e. it behaves as an acid. In the second stage of the reaction, the group A is now a proton donor while B is proton acceptor. After the complete series of reactions the enzyme is in its original state and ready to perform the catalysis of the hydrolysis of another P—O bond.

In a way the enzyme has brought together both acid and base hydrolysis and is consequently more effective than either would be on its own. Of course it is impossible to devise a conventional non-biological system containing both acid and base since neutralization would occur. The presence of these two ionizable groups at the active centre make the enzyme especially suited for its hydrolytic role.

1.4 Structure and biological function

This example of the mechanism of action of the pancreatic ribonuclease shows that there is a logical and strictly chemical explanation of the action of an enzyme. This has been found to hold true for all the enzymes whose mode of action has been studied in detail and there is no reason to believe that it is not universally applicable.

It is implicit in the reaction scheme given in Fig. 1–3 that the groups A and B are so arranged in space that they are in close proximity to the bond in RNA which is being hydrolysed. The groups, being part of a large molecule, are not free to diffuse through the solution of RNA as could OH^- ions and are thus dependent on being correctly spatially aligned with respect to each other and also with respect to the RNA molecule which must be bound to the enzyme in some way. The activity of the enzyme is dependent on the three-dimensional structure of the molecule and before the action of an enzyme can be fully appreciated it is necessary to consider the various chemical bonds and physical forces which together give the enzyme its structure and stability.

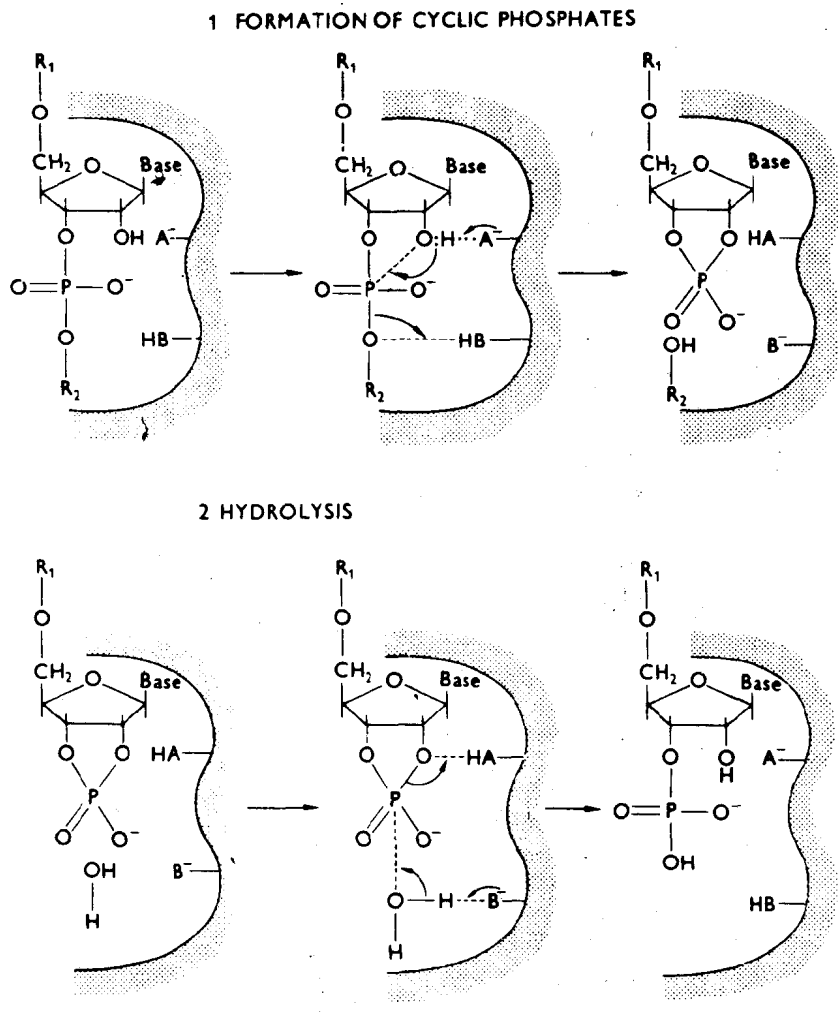


Fig. 1–3 A possible mechanism for the action of pancreatic ribonuclease on RNA. The enzyme with its two catalytic groups A and B is shown by shading. Only one repeating sequence of the RNA molecule is shown and R_1 and R_2 represent the remainder of the molecule.

1.5 The need for control

Over and above the need for catalysis of cellular reactions, there is a need for control of this catalysis. This is particularly important in the central area of metabolism, termed intermediary metabolism, where a

particular compound may be converted to many other compounds and the reaction favoured at any time will depend on the nutrition and the energetic and synthetic needs of the cell. Thus the carbon atoms of a molecule of dietary glucose may be found as expired CO_2, stored as fat or glycogen, or used to form the carbon skeleton of amino acids, sugars such as the ribose in RNA, heterocyclic bases such as uracil and a variety of other molecules. The choice of reaction route for any molecule of glucose is not an arbitrary one. If the cell is part of a tissue with excessive energy utilization at that instant, for example the heart during exercise or excitement, then the glucose will probably be oxidized ultimately to CO_2, thereby releasing its energy. On the other hand, cells undergoing rapid growth and division need the basic building blocks of the cell structure and the machinery for carrying out the building. In such a situation the glucose would tend to be converted to amino acids for protein production or to sugars and bases for nucleic acid synthesis. If both the energetic and sythentic needs of the cell are being provided for adequately, then it is probable that the glucose will be converted to a storage polymer – fat and glycogen in the case of mammalian cells.

One essential feature of enzymes is that their concentration will also affect the rate of reaction and so by varying the concentration of enzyme, subtle control of the extent of metabolic reactions may be made. While this control of metabolism by alteration of enzyme concentration is very important in some situations such as cell growth and differentiation, it is at best a coarse and sluggish control and completely unsuited to the fast accurate control demanded for cell efficiency. For this reason, the catalytic activity of the enzymes themselves is also subject to control. Thus the total concentration of enzyme may remain constant but the concentration of active enzyme may vary enormously. This variation in active enzyme may be achieved in two ways.

Firstly, the enzyme may exist in two forms, an active and an inactive form, these forms being interconvertible by covalent chemical modification. This modification is often catalysed by another enzyme. In recent years it has been found that many hormones exert their effect directly, or indirectly, on such modifying enzymes, and hence control the concentration of active enzyme within the cell. Such hormonal control of metabolism, while faster than the *de novo* synthesis of new enzyme, is still of necessity relatively slow because the secretion of the hormone usually takes place at a site in the body far removed from the target organ whose metabolism is to be controlled, and the transport of the hormone in the circulating blood can be relatively slow (see Chapter 5).

For the more instantaneous type of control, a second method of active enzyme concentration control is present: activators and inhibitors bind to the enzyme in an equilibrium system and hence determine the level of active enzyme. Such activators and inhibitors are usually small molecules and are often related to the products or reactants of the reaction catalysed

by the particular enzyme being controlled. As will be shown later, this method of control is rapid and fine and allows the precise and efficient use of available cell nutrient. It will also allow a tissue to vary its physiological activity rapidly. Thus, in the case of muscle, although the enzyme composition is essentially the same during contraction and relaxation, there are very different reactions proceeding during these two phases of muscular activity.

2 Protein Structure

2.1 Enzymes are proteins

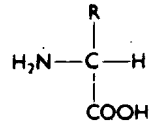
As soon as detailed analysis of the chemical structure of enzymes was undertaken, it was recognized that all enzymes were proteins. Today, even though over one thousand enzymes have been discovered and many of them fully characterized, there is no evidence to throw doubt on the validity of the identity of enzymes as proteins. In some cases there are additional features in the enzyme molecule such as metallic ions or carbohydrates and other small organic molecules, but the essential catalytic function of the enzyme is always dependent on the protein moiety, although these other non-proteinaceous materials may contribute, and in some cases be essential, to the catalytic activity.

The converse is not true, for some proteins are apparently devoid of catalytic activity. Collagen, the major protein of bone, skin and tendon is an example of such a protein. Collagen seems to play a structural role in the body and as such might not be expected to take part in the degradative and synthetic processes, which are collectively termed metabolism. Another example of a structural rather than a metabolically active protein is keratin which forms part of the exterior surfaces of the body such as hair, nails, horns and the outer layer of skin – the epidermis. Serving a different function are the proteins called γ-globulins. These are the antibodies which provide part of the body's defence mechanism against disease and all invasions by foreign matter. They have not been shown to possess any catalytic activity.

To understand fully the nature of enzymes it is necessary to consider the detailed structure of proteins. Proteins are polymers of amino acids, of which there are twenty which commonly occur in proteins. From this relatively small number of basic building units it must be possible to make at least a thousand different proteins and so obviously the nature and quantity of each amino acid, together with its arrangement within the protein polymer will be crucial to the structure of the enzyme. We must first consider the nature and variety of amino acids.

2.2 The nature and diversity of amino acids

The amino acids possess both amino and carboxyl groups and therefore exhibit both basic and acidic properties. They may be represented by the general formula:

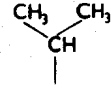
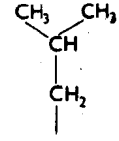
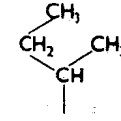


where R is different for each of the different amino acids. Except in the case of the simplest amino acid, glycine, where R is hydrogen, the central carbon atom is bonded to four different groups thus giving asymmetry to the molecule and hence the ability to rotate the plane of polarized light, i.e. optical rotation. Proteins also show optical activity, both as a result of their constituent amino acids and also as a result of the further asymmetry which is produced in the molecule of polymer by the linkage of the amino acids. This in part accounts for the extreme selectivity in the reactions which they will catalyse and also the precision of the products formed.

Later in this chapter we shall see that the amino and carboxyl groups are involved in the polymerization of amino acids to form protein. Thus it is the nature of R which contributes most to the catalytic activity of proteins, and the diversity of R which allows the diversity seen in the variety of reactions catalysed. It is convenient to classify the various R groups in seven broad categories (Table 1). The seventh category, the

Table 1 Classification of amino acids by the nature of their side chains (R).

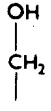
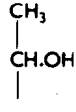
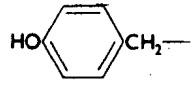
(1) *Non-polar aliphatic groups*

Glycine (Gly)	Alanine (Ala)	Valine (Val)	Leucine (Leu)	Isoleucine (Ile)
				

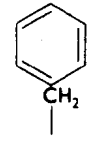
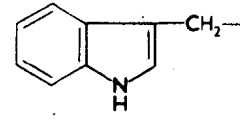
Inert and Hydrophobic

Aliphatic are neutral but tyrosine is weakly acidic. Less hydrophobic than the non-polar aliphatic amino acids.

(2) *Hydroxyl-containing aliphatic and aromatic groups*

Serine (Ser)	Threonine (Thr)	Tyrosine (Tyr)
		

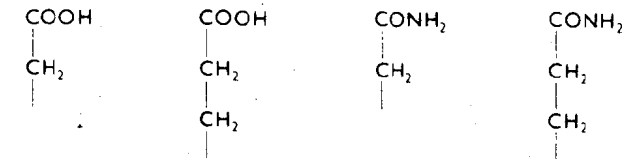
(3) *Aromatic*

Phenylalanine (Phe)	Tryptophan (Try)
	

Tyrosine is also aromatic but is conveniently classed with the other hydroxyl-containing amino acids. Hydrophobic.

(4) *Acidic*

Aspartic acid (Asp) | Glutamic acid (Glu) | Asparagine (Asn) | Glutamine (Gln)

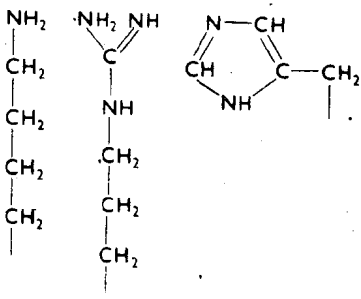


Asparagine and glutamine are the amides derived from aspartic acid and glutamic acid respectively

Hydrophilic.

(5) *Basic Groups*

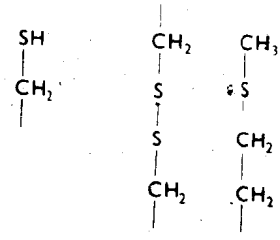
Lysine (Lys) | Arginine (Arg) | Histidine (His)



Hydrophilic

(6) *Sulphur containing groups*

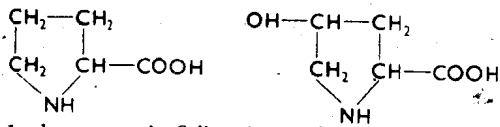
Cysteine (Cys) | Cystine (Cys-Cys) | Methionine (Met)



Cysteine is weakly hydrophilic while methionine is hydrophobic. Cystine is two cysteine residues linked by a S—S bond

(7) *Imino groups*

Proline (Pro) | Hydroxyproline (Hyp)



In these cases the full amino acid structure is shown.

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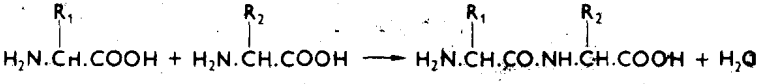
amino acids, are not true amino acids since in this case the amino group is not free but forms part of a ring structure. This difference leads to important modifications of protein structure as we shall see later. The important physical properties of the group are summarized at the end of

each group. Of particular note is the interaction of the group with water. It is possible to divide groups roughly into two categories:

(1) *Hydrophilic – literally 'water loving'* Such groups confer the property of easy solubility in water. Acidic and basic groups are very hydrophilic.
(2) *Hydrophobic or 'water hating'* Such groups are not easily soluble in water and other polar solvents such as alcohol, but are readily soluble in ether, benzene and similar non-polar solvents due to the hydrocarbon nature of the group.

2.3 Primary structure – the peptide bond and the sequence of amino acids

Elimination of water between the carboxyl group of one amino acid molecule and the amino group of another results in the formation of a linkage similar to the type found in amides and called in this case the peptide bond:



where R_1 and R_2 represent the same or different substituent groups. The molecule formed is termed a dipeptide. Because the amino acid is bifunctional (i.e. it contains both an amino and a carboxyl group) this process may continue leading to the formation of a tripeptide, tetrapeptide, etc. After the condensation of a large number of amino acids the product is known as a polypeptide. The precise distinction between a polypeptide and a protein is not clear. Most proteins have at least 100 amino acids linked together (commonly expressed as amino acid residues) and in addition have a definite higher order structure. Polypeptides, on the other hand, is a term usually confined to molecules containing of the order of twenty amino acid residues and lacking a higher order of structure. Each polymeric molecule will only have one free α-amino and one free α-carboxyl group. Thus the properties of these two groups will play little part in the properties of the protein molecule, which will depend very largely on the properties of the R group. As can be seen from Table 1 some amino acids contain additional amino and carboxyl groups and these will of course be free and contribute to the protein's properties.

The order in which the amino acids are joined together is called the primary sequence of a protein and this, together with the structure of the peptide bond, make up what is termed the primary structure of the protein. The sequence of amino acids in many proteins has now been determined. The full structure of the hormone corticotrophin as isolated from sheep is:

Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val.Gly.Lys.Lys.
Arg.Arg.Pro.Val.Lys.Val.Try.Pro.Ala.Gly.Glu.Asp.Asp.Glu.Ala.Ser.Glu.
Ala.Phe.Pro.Leu.Glu.Phe.

The abbreviations of the amino acids are usually used in writing the sequence of a protein or polypeptide and it is convention that the sequence should be written from the N-terminal end, i.e. from the end that has the free α-NH_2 group.

What would happen if the same 39 amino acids were joined together in a different order? In general the molecule would lose its biological activity. Thus the nature and sequence of amino acids are always specific for a particular protein. When one considers proteins serving identical functions for different species then some slight variations in the nature and sequence of the proteins is seen. For the corticotrophin the residues 1–24 and 33–39 appear to be invariable for all species studied. However, minor modifications are apparent in the residues 25–32 when the primary sequences of pig, human, ox and sheep corticotrophins are compared. Deviations from the sequence of pig corticotrophin are underlined. Very

	25	26	27	28	29	30	31	32	33
Pig	Asp	Gly	Ala	Glu	Asp	Gln	Leu	Ala	Glu
Man	Asp	Ala	Gly	Glu	Asp	Gln	Ser	Ala	Glu
Ox	Asp	Gly	Glu	Ala	Glu	Asp	Ser	Ala	Gln
Sheep	Ala	Gly	Glu	Asp	Asp	Glu	Ala	Ser	Gln

often the alterations are of a minor nature and amino acids with similar properties are involved. Thus alanine has replaced glycine to which it is very similar, and glutamic acid has replaced the other acidic amino acid, aspartic acid.

2.4 The active centre concept

It is now well established that only a few specific amino acid residues in a protein molecule are involved directly in the functioning of that protein. In the case of enzymes, these specific residues are responsible for the catalytic activity of the molecule. The other amino acid residues help to form and stabilize the three-dimensional structure of the molecule. In the case of corticotrophin, residues between 1 and 24 and/or between 33 and 39 are probably responsible for the hormonal function, while the variable residues between 25 and 32 help to direct the general shape of the molecule and provide species individuality.

Figure 2–1 shows the primary sequence of the protein, ribonuclease, which was introduced in the first chapter. Ribonuclease contains 124 amino acids and it has been shown that the histidines at 12 and 119 (written His 12 and 119) together with Lys 7 and 41 are among the