Protein Structure and Properties[1]

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ABSTRACT

Proteins are the most versatile of the bipolymers with respect to structure, properties and function. This versatility is a consequence of the chemical diversity of their amino acid monomers and of the infinite number of ways in which the amino acid composition, linear sequence and three-dimensional folding may be varied. The constituent amino acids include hydrophobic and hydrophilic, reactive and inert forms. The *trans*, planar nature of the amido (peptide) linkage between amino acids limits the conformational freedom of the resulting polypeptide chain. Portions of the chain usually occur as one of several regular forms such as helixes, stabilized by hydrogen bonds. Overall conformation of the molecule is maintained largely by noncovalent forces such as hydrogen bonds and hydrophobic interactions. Conformation of the protein is determined by the linear sequence of amino acids in the chain, but is readily interrupted by a variety of nonphysiological agents, with concurrent loss of biological function. This rearrangement of the polypeptide chains, denaturation, usually results in an alteration of the characteristic chemical and physical properties of the molecule. The observation that denaturation can sometimes be reversed leads to new concepts of protein structure and properties.

INTRODUCTION

In this review fundamental aspects of protein structure and properties will be presented by a somewhat unusual approach. A vast amount of evidence bearing on protein structure and properties will not be covered so that a few generalizations could be made and an exciting look into the future would be possible.

Because the structure and properties of oil seed proteins have not been intensively investigated, most of the present discussion will involve examples of proteins from other sources, which can be usefully applied to oil seed proteins. Moreover, the properties described will be usually of a single pure protein, in contrast to the bulk mixture of oilseed proteins commonly available.

One of the unique characteristics of living cells is that their most abundant organic constituents are macromolecules, high molecular weight polymers of several different types. The structural complexity of these biopolymers is closely related to the complexity of their cellular functions. In plant cells, the most abundant carbohydrate is cellulose, a polymer of glucose units. The cellular function of cellulose is that of a structural component, and a polymer of only one kind of monomer units is sufficient, perhaps even advantageous, to perform that function. The function of storage of genetic information is performed by deoxyribonucleic acid, a biopolymer composed of four different monomers. Although these four monomers are sufficiently different from each other to be readily recognized by the enzymes which transcribe and translate the information contained in the sequence of the monomers, all of the monomers contain rather chemically similar heterocyclic bases.

Functionally, proteins are far more versatile than other biopolymers. Protein enzymes specifically catalyze each of the thousands of chemical reactions ocurring in a living cell; proteins convert chemical energy into mechanical energy in contractile tissue, serve as structural supports in connective tissue, serve as hormones to regulate metabolic processess, and transport O_2 in the blood. Many less noticeable functions of proteins in seeds include storage of nitrogen and amino acids. This versatility of protein function is reflected in the high degree of variability observed in the composition, structure and properties of the proteins, and somewhat limits generalizations which can be made about them. To a certain extent every protein is unique. At present the structures and properties of proteins are not systematically predictable from each other or from other parameters, but the situation is improving.

AMINO ACIDS

Twenty different monomers, 19 α-amino acids and the imino acid proline, are the fundamental units of protein biopolymers. In contrast to the essentially uniform nature of the monomers from which cellulose and nucleic acids are polymerized, amino acid monomers differ widely in chemical and physical properties, thus contributing to the extraordinary variability of protein properties. They differ from each other in the nature of the substituent on the α-carbon atom, which ranges in size from a hydrogen atom (glycine) to the heterocyclic indole ring system of tryptophan. Hydrophilic substituents differ and acids, amines and alcohols are common. Hydrophobic substituents include straight and branched-chain hydrocarbons, and aromatic forms. The aromatic amino acids are the major contributors to characteristic protein ultraviolet absorption spectra, which usually have a peak around 280 nm (1). The relative chemical reactivity of the substituents range from the inert hydrocarbons to the highly reactive sulfhydryl group of cysteine and the imidazole group of histidine. Other substituents include amines, phenolic hydroxyl (tyrosine), thioether (methionine), guanidino group (arginine), etc. In fact, most of the common types of organic chemical groups, with the notable exception of carbonyl and unsaturated forms, are represented in the side chain substituents of the amino acids. However, of countless variations of chemically feasible and essentially equivalent forms actually found in amino acids from nonprotein sources, only 20 are present in proteins from all sources, which indicates a sufficient degree of variability to provide the necessary functional versatility of proteins. Some proteins, notably enzymes, often occur conjugated with some other nonprotein material and provide even greater functional heterogeneity.

Large differences in the properties of amino acids have been emphasized as an important aspect of the unique nature of proteins. Protein structure and properties are also a function of the number of amino acids present (the length of the chain), the amino acid composition (a protein consisting largely of hydrophobic amino acids would be expected to be much less soluble in water than a protein with a high proportion of hydrophilic residues), as well as the sequence of amino acids within a chain of defined

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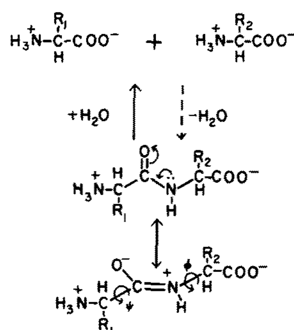

FIG. 1. Chemistry of the peptide bond.

length and composition. Are these parameters sufficient to account for the extraordinary variety observed in proteins? For example, in the human organism there may be as many as 5 million different kinds of protein molecules, none of which are identical with the hundreds of thousands of different kinds in corn plants or yeast cells.

To indicate the variability in protein structures let us hold constant one of these parameters, the number of amino acids present, and vary the amino acid composition and sequence. Assume the number of amino acids present in the single chain is 400, an average size protein of molecular weight about 50,000. At each of the 400 positions in the chain any one of the 20 amino acids can occur, so that the total number of different combinations is 20^{400}, or 10^{520}. To appreciate the incomprehensible number 10^{520}, consider the size of a single molecule of each of the different proteins of this size. Assuming a density of 1g/cc, which is slightly lower than that of most proteins, the resulting collection of molecules, each different from others of the same length, would form a cube of 10^{149} light years on one edge. Obviously only an infinitesimal fraction of the latent variability is expressed in actual protein structures (2).

Several properties of amino acids and the peptide bond by which they are polymerized into proteins are illustrated in Figure 1. First, all amino acids except glycine have an asymetric carbon atom and are therefore optically active. All amino acids obtained from proteins have the "L" configuration. Also, when joined with folded and twisted chains, additional asymmetry is imposed upon the molecule; this activity can be used to characterize the protein, although the relationship between optical activity and protein structure is not direct and explicit (3). For example, alteration of the sequence of amino acids in a short peptide produces large and unpredictable changes in optical activity (4). Second, formation of the peptide bond is not simply the reversal of its degradation by hydrolysis, which is readily accomplished by a simple enzymatic reaction, or in a strong acid or alkaline solution. The former, whether done enzymatically or synthetically, requires a source of chemical energy and precise direction for the process (5). Third, the peptide bond itself has two properties which contribute to the unique structure of protein molecules. It has approximately 40% double bond character due to resonance, and therefore has the possibility of *cis-trans* isomerism. In all cases examined peptide bonds have been of the *trans* variety. Indeed, with models, it can be deducted that bulky substituents on the peptide chain would make the *cis* double bond highly unstable. Moreover, as a consequence of its double bond nature the six atoms involved in the peptide bond lie on a plane, and there is no rotation about the bond. Therefore one of every three bonds in the backbone of the polymer is not free to rotate, and a polypeptide chain is not analogous to some other types of polymers such as uncrosslinked polyethylene in which all bonds rotate freely. Since there are three bonds

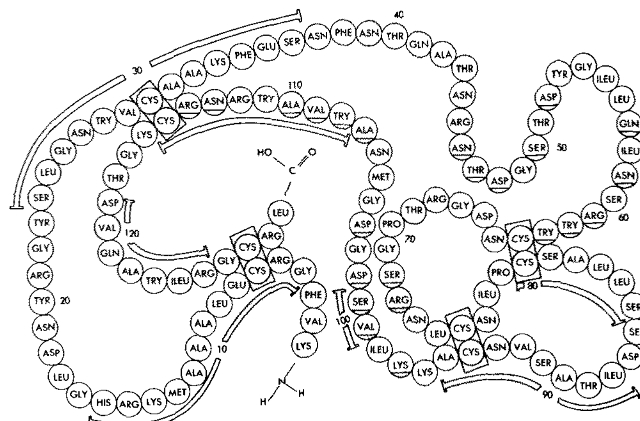

FIG. 2. Primary structure of hen egg white lysozyme (9).

in the peptide backbone of the protein polymer for every amino acid, and the bond lengths and angles have been well defined from model compounds, once one end of the peptide chain is fixed, the three-dimensional position of all the atoms in the backbone chain can be described by specifying the degree of rotation of each of the pair of rotable bonds in every amino acid (6). Additional constraints on the degree of flexibility of the polypeptide chain are imposed by the bulky side chain substituents of the amino acids. Thus, the three-dimensional arrangement of a polypeptide chain in a protein is not random, but is constrained in several different ways.

ORDERS OF PROTEIN STRUCTURE

For convenience, we shall follow the commonly accepted convention of describing protein structure in orders of increasing complexity, primary through quaternary. Primary structure is the sequence of amino acids polymerized into a linear chain by formation of an amide linkage between the α-carboxyl group of one amino acid with the α-amino group of the next. Figure 2 shows the primary structure of chicken egg white lysozyme, an intensively studied enzyme protein. Note that it is a single linear chain of 129 amino acids intramolecularly crosslinked by four disulfide bonds. Sanger and associates were the first to successfully determine the primary structure of a protein, insulin, twenty years ago, a tremendous feat which fully justified the awarding in 1954 of the Nobel Prize. Since that time the amino acid sequence has been elucidated for scores of proteins (7).

This basic primary structure of proteins is the result of strong covalent bonds between amino acids, and superimposed upon it are the higher orders of protein structure, which are due to relatively weak noncovalent forces. Secondary structure is the uniform periodic formation of hydrogen bonds between the components of the peptide linkage itself, the carbonyl oxygens and amide hydrogens. These structures may be arranged as coiled helixes or sheets, first suggested by Pauling et al. (8). The α-helix structure is shown in Figure 3. Due to the repetition of the elements of the peptide bond along the chain, secondary features consist of ordered repeated units of the peptide chain with the amino acid substituents sticking out into space outside the helix or outside the plane of the sheet. In many cases, only a small portion of the protein molecule is involved in secondary structures, with the remainder folded in what appears to be an irregular or random arrangement, but which may be quite precise. For example, irregular regions may connect several helical portions which are bent back on each other to give a much more compact form than a completely helical molecule would assume.

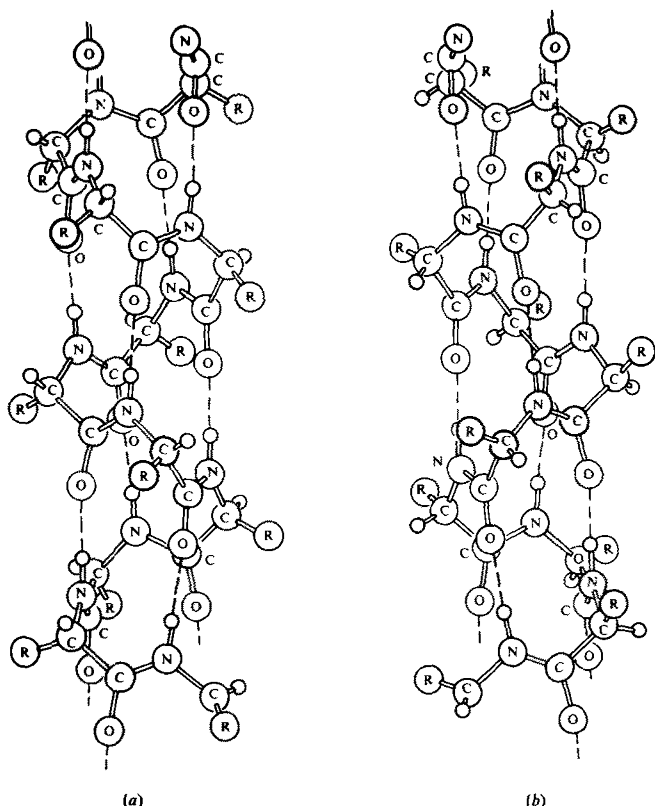

FIG. 3. The α-helix (26).

The overall shape of the folded protein chain is considered to be the tertiary structure. Some of the forces which stabilize the tertiary structure are illustrated in Figure 4. These include irregular hydrogen bonds involving appropriate amino acid chains as well as elements of peptide bonds, nonpolar interactions and salt bonds. Nonpolar interactions are not true chemical bonds at all but are simply hydrophobic residues clustered together to form a nonaqueous region similar to an oil droplet, rather than being dispersed and exposed to the aqueous solvent in which they are essentially insoluble. Salt bond refers to electrostatic attraction between oppositely charged ionized groups; such forces are probably relatively unimportant in maintenance of protein structure because of electrostatic isolation by hydration and by counter ions from the medium.

Primary, secondary and tertiary orders of protein structure refer to interactions within a single polypeptide chain. However, many proteins consist of two or more polypeptide chains, and the manner in which these chains associate is considered to be the quaternary structure. Table I demonstrates that, in general, the number of chains within a protein molecule is some multiple of 2, especially for proteins which are composed of identical chains. Thus, quaternary structure usually involves some degree of symmetry, which may be so highly structured as to approach that of the crystalline state. The forces which maintain the associated peptide chains in quarternary structure are probably similar to those which maintain the precisely folded tertiary structure within a single chain.

In general the bonds responsible for maintenance of secondary, tertiary and quarternary orders of protein structure are noncovalent in character, and therefore may be readily disrupted by mild alterations in chemical or physical conditions. The single common exception to the generalization of noncovalent stabilization of the higher orders of protein structure is the disulfide bond, which is formed by oxidation of two cysteine residues to form a

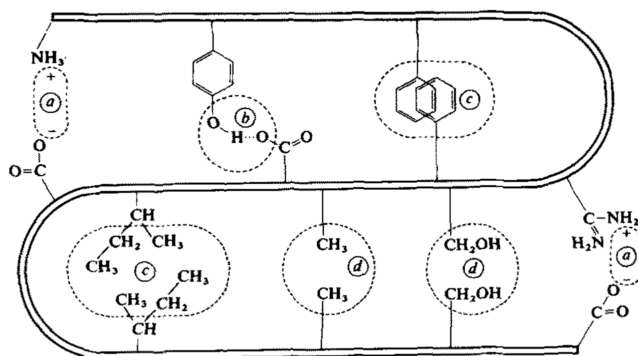

FIG. 4. Forces which stabilize tertiary structure of proteins. (a) Salt linkages, (b) hydrogen bonds and (c) hydrophobic interactions.

strong covalent crosslink between two peptide chains or between two areas within a single peptide chain. Disulfides, therefore, introduce a highly stable chemical crosslink which is of great importance in maintaining tertiary and possibly quaternary structure for some proteins. However, some proteins have no disulfide bonds (Table I), so the maintenance of protein structure can be independent of covalent interactions at all degrees of organization higher than the primary structure.

Probably the greatest advance in recent years in the elucidation of protein structure has been due to the technique of x-ray chrystallography. In favorable cases this technique allows the determination of the three-dimensional position in space of every atom in the molecule. Thus it is possible to build an accurate model, and for the first time to see what a protein molecule looks like. Figure 5 is a two-dimensional representation of a model of the three-dimensional structure of the polypeptide backbone of lysozyme, as determined by David Phillips and associates at The Royal Institution in London (9). The disulfide bonds and several short helical sections joined by nonhelical segments are readily apparent. Note the much more complicated arrangement in three dimensions than could be visualized from the amino acid sequence of the same molecule shown in Figure 2. Correlation of the x-ray data with chemical data on the structure of the lysozyme molecule and the reaction it catalyzes has resulted in a comprehensive and satisfying explanation of how the enzyme participates chemically in the catalytic process (10).

A few pertinent generalizations can be made about the protein structures which have been solved by x-ray crystallography (11). Space-filling models indicate that the structure is compact, with essentially no space inside for solvent water. In general the hydrophobic residues are on the inside, creating an environment of low dielectric constant, and the hydrophilic residues are on the outside in contact with water. There is no obvious symmetry within a single chain, as expected from the observed absence of repeating sequences in the primary structure. There are intriguing similarities between the structures of proteins which are highly homologous, i.e., that have significant portions of the chain in which the sequence of amino acids are identical (12).

The question of whether protein structure in solution (physiological conditions) corresponds to the structure in the nonphysiological conditions necessary for crystallization is of great importance (13). The question has been answered in a few cases in the affirmative, at least to the extent of demonstrating that the crystal structure possesses biological activity and is therefore of some intrinsic interest, whether or not it exactly conforms to the solution structure (14).

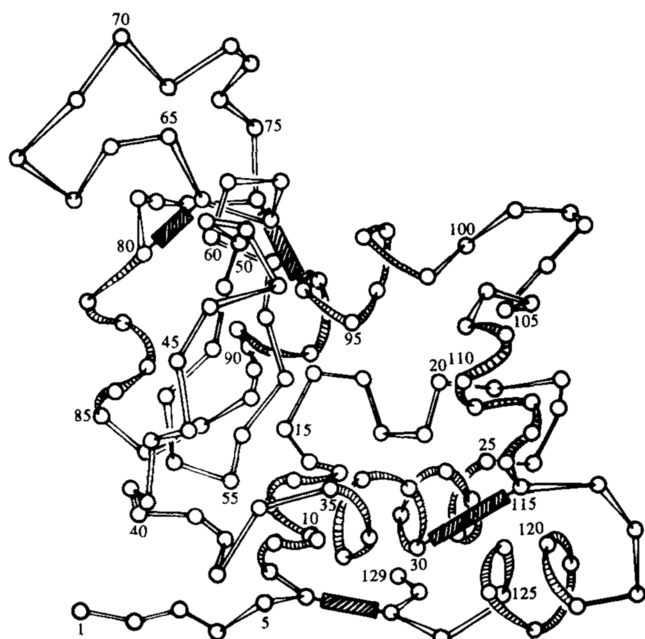

FIG. 5. Schematic representation of the polypeptide conformation of lysozyme. The shaded rectangles represent disulfide cross-links (9).

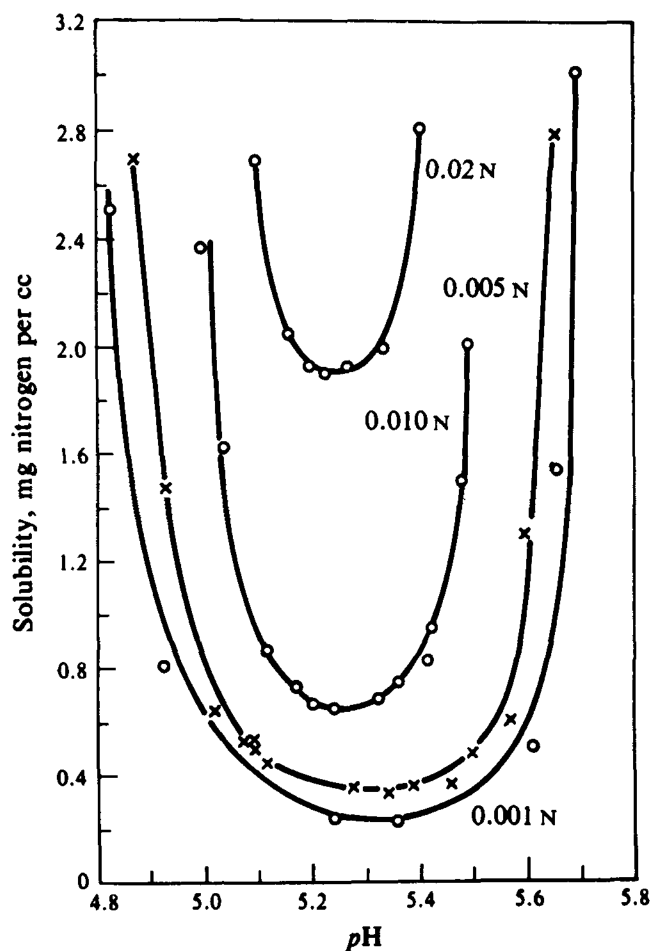

FIG. 6. Solubility of β-lactoglobulin as a function of pH at four different concentrations of sodium chloride (27).

PROPERTIES OF PROTEINS

Proteins possess unique and varied physical and chemical properties, not all of which are predictable from their structures. A property especially useful for classification of the seed proteins is that of solubility in various types of aqueous solvents. Simple proteins which are soluble in pure water, called albumins, are a rather minor component of seed proteins. Proteins which can be dissolved in dilute salt solution but are insoluble in pure water are called globulins. The seeds of soybean and peanuts contain considerable globulins. In general, the enzymes and the hormonal proteins are either albumins or globulins. Proteins more characteristic of plant seeds are the glutelins, which are soluble in dilute acids and alkalies but not in neutral solvents, and the prolamins, which are soluble in 70-80% alcohol but insoluble in water or neutral solvents. Other solvent systems are of interest for special purposes. In our laboratory we have found that phenol is an extraordinary protein solvent. Essentially all proteins of animal and bacterial extracts are quantitatively and irreversibly extracted into phenol when partitioned between phenol and neutral aqueous solutions (15,16). Although this usually results in loss of catalytic activity of enzyme proteins, we find that the technique is quite useful for separating proteins from phenol-insoluble materials such as phosphorylated metabolites.

In general, we expect that the solubility of proteins should be predictable from their composition; that is, proteins containing a high proportion of hydrophobic residues should be only slightly soluble in aqueous solutions and proteins containing a high proportion of hydrophilic residues would be expected to be much more soluble in aqueous solutions. The relative insolubility of the glutelins and the prolamins makes them difficult to characterize adequately; the structures of the albumins and globulins has been studied in much greater detail. The information discussed here has been obtained primarily from studies of albumins and globulins, and may pertain much less directly to glutelins and prolamins.

The solubility as a function of pH and ionic strength of β-lactoglobulin, a well characterized milk protein, is shown in Figure 6. At any pH value solubility is enhanced by increasing the salt concentration. This is conventionally explained by the electrostatic isolation of protein molecules from each other by the salt, under conditions where they would otherwise tend to aggregate and precipitate out of a solution. At very high concentrations of polyvalent salts, the solubility of most proteins is greatly lowered. This salting out process is probably due to competition between the salt and the protein for solvent water to maintain the stable highly hydrated state.

All proteins contain amino acids with ionizable side chains and therefore have the properties of electrolytes. Because the ionizable groups include both acidic and basic forms with pK values which range from 3 to 12, except under unusual conditions proteins bear both positive and negative charges. The net charge on the protein molecule is a function not only of the pH but of the composition (relative abundance of basic and acidic amino acids). There is a pH value, the isoelectric point, characteristic for each protein, at which it possesses equal numbers of positive and negative charges. At pH values lower than the isoelectric point, the protein bears a net positive charge and at pH values higher than the isoelectric point the protein has a net negative charge. As can be seen from Figure 6, the solubility is a function of pH, with a minimum solubility near the isoelectric point where due to the lack of net charge on the molecules, electrostatic repulsion is minimized.

The rate and direction of migration in an electric field is a function of the net charge on the molecule. It is important to point out that at the isoelectric point where the net charge on the molecule is O, and therefore there is no migration in an electric field, the protein nevertheless is

TABLE I

Structural Parameters of Several Proteins

Protein	Molecular weight	Number of chains	Number of disulfides
Insulin	5,800	2	3
Ribonuclease	13,700	1	4
Lysozyme	14,400	1	4
Myoglobin	17,000	1	0
Papain	20,900	1	3
Chymotrypsinogen	25,000	1	5
Carboxypeptidase	34,300	1	0
Hexokinase	45,000	2	0
Taka-amylase	52,000	1	4
Bovine serum albumin	66,500	1	17
Yeast enolase	67,000	1	0
Hemoglobin	68,000	4	0
Alkaline phosphatase	80,000	2	4
Hemerythrin	107,000	8	0
Lactic dehydrogenase	140,000	4	0
Muscle aldolase	160,000	4	0
γ-globulin	160,000	4	25

a charged molecule, containing equal numbers of positive and negative charges. There is no pH to which one can adjust a protein solution such that ionization is suppressed and the molecule is uncharged. As a consequence of this charge on the molecule, proteins electrostatically bind ions of opposite charge; not only hydrogen ions but anions and other cations. Of course, binding of any ion alters the net charge on the molecule and modifies its rate of migration in an electric field. The phenomenon of binding of small molecules to the macromolecular proteins is a general one and often involves forces other than electrostatic attraction. Substrates bind in a highly specific manner to enzymes before they are acted upon (*10*) and many materials are transported by being bound to proteins in the circulatory system.

PROTEIN DENATURATION

We have seen that the bonds which maintain the folding of the polypeptide chain and thus define the shape of the molecule are largely noncovalent in character. Thus, relatively small fluctuations in the chemical or physical environment may result in relatively large changes in the folding of the chain. It is unlikely that a protein molecule has a particular fixed conformation; globular proteins are thought to be in a state of dynamic flux, with portions of the molecule opening up and closing, or helical sections uncoiling and recoiling. Various portions of the molecule may interact in concert with each other. As the temperature is raised, for example, these motions may increase so greatly that disorganization of the structure can occur through disruption of the previously mentioned noncovalent forces which stabilizes the characteristic shapes and forms of the molecule at lower temperatures. When this occurs, whatever biological activity the molecule possesses is usually lost and only rarely can be regained by lowering the temperature. This process of denaturation is the result of disruption of noncovalent bonds, without breaking covalent bonds. Thus, a characteristic property of proteins is their susceptibility to denaturation, or their lability. Many other types of physical treatments such as freezing, radiation, extreme dilution and exposure to an air-water interface can result in denaturation. A wide variety of materials are effective chemical denaturants (*17*). For example, organic solvents tend to disrupt hydrophobic bonds by promoting solubility of hydrophobic residues in the solvent medium, decreasing their tendency to coalesce to avoid the aqueous environment, with resulting denaturation of the protein. The effectiveness of classical denaturants such as urea had previously been considered to be due to their ability to disrupt hydrogen bonds, but it has been recently shown that urea and guanidine hydrochloride promote the solubility of the hydrophobic residues in the aqueous medium and may therefore also disrupt the hydrophobic interactions (*18*). Detergents are good denaturants because of their ability to provide a chemical bridge between hydrophobic and hydrophilic environments, thus diminishing the effectiveness of hydrophobic interactions.

TABLE II

Reconstitution of Reduced Denatured Proteins

Protein	Disulfide bonds	Observed recovery of activity, % (approx.)	Random recovery of activity, % (approx.)
Ribonuclease	4	100	1
Lysozyme	4	80	1
Poly-DL-alanyl trypsin	6	8	0.01
Alkaline phosphatase	2/chain	80	33
Pepsinogen	3	50	6.7
Serum albumin	17	50	?

The properties of a protein are altered considerably on denaturation. For example, the solubility of denatured proteins is far lower than that of undenatured native proteins. This is probably due to the increased opportunities for intermolecular protein-protein interactions in denatured proteins. Indeed, the process of denaturation can be considered to result in turning the molecule inside out, exposing numerous hydrophobic residues for interaction with those of other similarly deformed molecules, resulting in the formation of large, insoluble aggregates. The chemical reactivities of certain amino acid side chains can also be altered dramatically on denaturation. Sulfhydryl groups, for example, may be completely unreactive in the native molecule because of being buried in the core, but in denatured proteins their reactivity usually approaches that of small model compounds (*19*).

Denaturation has usually been considered to be an irreversible process, but as shown in Table II, under the proper conditions the process can be completely reversible. For example, when the four disulfide bonds of lysozyme are reduced and the structure disrupted to that of a random coil, random reoxidation of the disulfide bonds leads to regaining of the correct pairing in less than 1% of the molecules. However, if the denaturant is removed and conditions returned to near physiological, the reformation of correct disulfide pairs is 50 to 80 times better than random, suggesting that the process is somehow directed. The protein ribonuclease gives even higher yields of reconstituted protein from denatured protein, and in both cases the reconstituted material is virtually identical to native protein. Reconstitution of denatured protein may require gradual rather than abrupt alteration of denaturing conditions (*20*).

Because under denaturing conditions all secondary and tertiary structure is destroyed (sometimes this is only assumed, and not rigorously proven), the implication is that the primary structure contains sufficient information within the sequence of amino acids to direct the proper recoiling and refolding of the chain into the unique structure of the biologically active native protein. An alternative way of stating this is that in cases where a high degree of reversibility of denaturation is observed there is a single unique structure which is thermodynamically more stable under suitable physiological conditions than all other arrangements, and that this most stable form is the

biologically active form. If this dogma universally applies to all proteins, then it can be concluded that the genetic information which specifies the amino acid sequence of a protein is sufficient to determine the complete three-dimensional structure of that protein, and that no bending or coiling enzymes or other materials are involved in protein biosynthesis. Complete chemical synthesis of an active ribonuclease completely independent of any such influences lends support to this conclusion (21,22).

Perhaps this concept can ultimately predict the secondary and tertiary structures of protein molecules by estimating the type and magnitude of all forces and constraints acting upon a polypeptide chain of a given amino acid sequence in solution. Putting all these parameters and the linear amino acid sequence into a properly programmed computer, the computer can generate three-dimensional structures and can search for the structure of lowest energy. In some simple peptides, at least, the structures generated by the computer have striking similarities to those found by analysis of the molecule by conventional x-ray techniques (23). Complete success of this procedure would tend to reduce structural protein chemistry to elucidation of primary structure, which is also being automated (24), and to computer analysis of the resulting higher orders of structure. There seems to be no theoretical reason why the analysis could not be extended to computation of the various properties of the protein.

We began with consideration of protein structure and properties by emphasizing the diversity of protein structure and function, and have ended with a look into the future at a unifying concept which may allow, in favorable cases, prediction of protein structure and at least some simple properties. Analyses of structural factors in enzyme catalysis may not be susceptible to this treatment, but recent theories on the nature of enzyme catalysis give hope that even these subtelties may yield to analysis (25).

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