Proteins and Proteases

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Introduction

Proteins are among the most versatile and active macromolecules in our living system. They are the work horses for critical functions in most biological processes, and are key in affording mechanical support, generating motion, controlling the processes in metabolism, providing defenses (as in our immune system), managing genetic information, and acting as hormones. To this end, proteins interact with one another and with each of the other three biomolecular classes: nucleic acids, lipids, and carbohydrates. Most importantly, proteins that are enzymes catalyze an enormous number of highly regulated and integrated chemical reactions in life. Proteins are built when needed and broken down when not needed. This process continues constantly in our bodies, where we maintain a “dynamic steady state” of these biological molecules. Proteins, therefore, are key components of our diet, providing much-needed nutritive value.

Amino Acids

Proteins are polypeptides built of amino acids, which are linked by covalent peptide bonds, to form long unbranched polymers. The general structure of an amino acid is depicted in Figure 68.1 and consists of an amino group (NH₂), a carboxyl group (COOH), a hydrogen atom, and a distinct R group, all bonded to a single carbon atom, called the alpha-carbon.

There are 20 amino acids in living systems, and each has its own unique chemical and structural characteristics defined by the R group. The R groups, also known as the amino acid side chains, can be classified into four main sub-families according to their chemical character: charged amino acids, hydrophilic amino acids, and hydrophobic and aromatic amino acids (Figure 68.2). Hydrophilic R groups are soluble in water, while hydrophobic groups are not soluble in water. Charged amino acid side groups that are acidic are aspartic acid (Asp) and glutamic acid (Glu). These have a negative charge at neutral pH. Charged amino acids with a basic R group (lysine (Lys), arginine (Arg), and histidine (His)) have a positive charge at neutral pH.

Especially interesting amino acids for molecular gastronomy are glycine (Gly), proline (Pro), and cysteine (Cys). Glycine is the smallest amino acid, with a single H for the R group, and is classified in a hydrophobic sub-group; it is a prominent amino acid in collagen and is responsible for the tight packing of the coils of that molecule. Besides contributing to the structure of proteins, glycine is necessary for our bodies to produce heme, which is necessary for the function of our iron-carrying molecules, hemoglobin, and myoglobin.

Proline exhibits a structure with a pyrrolidine ring that incorporates the amino nitrogen. When incorporated into a polypeptide chain, the nitrogen of the ring lacks a hydrogen atom and therefore cannot participate in hydrogen bonding. The ring also reduces flexibility, which is the reason why the structural form of collagen is a stretched-out left-handed helix.

Cysteine contains an ionizable sulfhydryl group and is the most highly reactive amino acid side group. When two cysteine residues on a protein are located near each other in an oxidizing environment, they form a disulfide bond or disulfide bridge, also called a cystine. The bond can be broken by a reducing agent and extremely high heat (Figure 68.3). This amino acid contributes to the coagulation phenomena in eggs, discussed in several other chapters of this book, and many other food systems, including whey proteins.

Amino acids in their own right are also important, as they serve as precursors for a number of metabolites used in many life processes, including hormones such as epinephrine. The food industry has utilized a methyl ester dipeptide of aspartic acid and phenylalanine to synthesize an artificial, non-carbohydrate sweetener named aspartame.

FIGURE 68.1 General structure of an amino acid. The R group refers to the side groups of amino acids.
**Protein Structure**

*Primary structure:* The precise sequence of the amino acid residues in the protein chain is responsible for the final three-dimensional structure of that protein, which, in turn, dictates the function of the protein. DNA contains the informational code for the sequence of the amino acid residues in a protein. This information is transcribed into mRNA, and then the translation of this code occurs during protein synthesis. Most natural proteins are 50–2000 amino acid residues in length. Proteins are very diverse in nature, and the different combinations of these 20 amino acids allow trillions of different entities to be formed. However, the actual number of biologically relevant proteins is much smaller than the theoretically predicted number, because not all combinations of amino acids have useful functions.

Peptide bonds are formed by a condensation reaction between the backbone carboxylic acid group and amino group of two amino acids, leading to the release of a water molecule (Figure 68.4). By convention, the amino acids in a protein are specified from the amino end, which is designated amino acid 1, to the carboxyl end. The backbone sequence of atoms is \(-\text{N-}C\,\text{r-}C\,\text{r-}C\,\text{r-}\). This peptide bond is extremely stable, and degradation of this bond, which on average has a half-life of 10 years in water, can only be accomplished by biological catalysts called proteases. Proteases are inherent in our digestive system, where they act on dietary proteins, reducing them to amino acids to be recycled.

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**FIGURE 68.2** Structures of the 20 different amino acids with their R groups shown in red. The R groups are named by their three letter codes.

**FIGURE 68.3** Illustration of the cystine bond between two cysteine (Cys) R groups.

![Image of amino acids and peptide bonds]
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in subsequent protein synthesis. The significance of proteases in gastronomy is discussed later in this article.

It is imperative that we take in dietary protein, as our bodies can only synthesize 11 of the 20 amino acids necessary for life. Those not included are called essential amino acids: histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val).

**Secondary structure:** The polypeptide chains can fold into regular formations with stabilizing hydrogen bonds between backbone peptide NH and CO groups of amino acids, often near one another on the chain. These secondary elements are largely independent of primary amino acid sequence. The first type of structural unit is the alpha helix, which is a coiled structure stabilized by intra-strand hydrogen bonds between NH and CO groups on the backbone four residues apart (Figure 68.5).

Most amino acids are compatible with this secondary structure except for proline (*vide supra*), which usually ends the helical structure. The prominent feature of this helix is that the R groups are on the outside of the helix. This allows the helix faces to adopt the chemical character of the specific R groups on that side. Helices have been known to be amphipathic, in that

![FIGURE 68.4 Depiction of formation of peptide bonds with the release of a water molecule. As illustrated, the first amino acid in a protein chain is located on the amino terminus and the subsequent amino acids are added to the carboxyl terminus in the growing protein chain.](image)

![FIGURE 68.5 Depiction of the alpha helix. The ribbon form of the helix is shown in A. The ball and stick form is shown with the ribbon in B. As illustrated, the R groups of the protein are on the outside of the coiled structure. The backbone atoms including NH and CO involved in hydrogen bonding are on the inside of the helix. The atomic form of the alpha helix in ball and stick form is shown in C. The three-dimensional alpha helix figure was generated using amino acids 44-59 of the glucose and galactose binding protein using the protein data base file 2GBP.pdb. This figure was generated using amino acids 444-59 Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2016, San Diego.)
one side of the helix has charged R groups – hydrophilic – and the other side of the helix has hydrophobic groups. This allows the helix to face inwards or outwards depending on the cellular environment.

Beta sheets are stabilized by inter-strand hydrogen bonding between polypeptide strands and are markedly different from the alpha helix. The beta sheet consists of two or more strands of polypeptides that are more fully extended rather than tightly coiled as in the alpha helix. The beta sheet is formed by linking two or more strands lying next to one another through hydrogen bonds. Adjacent strands can be running in the same direction (parallel) or the opposite direction (antiparallel) (Figure 68.6). Antiparallel sheets are more stable, as the hydrogen bonds are in a straight line; this is illustrated in Figure 68.6 (top).

The third type of secondary structure in proteins is beta turns and loops. Since most proteins are globular in shape, containing both beta sheets and alpha helices, there must be polypeptide strands that connect the structural units. Beta turns are common for connecting two beta strands in an antiparallel beta sheet. The turns contain four amino acids, two of which are hydrogen bonded. In beta loops, there are six to ten amino acids, and they are located mainly on the surfaces of proteins. The loops are more flexible because they are held together by multiple weak interactions within the structures. In Figure 68.7, loop structures are shown as thin licorice structures in the myoglobin molecule.

### Tertiary Structure

Another level of protein structure, namely tertiary structure, refers to the spatial arrangement of all atoms in the protein – the three-dimensional view of the protein, for example, myoglobin, shown in Figure 68.7. On this level, we observe the arrangement of the secondary structures, the interaction of the R groups, and the role of disulfide bonds. The overall structure of any protein is the most stable arrangement of elements that generally minimizes solvent contact and overall free energy. The tertiary structure is held together by a combination of four non-covalent weak interactions, which play a role in stabilization of the overall protein structure, and in some cases disulfide bonds and/or metal ion interactions. The following are descriptions of these interactions.

1. Weak interactions between oppositely charged atoms or groups are called ionic interactions and are a type of electrostatic interaction. An example of this would be the attraction of a positively charged amino group \( \text{NH}_3^+ \) and a negatively charged carboxyl group \( \text{COO}^- \). The strength of the interaction depends on the environment of the ions and the distance between them. These interactions are often referred to as salt bridges.

2. Hydrogen bonds are not just reserved for water molecules. These bonds can form between a hydrogen atom and an electron donor group such as oxygen or nitrogen and sometimes sulfur. The strength of the bond is dependent on the distance and the angle between the atoms. Although these hydrogen bonds are the main stabilizing factors in secondary structure,
they play an equally important role in tertiary and quartenary structures.

(3) Hydrophobic interactions occur when non-polar molecules tend to cluster together in water, not because they are attracted to one another but because, when they associate, they release the water clustered around them. The water released is then less ordered and the entropy of the system is higher. This is an entropy-driven association, and it forms spontaneously. In the overall scheme of protein folding, this entropy effect is the driving force for hydrophobic amino acids to be buried in the center of a globular protein molecule.

(4) van der Waals interactions, the weakest of the four discussed, depend on asymmetry in electrical charge around atoms in the R group. Many molecules are neither charged nor polar but can interact with each other electrostatically due to asymmetry of the electron cloud distribution about an atom or group causing a temporary dipole moment. At any instant, the cloud can have transient regions of positive and negative charges. These charged regions can induce a complementary asymmetry on a neighboring atom, which in turn does the same to another neighboring atom. The overall results within the biomolecule can be additive and provide a stabilizing force in the tertiary structure.

(5) A covalent interaction that plays a major role in tertiary structure is disulfide bond formation between two cysteine residues in the protein. This interaction is also very important in the quaternary structure, to be discussed later.

(6) Coordinated metal ions such as iron and zinc are another way to stabilize tertiary structure through cysteine and histidine residues. A common protein using this configuration is the zinc finger protein which binds DNA, while coordinating metals are also found in lactoferrin.

**Quaternary Structure**

Proteins that consist of more than one polypeptide chain display quaternary structure, with each individual protein chain being called a subunit. Quaternary structure can be as simple as two identical subunits or as complex as dozens of different subunits. Figure 68.8 shows the subunits in the molecule hemoglobin. In most cases, and as in hemoglobin, the subunits are held together by the four weak forces described earlier, and in many other cases, such as in insulin, disulfide bonds at the interface of the subunits. The shared surfaces are chemically and physically complementary to each other, providing a stabilizing environment for their interaction. The multi-subunit array in quaternary structured proteins provides increased functionality to the proteins not found in the individual units. This may be in terms of structural properties not present in the individual units, or it may be a means for regulation of protein function. This is evident in the four-subunit hemoglobin protein that uses oxygen binding to one subunit to increase binding action to the other three. This cooperative binding is observed only with quaternary structure. Multiprotein complexes also increase the efficiency of biochemical processes by bringing linked chemical reactions into close proximity.

Not all proteins have quaternary structure. For example, myoglobin has tertiary structure but not quaternary structure, because it consists of only one subunit. On the other hand, collagen and keratin have quaternary structure, as they are both multi-subunit proteins. The structure of collagen is shown in Figure 68.9.
Since the three-dimensional structure of proteins is held together by weak forces, they are quite susceptible to denaturation or unfolding. The weak forces can be disrupted by a number of methods that interfere with the above-mentioned non-covalent interactions (without peptide bond cleavage) holding the molecule in its native form. The unfolded form is usually an outstretched chain with all the internal amino acid residues exposed to the solvent. When the protein loses its native form, it becomes denatured or unfolded, and if it is impossible for it to regain the native structure, then it is considered to be irreversibly denatured. Protein unfolding usually results in a loss of solubility due to the exposure of the hydrophobic core regions in the center of the protein. Exposed hydrophobic regions of one protein are likely to associate with hydrophobic regions of other unfolded proteins, causing aggregation and subsequent precipitation or coagulation.

Denaturation can be induced by heating, changes in salt concentrations, pH changes, mechanical treatments such as shearing (as in whipping, kneading, or rolling), exposure to organic solvents, or exclusion or chelation of metals needed for structural stability. Heating causes denaturation mainly by providing heat energy, which breaks hydrogen bonds and van der Waals interactions. pH changes will affect the charged R groups, either adding or removing H+ ions and disrupting the necessary ionic interactions to keep the protein in the native form. Shearing forces disrupt alpha helices and cause loss of the tertiary structure. Organic solvents or hydrophobic substances can change the solvation pattern of the protein. The outside of the protein is usually populated by hydrophilic groups, and if the solvent is organic in nature, those hydrophilic groups will want to be buried inside the protein, while the hydrophobic groups will be more stable on the outside of the protein immersed in the hydrophobic solvent. Basically, the organic solvent will turn the protein “inside out”.

A simple illustration of the denaturation process is shown in Figure 68.10.

Each protein has a different chemical make-up, and thus different weak forces are unique to each individual protein. An example of a denaturation curve is shown in Figure 68.11, in which the relative amount of unfolded protein increases as the denaturing condition increases. The conditions could be an increase or decrease in pH or salt or an increase in temperature. The midpoint of the curve is called the \( T_m \), which is defined as the point where half of the proteins are unfolded.

A practical example of this is the denaturation of an egg by heat. The egg white will denature at a lower temperature than the yolk, because the white part of the egg has different proteins than the yolk. The egg white proteins have lower \( T_m \)s than the yolk proteins. In another example, whey proteins from milk denature, unfold, and aggregate when heated, and, depending on the specific heating applied, plus environmental conditions such as protein concentration, pH, and ionic environment, can form aggregates or gels.

The denaturation of an enzyme results in loss of activity because it is not in its native form. This is very useful for destroying the
action of enzyme proteins with catalytic activity. The actions of these enzymes can be halted by denaturation by heat, pH, or other means depending on the cooking application. For example, the protease activity of bromelain from pineapple will break down gelatin, which is why pineapple is heated first before it is used in Jello (jelly) preparation. Also, enzymes called phenolases cause chemical color changes in a food, such as the browning of an apple, but pH changes, for example by adding lemon juice, will slow the enzymes in fruits so they do not brown.

Another application of this phenomenon is in reducing food spoilage by bacteria. Heating, pH change, or irradiation will denature bacterial proteins, rendering them inactive and killing the bacterial cells.

### Proteases and Proteins

As discussed, the structure of many food products and dishes is greatly influenced by the properties of proteins, and changes on cooking or preparing food often result from changes such as heat-induced denaturation or changes in structure due to changing acidity or salt concentrations. Examples such as the gel structure of cheese and yoghurt, transformations on cooking or marinating meat, and the range of ways in which the texture of a raw egg can change on cooking in different ways all represent different aspects of the behavior of food proteins.

For this reason, it is not surprising that reactions which result in the breakdown of proteins have significant impacts on the culinary properties of food. The key biological process through which such breakdown can occur is proteolysis, whereby enzymes from a range of sources, either naturally present in the food (indigenous) or added to the food (exogenous), result in desirable (or, occasionally, undesirable) changes in the food.

There are four main types of protease, depending on their mode of catalytic action: serine, aspartic, metallo, and cysteine. These differ in their requirements for optimal activity and so may be more or less active in different food systems depending on factors such as temperature, pH, ionic environment, and presence of inhibitory substances. The activity of other enzymes is routinely controlled by manipulation of such factors (e.g., the inhibition of enzymatic browning of fruit by oxidases by reducing pH through the use of acids such as lemon juice), while the inactivation of most proteases depends on heat treatment.

For example, fresh pineapple, or pineapple waste, can be extracted to yield a potent protease mixture called bromelain, but in canned pineapple, which has been subjected to significant heat treatment (retorting), the enzyme activity has been inactivated. This becomes relevant when either fresh or tinned pineapple is added to another product, such as gelatin-based desserts, which the former can rapidly soften due to the residual activity present. As the fresh fruit ripens, increasing enzyme availability can give pineapple a very sharp taste and even give rise to a tingling sensation as the enzyme actually attacks the eater’s mouth during consumption.

Proteases are also used in the preparation of many foods and beverages, for example to reduce haze in beer and wine or to clarify blackcurrant juice (Mamo and Assefa, 2018), and in baking to control dough structure and firmness. In baking, proteases can be targeted to hydrolyze gluten and increase dough extensibility, improve gas-holding capacity, and enhance swelling of starch granules (Hamada et al., 2013; Bender and Schönlecher, 2020).

### Enzymes and Cheese

To take a beneficial example of very limited proteolysis, cheese has been made for millennia using an extract from calf stomach (rennet), which contains an enzyme called chymosin, which very specifically cleaves one bond within aggregated protein structures in milk called casein micelles, destabilizing them in such a way that they form three-dimensional networks that transform the milk from a liquid to a solid. This solid matrix is then dehydrated by cutting, stirring, and cooking to expel whey, and pressed into a curd, which is stored for periods from weeks to years to allow other enzymes, including proteases, to slowly break down the proteins and other milk constituents to give textures and flavors characteristic of the variety in question (Uniacke-Lowe and Fox, 2017).

### Fruit Enzymes

As mentioned earlier, many fruit products are potent sources of proteases. Bromelain extracted from pineapple (Ananas comosus L.) is a crude mixture containing several proteases as well as other enzymes, including phosphatases, glucosidases, and cellulases. Bromelain can be extracted from various parts of the pineapple, including stem, peel, core, and crown, and methods for extracting and purifying bromelain were reviewed by Vasiljevic (2020). As well as bromelain, another potent cysteine protease is papain, which can be extracted from the latex of the papaya tree, while fig tree latex contains a protease called ficin. Papain is quite heat-stable and can act at temperatures of 60–70 °C, which means that cooking under mild conditions will be insufficient to inactivate the enzyme, and stored cooked meat may lose texture due to continuing enzyme action.

### Marinades and Meat

Proteases have substantial effects on the properties of meat. Many of the post mortem changes in meat texture and tenderness, for example, arise due to the action of proteases within the tissue breaking down proteins such as collagen and myofibrillar proteins; different meat types and characteristics depend on the exact handling of the carcasses and treatments at this point.

There have been many studies about increasing the tenderness or palatability of meat using fruit-derived enzymes such as papain and bromelain (Bekhit et al., 2014; Botinstein et al., 2018). Enzymes may be introduced to meat through a number of means. One common approach is to include them in a marinade (either as a powder or as the fruit or other source), while they may also be sprinkled onto meat that has been pierced, e.g., with a fork, to allow the enzyme access to the interior of the meat.

One of the most notable references to the culinary use of proteases was in the book *But the crackling is superb* (Kurti and Kurti, 1988), in which the use of a hypodermic syringe to inject fresh pineapple juice (in which, critically, the enzyme bromelain remains active) into cuts of meat (e.g., pork chops) with a view to tenderizing them is described; the injected meat is left to stand for
two hours, to allow the enzyme to act, before being cooked. For a larger piece of meat, such as a gammon joint, longer standing periods were recommended to allow percolation and action.

Many herbs and culinary ingredients also contain proteases, and their application in certain dishes may actually be favored for this reason; for example, ginger contains proteases, which can help to tenderize meat. In addition, culinary preparations containing papain and bromelain are available (e.g., in powder form), which can be used as meat tenderizers.

**Conclusions**

Biochemical processes are central to food applications, and their causes and effects are ubiquitous in food and cooking systems. Many transformations during food preparation and cooking are dependent on the properties of proteins, and phenomena such as denaturation, while the controlled breakdown of proteins using proteases is likewise a key aspect of many culinary processes. A good understanding of proteins with respect to their structure and function is critical for the future of foods, agriculture, processing, and design.

**REFERENCES**


