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Molecular Biology and Biotechnology

A Comprehensive Desk Reference

3 PERSPECTIVES

Numerous additional applications of fluorescence in the biomedical sciences are being developed because of the high sensitivity of fluorescence detection and the desire to eliminate the use of ionizing radiation (X-rays and radioactivity) in the laboratory and in clinical practice. Additionally, there is growing recognition of the value of using longer wavelength probes (red or near-infrared), because tissues are nonabsorbing at these wavelengths, and there is less autofluorescence to interfere with the measurements. These facts suggest the possibility of noninvasive diagnostics based on red/near-infrared probes, which can be excited with simple laser diode sources of the type used in everyday CD players. We have all observed the possibility of noninvasive diagnostics, most simply perhaps when seeing the red light of a flashlight transmitted through our fingers. However, only recently has technology enabled the use of this observation for research and clinical purposes.

See also GENE MAPPING BY FLUORESCENCE IN SITU HYBRIDIZATION; GENE ORDER BY FISH AND FACS; WHOLE CHROMOSOME COMPLEMENTARY PROBE FLUORESCENCE STAINING.

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Folding of Proteins: see Protein Folding; Protein Modeling.

FOOD PROTEINS AND INTERACTIONS

Nicholas Parris and Charles Onwulata

Key Words

- Emulsion** Thermodynamically unstable dispersion of micellar particles or globules in a liquid medium.
- Foaming** The trapping of air at the surface of a liquid resulting from the rapid diffusion of protein to the air-water interface and the subsequent reduction of the interfacial tension.
- Food Protein Interaction** Interaction of protein molecules and other compounds within their domain which affects their behavior in food products.
- Gelation** Formation of an ordered matrix of functional networks by balancing intramolecular and intermolecular forces between protein aggregates.

Phospholipids Class of membrane lipids that possesses polar groups attached to the glycerol residue by a phosphodiester bridge.

Protein Micelles Protein aggregates that are formed by the reversible interaction of protein monomers.

Sedimentation Coefficient(s) Proportionality constant that defines the sedimentation of a protein as a function of its size and shape.

Syneresis Spontaneous exudation of liquid and shrinking of a gel.

Proteins are the most abundant macromolecules found in living cells and account for approximate half of the cell's dry weight. They are required in the food of humans, fish, and most higher animals. Historically, food proteins have been selected for their nutritional value and can be obtained from a wide variety of naturally occurring sources. Proteins undergo a wide range of structural and conformational changes through a variety of complex interactions during processing and storage. Such changes can affect the principal purpose of dietary protein, which is to supply nitrogen and amino acids for the synthesis of proteins in the body. It is through an understanding of these interactions and their effects on functionality that food proteins have played a major role in the food supply.

1 PROTEIN STRUCTURE AND CONFORMATION

Proteins are natural compounds composed of amino acids organized at four different levels of structure: primary, secondary, tertiary, and quaternary. The primary structure consists of amino acids that are sequenced in a linear polypeptide chain and constitute the basic building blocks. The secondary structure describes the conformational arrangement of the proteins in space, which is due primarily to hydrogen bonding of the polypeptide chain resulting in stable conformations. The secondary structure of the polypeptide of the protein is composed of α -helices, β -sheets, and random coils. The tertiary structure refers to the overall architecture of the polypeptide chain whose folding brings into proximity parts of the molecule otherwise widely separated along the backbone. The schematic tertiary structure of carbonic anhydrase is shown in Figure 1 with helices and β -sheets. Noncovalent association of protein subunits describes their quaternary structures, which are stabilized by hydrogen bonds and van de Waals forces. Systematic denaturation of the organized structures and forces changes the functionality of food proteins.

1.1 FOOD PROTEINS

Food proteins are derived from a number of sources: plants, meat and fish, milk, eggs, and microbial proteins from unicellular or multicellular organisms (e.g., bacteria, yeast, molds, algae). Plant proteins are broadly classified according to solubility, shape, prosthetic group, and regulatory properties, as well as biological activity. They were first classified on the basis of solubility as albumins, globulins, glutenins, and prolamines. Albumins are the most water-soluble globular proteins. Soybean globular proteins contain globulins such as conglycinin (7S) and glycinin (11S), that are soluble in dilute salt solutions at neutral pH. Glutenins include wheat

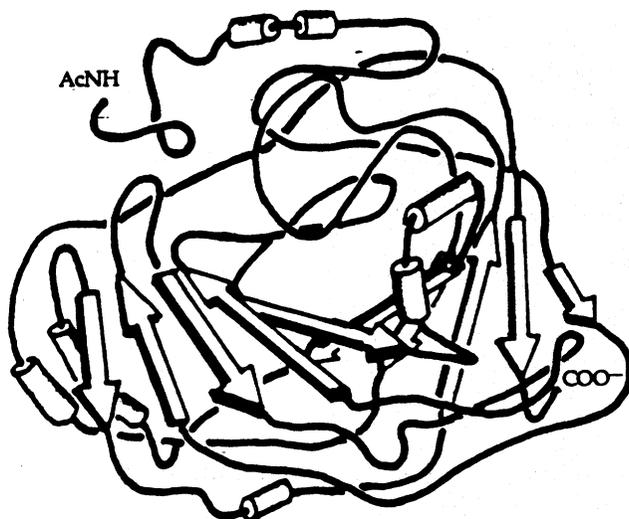


Figure 1. Schematic tertiary structure of carbonic anhydrase. Helices are drawn as cylinders; β -sheet strands as arrows with N at the tail and C at the point of each arrow. (From O. R. Fenema, *Food Chemistry*, 2nd ed., Dekker, New York, 1985; reproduced by permission of the author.)

glutenins and are soluble in dilute acid or alkali. Prolamines are corn or wheat storage proteins, are soluble in 70% ethanol.

Muscle or contractile proteins are derived directly from animal tissue and are the most conspicuous food protein in the human diet. Muscle proteins are generally classified into three groups based on their solubility in water: sarcoplasmic proteins, which are soluble in water or dilute salt; myofibrillar proteins, which are soluble in salt solutions $>0.6\text{M}$; and stromal proteins, which are the least soluble class of muscle proteins. Fibrous proteins, actin and myosin, have polypeptide chains arranged in long strands, are generally insoluble, and serve a structural or protective role. Collagen and elastin, the major proteins of connective tissue, occur in several polymorphic forms consisting of three polypeptide chains.

Egg proteins are primarily globular proteins found in the albumen; they contain ovalbumin, conalbumin, ovotransferrin, ovomucoid, and lysozymes. Milk proteins consist of a colloidal dispersion of casein micelles and soluble whey proteins; their stability is of tremendous practical importance in the dairy field. Casein micelles are extremely sensitive to changes in ionic environment and readily aggregate with increased concentration of calcium and magnesium ion.

Other proteins classified according to their prosthetic groups (tightly associated non-amino acid portion) are lipoproteins, glycoproteins, caseins, hemoglobin, and myoglobins. Proteins derived from unicellular organisms are grown on food processing by-products from which the protein is harvested and subsequently purified.

1.2 ENZYMES

Enzymes are globular proteins that function as specific biological catalysts for chemical reactions in living systems. Given the restricted conditions of temperature and pH in which they operate, enzymes are much more efficient and specific than other catalysts. Enzymes are frequently used in the food industry to modify the functional behavior of food proteins. For instance, proteases from

plant sources, such as papain, are used as commercial meat tenderizers. It is important, however, that the nutritive values of the product not be significantly affected by the hydrolysis of peptide bonds and the subsequent loss of essential amino acids.

1.3 ENERGY AND NUTRITIVE VALUE

The nutritional quality of a protein is determined by its amino acid composition. Nine of the 20 identified amino acids are classified as essential because they cannot be synthesized in the human body. The food value of protein is evaluated by quantifying the ratio of essential to nonessential amino acids and calculating protein efficiency ratio (PER), which is determined by the weight gain in the animal divided by the protein intake. Nutrient availability and food protein quality depend on the nature of the cross-linking and denaturation. Protein requirements for maintaining good health and growth are listed as the recommended dietary allowance (RDA) set by the U.S. National Academy of Sciences.

2 PROTEIN INTERACTIONS

Molecular forces governing protein interactions determine the relationship of the structure of individual proteins to their functional properties as well as the association of a protein with other compounds in the cell. Forces involved include covalent bonds, ionic interaction, hydrogen bonds, hydrophobic interactions, hydration, and steric repulsion. Covalent bonds include the peptide linkage of the primary structure of the protein as well as disulfide bonds, which are formed between cysteine residues and depend on the conformation of the peptide chain. Bonds or interactions that determine secondary and tertiary structure of proteins are shown in Figure 2. Protein denaturation results in changes in the secondary and tertiary structures. Breaking the peptide bonds is achieved by means of proteolytic enzymes or hydrolysis with strong acid or base. Of the covalent forces, disulfide bonding is the most important in protein interactions. Noncovalent molecular forces, which are one to three orders of magnitude smaller than covalent bonding energy, include hydrogen bonds, hydrophobic interaction, and repulsion forces. Association and disassociation of the molecular forces maintain the integrity of food proteins.

2.1 PROTEIN-PROTEIN

Protein-protein interactions often occur as a result of food processing designed to improve the functional properties of proteins for new product application. These interactions occur in two-stage processes consisting of unfolding of the native protein and exposing active sites, followed by association of the polypeptide chain by covalent and noncovalent forces. Gelation is an association of aggregated proteins existing in a three-dimensional network with trapped water molecules. Protein cross-links are formed through sulfhydryl groups or through hydrophobic interactions. To maintain a stable gel, there must be a balance between attractive forces required to form the network and the repulsive forces needed to prevent its collapse or syneresis.

2.2 CARBOHYDRATES

Protein and carbohydrates form irreversible complexes in nonoxidation reactions. Food protein heated in the presence of a reducing sugar results in the reaction of the carbonyl groups of the carbohy-

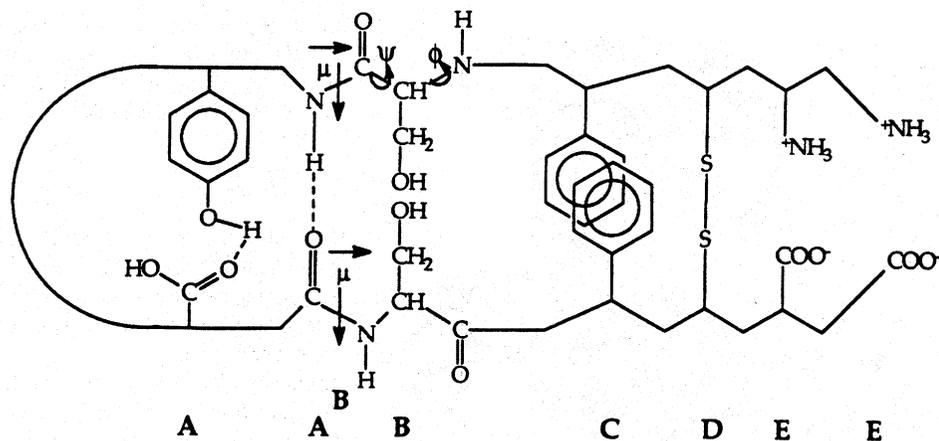


Figure 2. Bonds or interactions that determine secondary and tertiary structure of proteins: (A) hydrogen bond, (B) dipolar interaction, (C) hydrophobic interaction, (D) disulfide linkage, and (E) ionic interaction. (From O. R. Fenema, *Food Chemistry*, 2nd ed., Dekker, New York, 1985, reproduced by permission of the author.)

drate with the free amino group of protein followed by a cascade of reactions leading to brown polymers. These Maillard reactions occur in preference to other types of protein damage in the drying of milk at moderate temperatures. However, more severe heating, required in the preparation of toasted breakfast cereals, bread, and biscuits, results in late Maillard-type and protein-protein damage.

2.3 LIPIDS

Protein-lipid interactions in nature result in lipoproteins in food systems such as milk and eggs. Proteins stabilize emulsions by assuming the form of lowest free energy at the interface between the two immiscible substances. Interactions between milk proteins and lipids occur through their polar groups; there is no significant modification of the conformation of the proteins or organization of the lipid monolayer at the interface between the water and the oil droplets. Stronger interactions occur in flour-water mixtures, since lipids can bind with gluten proteins to form highly stable lipoglutenin complexes.

2.4 HYDRATION

The native structure and normal functionality of protein is facilitated by the presence of water. Water in the vicinity of protein determines its intrinsic properties in such functions as solubility, swelling, dispersibility, and wettability. Water absorption is considered the most important step to imparting desired functional properties to proteins. Proteins interact with water through their peptide bonds or their amino groups, and their solubility depends on conformational forces (e.g., hydrogen bond, dipole-dipole and ionic interactions, pH, and temperature). The ability of proteins to absorb and retain water plays a major role in the textural stability of food systems that employ swelling of protein matrices. Texturized proteins employing the protein-water balance are designed as analogues that resemble meat and fish patties.

2.5 SOLUBLE IONS

Zinc, magnesium, and sulfur are some of the ionic components associated with proteins that increase the proteins' activity and

stability. The reactive nature of proteins allows for the manipulation of the ions to accomplish food preservation by controlled denaturation. Proteins are stable within a defined range of pH and have either positive, negative, or neutral charges, depending on the reactive group of amino acids that determine the stability, conformation, and function of the protein in the medium. At extreme pH, proteins aggregate. Small amounts of polyvalent cations, such as calcium and magnesium, are very effective in destabilizing the casein micelle, particularly in conjunction with heat and reduced pH. Polyvalent cations also act as cofactors for enzymes to be catalytically active. Salts used in food processing can denature proteins by charge neutralization or by changing the isoelectric point of the protein.

3 FUNCTIONAL PROTEIN INTERACTIONS

Physicochemical properties that enable proteins to affect the characteristics of foods during processing, storage, preparation, and consumption define the functional properties of proteins. Protein functions that affect food utilization are water absorption (viscosity and gelation), surface effects (gelation and foaming), and chemical reactions (textural properties). Functionality of a food product is determined experimentally, since the study of protein structure provides information on physicochemical properties but is not an accurate method of predicting functionality.

3.1 GELATION

Gelation is the formation of an extended network of denatured protein molecules in which the intramolecular and intermolecular forces are in an ordered matrix. Gel matrices hold water and other ingredients, which improves the swelling properties of the gel. Strong gels hold water and other ingredients, producing such foods as yogurt, gelatins, and doughs. Gels degrade over time, leading to syneresis or shrinkage of the gel matrix. Gel formation results from controlled protein denaturation and unfolding, formation, and realignment of protein matrices and balance in protein interactions. The cross-linking of proteins in the matrix via disulfide bonds

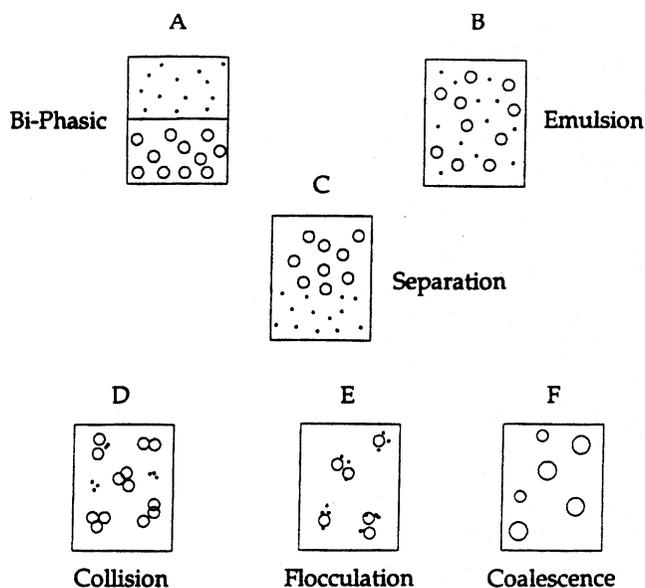


Figure 3. Process of emulsion formation, stabilization, and breakdown: (A) biphasic medium of liquid and semisolid emulsion, (B) thermodynamically unstable emulsion, (C) gradual migration of emulsion based on charge separation, (D) particle collision with increasing electrostatic charges, (E) flocculation (i.e., clear aggregation of collided particles), and (F) coalescence and syneresis of emulsion.

results in heat-irreversible gels stabilized by hydrogen bonds. Higher molecular weight proteins tend to form stronger gels.

3.2 EMULSIFICATION

A stable protein emulsion is produced when proteins in a biphasic medium unfold slightly at the interface and align their nonpolar regions toward an oil phase with their hydrophilic regions toward the aqueous phase, at a minimum of free energy. Force imbalances cause emulsions to separate over time. The breakdown is a result of large positive free energy at the emulsion interface, leading to flocculation, followed by coalescence of the native protein as depicted in Figure 3. Globular proteins with a highly ordered and stable tertiary structure (e.g., β -lactoglobulin, bovine serum albumin, lysozyme) are more likely to unfold and are considered good emulsifiers. Emulsification activity continues to increase with increasing protein denaturation, provided solubility is not compromised. The balance of the hydrophilic and hydrophobic forces in the protein maintains emulsion stability.

3.3 FOAMING

Foams are emulsions in which gas is dispersed in a continuous aqueous semisolid phase of protein. Foods such as ice cream and whipped toppings are stabilized protein foams. Foam expansion is determined by the volume of foam formed after a known quantity of gas has been incorporated into a food system without breakdown of matrix. Factors that affect foam stability include electric potential, protein concentration, energy input, and presence of salts, sugars, lipids, and metal ions.

3.4 FOOD PROCESSING

Food proteins are thermally processed either to enhance functionality, to improve textural properties, or to minimize natural deterioration. Processing may lead to slight loss in nutritional quality; however, most processes improve quality by destroying antinutritional factors through inactivation of enzymes such as peroxidase. The interactions of proteins are enhanced; leading to formation of new complexes. Factors such as temperature and the presence of salts and oxidizing-reducing agents may be used to produce desirable, high quality foods. The removal or addition of thermal energy can result in denaturation of proteins. Thermal denaturation of food proteins generally occurs between 45 and 85°C, accompanied by exposure of hydrophobic groups to water, resulting in protein aggregation. Low temperature denaturation is mediated by a reduction of hydrophobic interactions in conjunction with enhanced hydrogen bonding, leading to aggregation and precipitation of proteins.

3.5 THERMALLY INDUCED MUTAGENS

Mutagens are formed in muscle foods as a result of industrial processing or home cooking (e.g., frying, broiling, boiling, baking). They are generated through decomposition and rearrangement or recombination of endogenous precursors in muscle such as amino acids and sugars, which have individually undergone recombination as in the Maillard browning reaction. Much of the mutagenicity found in meat cooked at moderate temperatures can be attributed to the formation of heterocyclic aromatic amines. *N*-Nitrosamines are readily produced at higher temperatures—for example, in the frying of bacon. These mutagens are consumed daily in beef, pork, poultry, and fish at the low parts-per-billion level. Extensive research efforts to develop methods to reduce or eliminate such compounds from the food supply have been moderately successful.

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