

LIFETIME MEASUREMENTS IN FLOW CYTOMETRY AND IMAGING

An advantage of lifetime measurements is that the lifetime is not dependent on the total intensity. Consequently, lifetime measurements can be useful even when the intensities are not informative or cannot be reliably measured. Such situations arise with flow cytometry and/or fluorescence-activated cell sorting. In these measurements the cells flow one by one through a laser beam, which excites the fluorescence. The total signal is somewhat variable as a result of the differing volumes of the cells and the unequal amounts of probe (Figure 15). The use of lifetime measurements, on a cell-by-cell basis, can circumvent these difficulties of cell volume and/or probe concentrations, as well as the limitations of a small number of wavelength-ratiometric probes.

Lifetime-based sensing also provides significant advantages in fluorescence microscopy. Suppose the cell contains two regions with different Ca^{2+} concentrations and that the probe lifetime depends on Ca^{2+} (Figure 16). An intensity image of the cell will reveal the localization of the probe (lower left). In the emerging technology of lifetime imaging, the image contrast will be derived from the lifetime in each region of the cell (Figure 16, lower right), rather than from the local probe concentration. Such lifetime imaging will be analogous to magnetic resonance imaging (MRI), where the contrast is obtained from the proton relaxation time (analogous to a fluorescence lifetime), not from the signal intensity (analogous to the fluorescence intensity).

While not yet conveniently available, lifetime-based flow cytometry and imaging will allow cell sorting and imaging independent of total intensity. Such measurements, which seemed technologically impossible just a few years ago, are now achievable because of advances in lasers, opto-electronics, and computer technology.

PERSPECTIVES

There are numerous additional applications of fluorescence in the biomedical sciences. These applications 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-IR), because tissues are nonabsorbing at these wavelengths, and there is less autofluorescence to interfere with the measurements. These properties suggest the possibility of noninvasive diagnostics based on red/near-IR probes, which can be excited with simple laser diode sources of the type used in everyday compact disk players. We have all observed the possibility of noninvasive diagnostics when, as children we saw 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; LABELING, BIOPHYSICAL; WHOLE CHROMOSOME COMPLEMENTARY PROBE FLUORESCENCE STAINING.

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Folding of Proteins: see Protein Folding; Protein Modeling.

FOOD PROTEINS, INTERACTIONS OF

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FLUORESCENCE SENSING METHODS

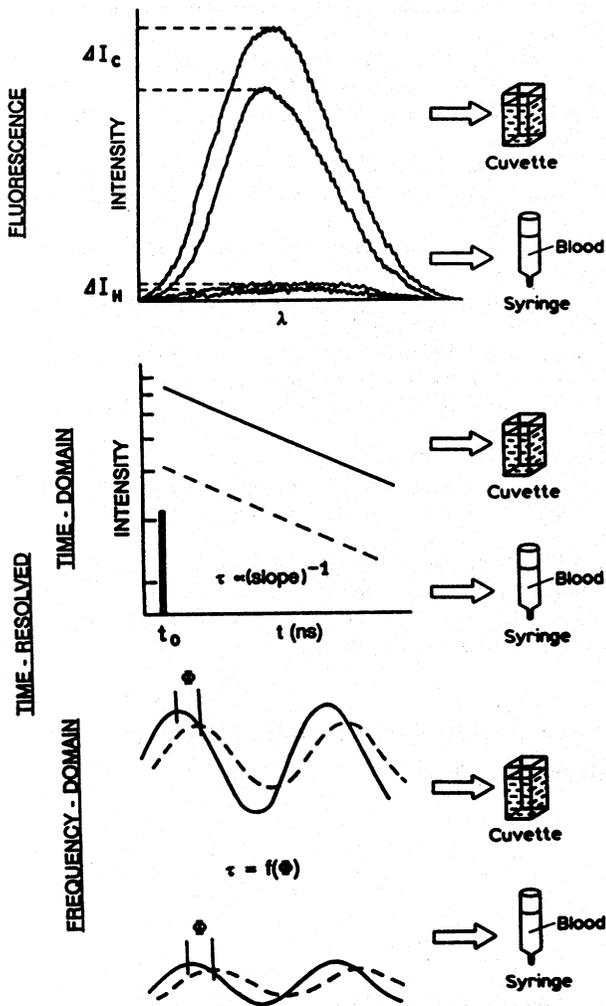


Figure 14. Comparison of intensity and lifetime sensing: λ , wavelength.

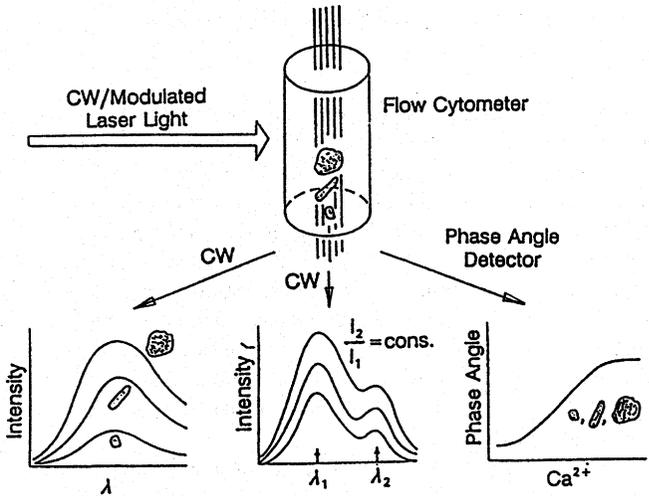


Figure 15. Schematic of a phase flow cytometer.

cur in environments that are not equivalent to optically clear and clean cuvettes. Instead, numerous factors can affect the intensity values: imperfections or misalignment of surfaces and light losses in optical fibers, to name just a few. Additionally, many desired applications, such as homogeneous immunoassays or transdermal sensing measurements, require quantitative measurements in highly turbid or absorbing media (Figure 14). Such factors preclude quantitative measurements of intensities, or even intensity ratios.

Lifetime-based sensing can be mostly insensitive to real-world effects, which are not expected to alter the rate at which the intensity decays (Figure 14, middle). In our opinion, phase modulation/phase modulation sensing provides additional advantages (Figure 14, bottom). The FD instrumentation uses of radio frequency methods to reject noise and filter signals, resulting in reliable data in electrically noisy environments.

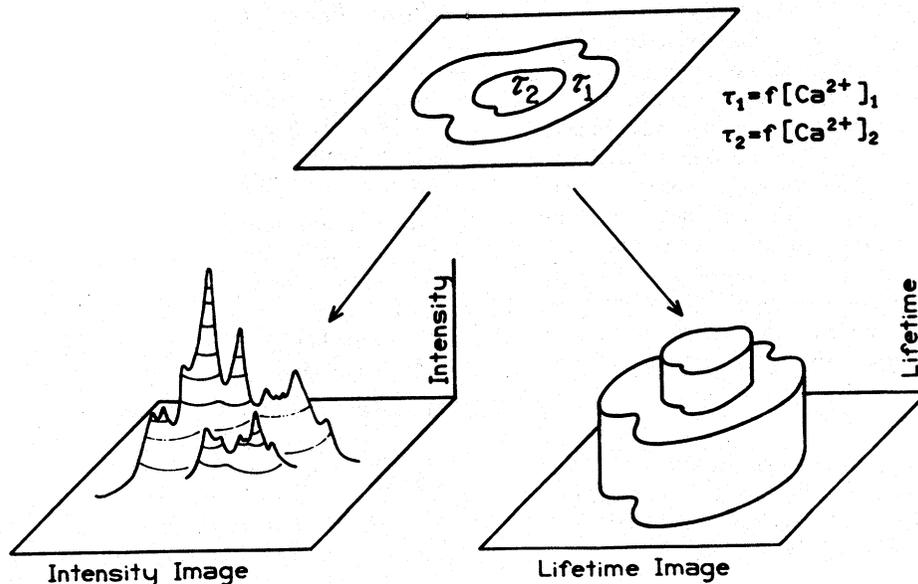


Figure 16. Intuitive description of fluorescence lifetime imaging (FLIM).

Key Words

- Emulsion** A thermodynamically unstable dispersion of micellar particles or globules in a liquid medium.
- Foaming** A coarse dispersion of a gas in a liquid: the bulk of the phase volume is the gas, and the liquid is distributed between the gas bubbles in thin sheets called lamellae, which are formed when the liquid and gas are agitated together in the presence of a stabilizing agent.
- Food Protein Interaction** The interaction of protein molecules and other compounds within their domain, which affects the protein's behavior in food products.
- Gelation** The formation of an ordered, continuous matrix, entrapping another component, in which attractive and repulsive forces are balanced.
- Hydrogen Bond** A highly directional noncovalent bond in which the hydrogen atom is shared with two other atoms.
- Hydrophobic Interactions** The association of nonpolar molecules or groups with each other.
- Phospholipids** Membrane lipids that possess polar groups linked to a diglyceride by a phosphodiester bridge.
- Protein Micelle** Protein aggregates that are formed by the reversible interaction of protein monomers.
- Syneresis** Shrinking of the gel with expulsion of trapped liquid.
- Van der Waals Forces** Nonspecific attractive forces between two atoms.
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Proteins, the most abundant macromolecules found in living cells, constitute approximate half a 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 they can be obtained from many 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.

The macrostructure of a protein is determined by its amino acid sequence. Amino acids are essential in basic nutrition, growth, and maintenance. Nine of the 20 identified amino acids are considered to be essential. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are so classified because they cannot be synthesized by humans and must therefore be supplied in the diet. When essential amino acids are not present in sufficient quantity, they are termed "limiting amino acids" because the utilization of other amino acids is restricted by the inadequacy of the supply of the former.

Processing of food proteins is designed to reduce microbial and enzymatic spoilage, inactivate antinutritional substances, improve the availability of perishable foods, and enhance the sensory quality of the food. Chemical changes that occur during food processing can influence nutritive value, sensory properties, and functional

properties of the food. Heat treatment of proteins under acidic conditions can result in unfolding or denaturation of proteins and inactivation of enzymes and antinutritional substances. More severe heating can result in cross-linking of proteins. Racemization can occur in food proteins heated under alkaline conditions, with the subsequent formation of lysinoalanine due to the reaction of dehydroalanine and lysine. The presence of lysinoalanine can significantly reduce the nutritive value of proteins. Process-induced chemical interaction, which can occur between proteins and carbohydrates, results in nonoxidative, nonenzymatic browning (the Maillard reaction). Lipids, especially when unsaturated, are susceptible to oxidation. They can form lipid peroxides, which interact with proteins to form lipid-protein complexes, resulting in a decrease in the nutritive value of the food.

In addition to the aforementioned purposes of food protein processing, proteins play an important role in improving the functionality of food ingredients. Whey proteins commonly are subjected to heat denaturation before spray-drying to improve water-holding properties for bread making. Soy proteins are treated with alkali to improve solubility and textural properties and to obtain desirable rheological properties. Viscosity and solubility measurements are commonly used to obtain information about the functional behavior and physicochemical nature of proteins.

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, which are sequenced in a linear polypeptide chain and constitute the basic building blocks. Amino acids are joined together through an amide linkage or peptide bond, which is formed through the removal of a hydroxyl group from the carboxyl group of one amino acid and a hydrogen atom from the α -amino group of the adjoining amino acid. The C—N bond of the peptide linkage exhibits partial double-bond character and is not free to rotate, which imposes some constraints on the number of conformations the polypeptide can assume (Figure 1). There is, however, a large degree of rotational freedom around the single C_{α} —C and C_{α} —N bonds of the polypeptide. The secondary structure of a protein describes the regular folding of a polypeptide chain, 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 -pleated sheets, and random coils (Figure 2). The α -helix structure exists in polypeptides that are tightly coiled in a rodlike structure, with side chains extending outward from the helix. In contrast, the polypeptide in the β -sheet structure formed of aligned loops in a plane with adjacent strands are stabilized by hydrogen bonds between the NH and CO groups. The arrangement of member strands can be parallel, antiparallel, or a mixture of the two. Many proteins have well defined combinations of β -strands and α -helices or β -strands alone and represent a form of supersecondary structure. Some amino acids cannot form α -helices because of electrostatic repulsion or bulky side chains. As a result, the polypeptide assumes a random coil structure having minimal electrostatic free energy. In general, re-

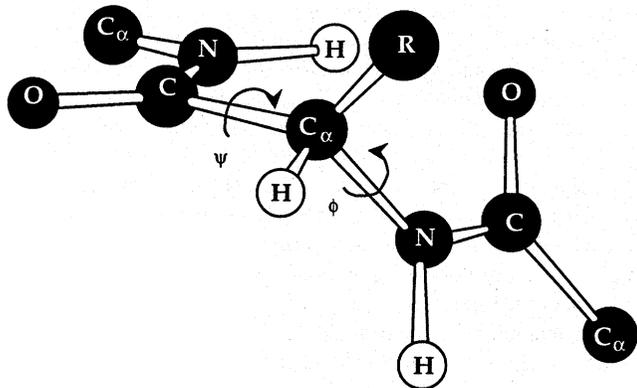


Figure 1. Polypeptide chain linking two peptide units; ϕ and ψ indicate rotation about the α -carbon. The two peptide bonds adjacent to the α -carbon are located in different planes. [From Stryer (1975); reproduced with permission of L. Stryer.]

regions of α -helices and β -sheets in the protein are well defined; random coils are not.

The tertiary structure refers to the steric relationships of amino acid residues and overall architecture in three dimensions of the polypeptide chain whose folding brings into proximity parts of the molecule otherwise widely separated along the backbone. Proteins containing more than one polypeptide chain exhibit another level of organization. This quaternary structure describes the packing of polypeptide chains, which are stabilized by hydrogen bonds and van der Waals forces. Structural changes at this level have unique biological applications, which are often mediated by enzymes. It is through systematic denaturation of the organized structures and forces of food proteins that changes in functional behavior can be achieved.

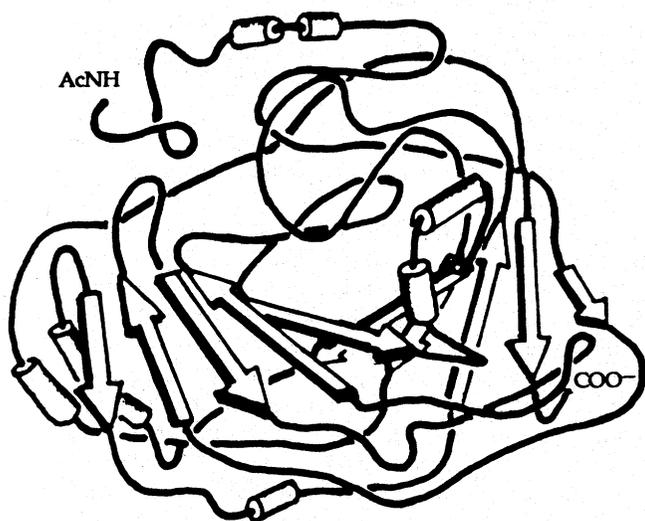


Figure 2. Schematic tertiary structure of the protein carbonic anhydrase. Helices are drawn as cylinders, β -sheet strands as arrows with N at the tail and C at the point of each arrow. [From Fennema (1985), reproduced by permission of O. R. Fennema.]

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 their solubility, shape, prosthetic group, and regulatory properties, as well as biological activity. They were first classified on the basis of their solubility as albumins, globulins, glutenins, and prolamines. Albumins are the most water-soluble globular proteins. Next most soluble are globulins such as conglycinin (7S) and glycinin (11S) from soybean, which are soluble in dilute salt solutions at neutral pH. Glutenins, such as wheat glutenins, are soluble in dilute acid or alkali. Prolamines are corn or wheat storage proteins and 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 (soluble in water or dilute salt), myofibrillar proteins (soluble in salt solution > 0.6 M), and stromal proteins, which are the least soluble class of muscle proteins. Fibrous proteins—for example, myofibrillar proteins such as actin and myosin, or stromal proteins, such as collagen and elastin—have polypeptide chains arranged in long strands and serve a structural or protective role. Collagen, the major protein of connective tissue, occurs in several polymorphic forms consisting of three intertwined polypeptide chains.

Egg proteins are primarily globular proteins found in the albumen (egg white) and include ovalbumin, conalbumin, ovotransferrin, ovomucoid, and lysozyme. The egg yolk contains a variety of lipoproteins, which act as emulsifying agents, as well as livetin (possibly derived from hen's blood) and phosvitin, an iron carrier.

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 in response to increased concentration of calcium and magnesium ion or below the isoelectric point (pI).

Other proteins classified according to their prosthetic groups (tightly associated non-amino acid moieties) are lipoproteins, glycoproteins, and myoglobins (including hemoglobin) with attached fat or oil, sugar, or iron groups.

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 proteins that function as specific biological catalysts for chemical reactions in living systems. Enzymes are much more efficient and specific than other catalysts, and they operate in restricted conditions of temperature and pH. They are frequently used in the food industry to modify the functional behavior of food proteins. For instance, proteases, which hydrolyze peptide bonds, can be recovered from plant sources such as papain and are used as commercial meat tenderizers. It is important, however, that the nutritive value of the product not be significantly reduced by the hydrolysis of peptide bond of the protein catalyzed by the enzyme.

Enzymatic action on free amino acids is of significance in food spoilage and production of flavors in fermented foods. For exam-

ple, enzymatic degradation of free amino acids by microorganisms of the genera *Staphylococcus*, *Pseudomonas*, and *Micrococcus* produce specific enzymes that are responsible for the degradation reactions. Enzymes are also used in the manufacture of beer, bread, cheese, coffee, vinegar, vitamins, and many other products.

1.3 ENERGY AND NUTRITION VALUES

The nutritional quality of proteins is determined by their amino acid composition. Nine of the most common amino acids are classified as essential because they cannot be synthesized in the human body. The nutritional value of a protein for human consumption is based on the content of essential amino acids compared with human requirements, and by protein efficiency ratio (PER), which is determined by dividing the weight gain of a rat by the protein intake. Nutrient availability and quality of food proteins depend on the processing treatments and interaction with other food components. 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. The RDA for a certain population varies depending on such factors as age, sex, level of activity, and individual metabolic factors. Protein requirements to meet growth and maintenance range from 2.0 g per kilogram of body weight for infants to 0.8 g/kg for adults. Energy needs increase during pregnancy and lactation as well as for work, stress, sickness, and aging. Proteins differ in nutrient value because of differences in amino acid composition. Protein quality is measured by chemical scores and biological assays that assess such qualities as content of essential amino acids, nitrogen value, limiting amino acids, and efficiency ratio. Cheese, eggs, fish, meat, and milks are sources of animal proteins. Vegetable proteins can be found in beans, grains, nuts, and a variety of vegetables.

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. These forces include covalent bonds (intermolecular disulfide linkages), ionic interactions (salt bridges), hydrogen bonding, hydrophobic interactions, hydration, and steric repulsion.

Bonds or interactions that determine secondary and tertiary structure of proteins are shown in Figure 3. Covalent bonds include all the bonds of the primary structure of the protein as well as disulfide bonds, which are formed between cysteine residues and are dependent on the conformation and structure of the peptide chain. Of the covalent forces, disulfide bonding is the most important in protein interactions. Protein denaturation results in changes in the secondary and tertiary structures. Proteolysis by appropriate enzymes or hydrolysis with strong acid or base is necessary to break peptide bonds. Noncovalent molecular forces (e.g., hydrogen bonds, hydrophobic interaction, repulsion forces) are one to three orders of magnitude smaller than covalent bonding energy. The integrity of food proteins is maintained by association and disassociation of the protein in secondary and tertiary structure.

2.1 PROTEIN-PROTEIN

Protein-protein interactions often occur as a result of food processing that is 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. Stable protein interactions occur through cross-linking and ionic, polar, and hydrophobic interactions. Gelation is an association of proteins existing in a three-dimensional network with trapped water molecules. Protein cross-links are established through the formation of disulfide links by sulfhydryl groups or through hydrophobic interactions. To maintain a stable gel, a balance must exist between forces that promote formation of the network and opposing forces.

2.2 CARBOHYDRATES

Proteins 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 carbohydrate with the free amino group of the protein, followed by a cascade of reactions leading to polymers. These Maillard reactions are the major cause 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

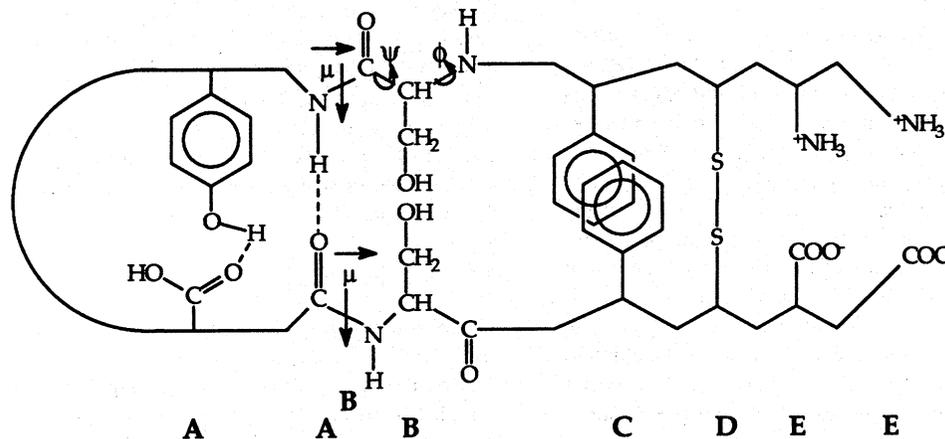
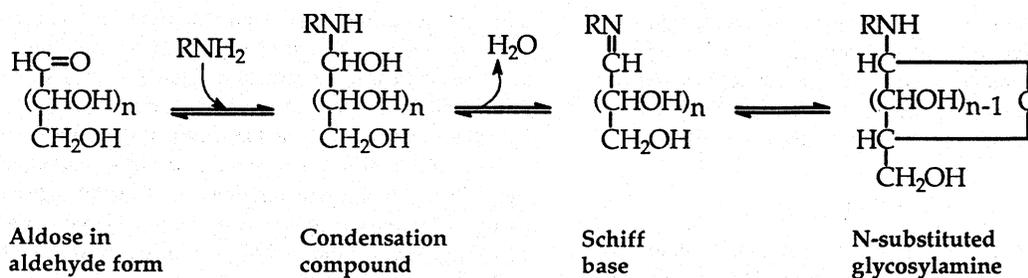
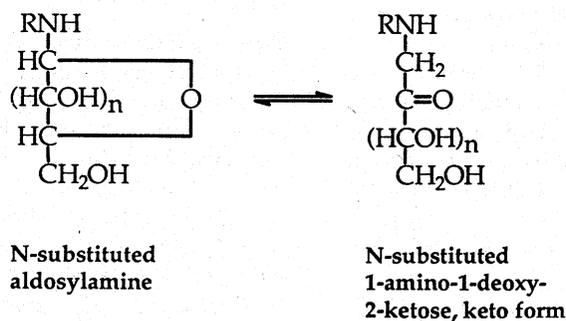


Figure 3. Bonds or interactions that determine protein secondary and tertiary structure: (A) hydrogen bond, (B) dipolar interaction (μ , dipole moment), (C) hydrophobic interaction, (D) disulfide linkage, (E) ionic interaction. [From Fennema (1985); reproduced by permission of O. R. Fennema.]



Initial steps of the Maillard reaction



Amadori rearrangement

Figure 4. Schematic representations of the Maillard reaction and the Amadori rearrangement.

in late Maillard-type damage and other undesirable reactions. In the early stages of the Maillard reaction (Figure 4), the carbonyl of the reducing sugar undergoes nucleophilic attack by the amine, followed by loss of water and ring closure to form the glycosylamine. The Amadori rearrangement follows with the formation of 1-amino-2-keto sugar, which has been isolated and identified in browned dried fruit.

2.3 LIPIDS

Protein-lipid interactions in nature occur in food systems such as milk and eggs. These interactions occur most notably at the cellular and intracellular membrane levels. Proteins that stabilize emulsions can adopt conformations that can interact with both lipid and aqueous phases by assuming the form of lowest free energy at the interface. Schematically the process can be represented in two steps: diffusion-controlled sorption at the interface, followed by protein unfolding (Figure 5). Emulsions are stabilized by hydrophobic interactions between the apolar region of the protein and the apolar aliphatic chain of the lipid. Confirmation of the importance of hydrophobic interactions has been demonstrated in model systems in which the energy of protein-lipid interaction reaches a maximum around the pI of the protein. Protein-lipid interaction can be increased by high pressure homogenization, which increases the number of lipid droplets and the interfacial surface area. Lipids protect proteins against thermal denaturation because of their high heat ca-

capacity and the absence of water. Strong interactions occur in flour-water mixtures, where lipids can bind with gluten proteins to form highly stable lipoglutenin complexes.

2.4 HYDRATION

Interactions of protein side chains with water determine the intrinsic properties of a protein (e.g., solubility, swelling, dispersibility, wettability). Water absorption is considered to be the most important step in imparting desired functional properties to proteins. Proteins interact with water through their side chains or backbone, and their solubility depends on factors such as hydrogen bonding, 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. Texturized proteins may be used to form meat or seafood analogues by taking advantage of hydration properties.

2.5 SOLUBLE IONS

The most important ionic components associated with proteins that affect solubility are sodium, potassium, and calcium. The reactive nature of proteins allows for the manipulation of the ions to accomplish food preservation by, for example, controlled dehydration. Proteins are stable within a defined range of pH; they may have net positive or net negative charges or be neutral. At pH values

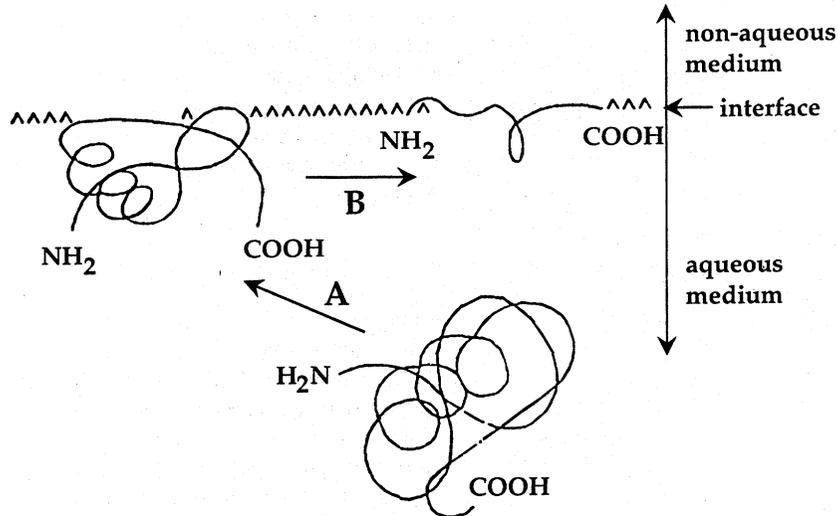


Figure 5. Schematic representation of the two-step process of protein denaturation at an interface: A, diffusion-controlled sorption at an interface; B, protein unfolding.

above their pI, proteins interact more readily with water molecules to improve solubility. Divalent cations also act as the cofactors that are essential for some enzymes to be catalytically active. Salts used in food processing can denature proteins by charge neutralization. Severe denaturation results in irreversible realignment of the native structure 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 activity (gelation and foaming), and chemical reactivity (textural properties). Functionality of a food product is determined experimentally because, while the study of protein structure provides information on physicochemical properties, the exact relationship between structure and function is not fully understood. Changes in functionality are the result of protein interaction with water and other proteins, or the result of changes in the surface characteristics of the protein molecule.

3.1 GELATION

Gelation is the formation of an extended network of denatured protein aggregates held together by intermolecular forces. Gels can result from controlled protein denaturation and unfolding (egg white); controlled folding and realignment (collagen); controlled unfolding, disulfide interchange, and enhanced hydrophobic interactions (gluten); and enzyme hydrolysis and hydrophobic interactions (rennet milk gels). Gel stability depends on the balance of hydrophobic interactions, hydrogen bonding, and electrostatic forces, which, in turn, are affected by pH and electrolyte content. Cross-linking of proteins in the matrix via disulfide bonds results in heat-irreversible gels stabilized by hydrogen bonds. Higher molecular weight proteins, with a larger percentage of hydrophobic amino acid residues, tend to form stronger gels.

3.2 EMULSIFICATION

A stable protein emulsion is produced when proteins in a two-phase medium, accompanied by energy input (usually a combination of shear and heat), unfold slightly at the interface and align their non-polar regions toward an oil phase with their hydrophilic regions toward the aqueous phase. The balance of the hydrophilic and hydrophobic forces in the protein maintains emulsion stability. Globular proteins with a highly ordered and stable tertiary structure, such as β -lactoglobulin, bovine serum albumin, and lysozyme, are more likely to unfold and are considered to be good emulsifiers. Emulsification activity continues to increase with increasing protein denaturation, provided solubility is not compromised. Surface hydrophobicity of proteins has been correlated with emulsifying activity. Emulsions are mechanically unstable and will separate over time. The breakdown in stability results in flocculation, followed by the coalescence of particles by forces depicted in Figure 6. Although emulsifiers can minimize interfacial surface energy and reduce coalescence, emulsions gradually separate into two phases as the repulsive electrical charges decrease. Gradual increase in free energy leads to more frequent collisions of similarly charged particles. These collisions produce flocculated globules or clusters, which will continue to grow unless conditions change. Complete breakdown of the emulsion is the result of coalescence of these particles.

3.3 FOAMING

Protein foams are emulsions of gas in a continuous aqueous protein phase containing various surfactants to prevent coalescence. Foods such as ice cream and whipped toppings are examples of stable foams formed by incorporation of a large volume of air during processing. Air is forced into the medium through mechanical mixing that increases the volume more than 1000%. Foam stability is maintained by surfactants that resist the influence of gravity, pressure, and temperature, which tend to rupture the stable emulsion. Controlled denaturing of the native protein by shear, temperature, or ma-

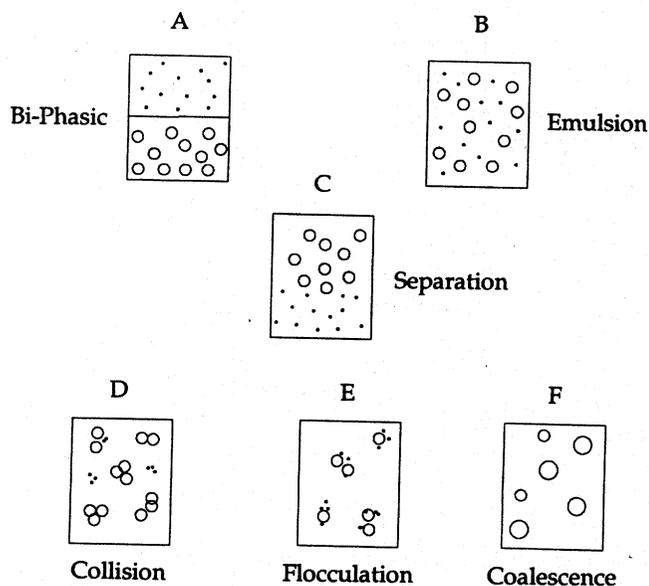


Figure 6. Process of emulsion formation and breakdown: (A) biphasic medium of liquid and semisolid emulsion, (B) thermodynamically unstable emulsion, (C) gradual migration of emulsion based on charge separation, (D) particle collision with increasing electrostatic charges, (E) clear aggregation and flocculation of collided particles, (F) coalescence and syneresis of emulsion.

nipulation of electrostatic balance increases hydrophobicity and improves foaming. Foam-stabilizing conditions include high surface hydrophobicity, low net energy charge, minimal electrostatic repulsion, good solubility, and protein concentration.

Foam expansion (overrun) is controlled by metering the mix and air that go into the equipment in proper proportions to form air cells of the desired size; overrun is computed as the percentage increase in volume or the percentage decrease in the density of the mix. Foam stability may be controlled by addition of combinations of stabilizers and emulsifiers: salts, sugars, lipids, cations, proteins, and energy input (heat and shear) during processing. As foams age during storage, they shrink, leading to loss of air and demixing.

3.4 THERMAL PROCESSING

Food proteins are thermally processed to enhance functionality, improve textural properties, or retard deterioration. In the preservation of fruits and vegetables (blanching), heat is used to denature enzymes. Processing may lead to a slight loss in nutritional quality; however, most processes improve quality by destroying antinutritional factors through inactivation of enzymes such as trypsin inhibitor or peroxidase. Complete denaturation of peroxidase (EC.1.11.1.7) is used as an indicator of adequate processing. Protein-protein interactions may be 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. Chilling or heating can denature protein. Protein structure can be altered as a result of property changes (in the electric potential, pH, etc.), and such changes can be used to enhance functionality in food. Thermal denaturation of food proteins generally occurs between 45 and 85°C, accompanied by exposure of hydrophobic groups that can lead to protein aggregation. More

severe treatments can result in the splitting of the disulfide cross-links with release of hydrogen sulfide, as well as alteration of amino acid residues with the formation of new intra- or intermolecular covalent cross-links. 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 can be formed in muscle foods as a result of industrial processing or home cooking, including frying, broiling, boiling, and baking. Cooking methods that involve direct contact with high temperature sources generate the most mutagens. Three general types of mutagen are found, arising from different sources. The IQ (imidazo-quinoline) types arise from endogenous nitrogen-containing compounds such as creatine and amino acids in combination with sugars; the *N*-nitrosoamine types arise from endogenous secondary amines and added nitrite; and the polycyclic aromatic hydrocarbon types arise from pyrolysis of fat due to smoke treatment or upon cooking (charring) on charcoal or direct flame. These mutagens are present in meat or seafood products at a low parts-per-billion level. Extensive research aimed at developing methods to reduce or eliminate such compounds from the food supply has seen moderate success.

See also LIPID AND LIPOPROTEIN METABOLISM; NUTRITION.

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