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Non-covalent interactions between proteins and polysaccharides

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Abstract

Foods with novel or improved properties can be created by utilizing non-covalent interactions between proteins and polysaccharides. In solution, either attractive or repulsive interactions between proteins and polysaccharides can be used to create microstructures that give foods novel textural and sensory properties. At interfaces, attractive electrostatic interactions can be used to create food emulsions with improved stability to environmental stresses or with novel encapsulation-release characteristics.

1. Introduction

Proteins and polysaccharides are natural polymers that are widely used as functional ingredients in food materials. These biopolymers may be part of complex natural foods (such as milk, flour, eggs or meat) or they may be isolated functional ingredients (such as gelatin, pectin or carrageenan). This brief review shows how an understanding of non-covalent interactions between proteins and polysaccharides can be used by food scientists to design and fabricate foods with novel or improved properties.

2. Functional properties of food biopolymers

The type, number, sequence and bonding of monomers within a food biopolymer determine its molecular characteristics in solution *e.g.*, chain length, branching, charge, flexibility and hydrophobicity (Cui, 2005). These molecular characteristics largely determine the functional attributes of biopolymers in foods, *e.g.*, their ability to thicken solutions, form gels, hold water, and to form and stabilize emulsions and foams. Individual biopolymers can be used to provide many of these functional attributes, however novel or improved functionalities can often be engineered into a system by using biopolymer blends. To utilize these interactions it is important to understand the basic principles of directed polymer self-assembly, rather than just empirically mixing different biopolymers together.

3. Biopolymer interactions

There may be synergistic or antagonistic interactions between different kinds of biopolymers that cause large changes in their functional properties (Schmitt et al., 1998; Benichou et al., 2002; de Kruif et al., 2004). Knowledge of the origin and nature of the interactions involved can often be used to engineer novel structures and physicochemical properties into food systems. The major non-covalent interactions between proteins and polysaccharides are:

- *Electrostatic interactions*. This interaction is important for biopolymers that have an electrical charge under the conditions where they are used (pH and ionic strength). They may be either attractive or repulsive depending on whether the charge groups

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involved have different or similar signs. The strength and range of these interactions decreases with increasing ionic strength.

- *Steric exclusion*. The relatively large volume occupied by many biopolymers in solution means that steric exclusion effects are important, *i.e.*, there is a reduction in the mixing entropy of the system due to the reduction in the volume available for the biopolymer molecules to occupy.
- *Hydrophobic interactions*. This interaction is important for biopolymers in aqueous solutions that have non-polar groups, and manifests itself as a tendency for the non-polar groups to associate with each other.
- *Hydrogen bonding*. This interaction is important for biopolymers that have segments along their chain that can form relatively strong hydrogen bonds with segments on other molecules, *e.g.*, through helical or sheet-like structures.

The relative importance of these interactions in a particular system depends on the types of biopolymer molecules involved (*e.g.*, molecular weight, charge density *vs.* pH profile, flexibility, hydrophobicity), the solution composition (*e.g.*, pH and ionic strength) and the environmental conditions, (*e.g.*, temperature, shearing). By modulating these parameters it is possible to control the interactions between the biopolymers and therefore create different functional attributes in a food system.

4. Creation of novel functionality based on biopolymer interactions in solution

When two different biopolymers are mixed together they may either form a one-phase or a two-phase system depending on the nature of the biopolymers involved, the solution composition and the prevailing environmental conditions (Fig. 1). In a one-phase system, the two biopolymers can exist either as individual molecules or as soluble complexes that are evenly distributed throughout the entire system. In a two-phase system, the solution separates into two distinct phases that have different biopolymer compositions. Phase separation can occur through two different physicochemical mechanisms: associative and segregative separation.

In *associative separation*, there is a relatively strong *attraction* between the two different kinds of biopolymers which causes them to associate with each other. The most common example of this type of interaction for food biopolymers is the electrostatic attraction between molecules with opposite electrical charges. The resulting two-phase system consists of a phase that is rich in both biopolymers and a phase that is depleted in both biopolymers (Fig. 1). The biopolymer-rich phase

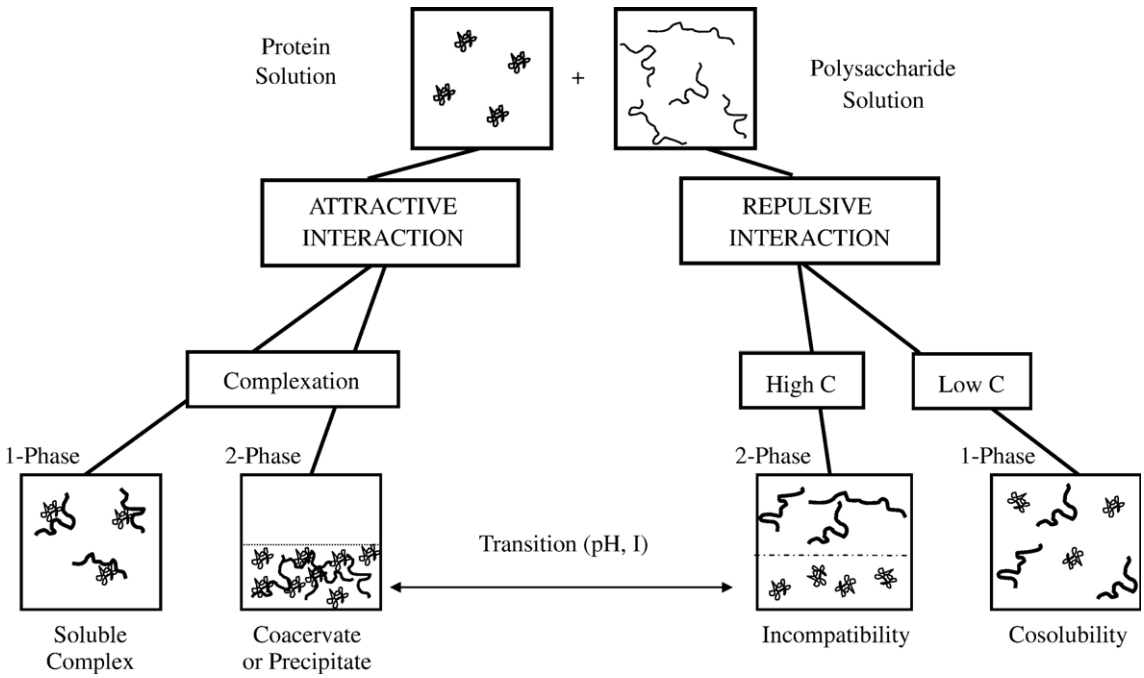

Fig. 1. Schematic representation of the kinds of structural arrangements of the molecules involved that can occur when proteins and polysaccharides are mixed together.

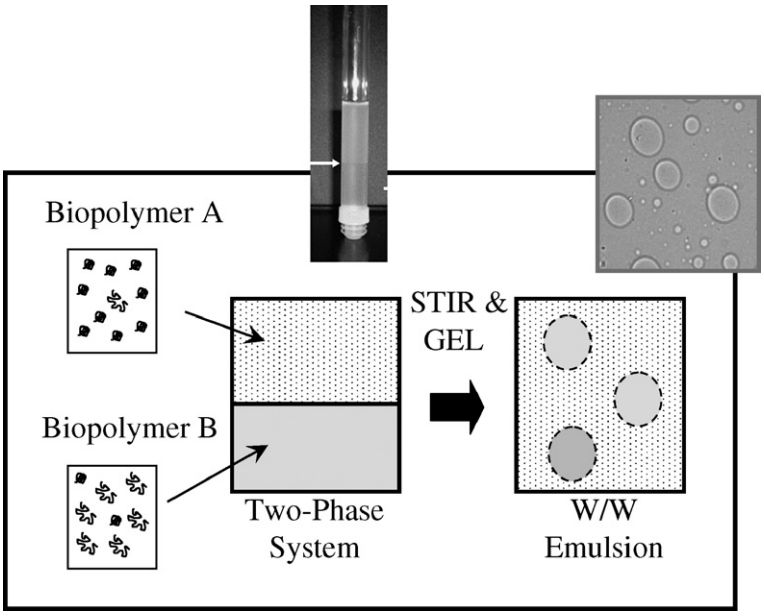

Fig. 2. Schematic representation of production of a water-in-water (W/W) emulsion from a two-phase system consisting of two aqueous phases.

may either be a *coacervate* or a *precipitate*, depending on the strength of the attraction and the nature of the polymers involved.

In *segregative separation*, there is a relatively strong *repulsion* between the two different kinds of biopolymers, *i.e.*, there is a relatively high positive (unfavorable) free energy of mixing. The molecular origin of this effect is usually the steric exclusion effect mentioned above. This type of phase separation often occurs when one or both of the biopolymers are uncharged, or when both biopolymers have similar electrical charges. At sufficiently low biopolymer concentrations, the two biopolymers are intimately mixed and form a one-phase solution, but once the biopolymer concentration exceeds a certain level phase separation occurs and a two-phase solution is formed with one of the phases being rich in one type of biopolymer and depleted in the other type, and *vice versa* (Fig. 1). The behavior of biopolymer

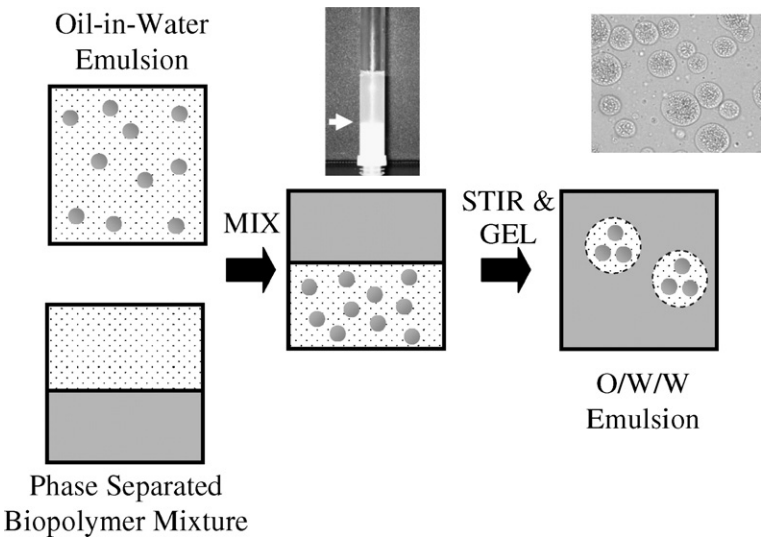

Fig. 3. Schematic representation of production of an oil-in-water-in-water (O/W/W) emulsion from a two-phase system consisting of two aqueous phases.

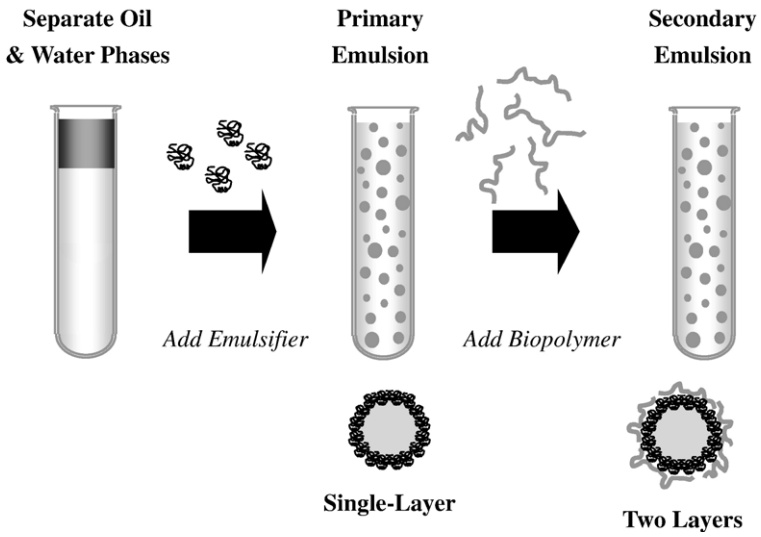

Fig. 4. Schematic representation of production of oil-in-water emulsions containing droplets stabilized by interfacial complexes formed by a protein and a polysaccharide.

blends under different solution and environmental conditions can be conveniently characterized in terms of phase diagrams (Walstra, 2003). These phase diagrams can often be used to optimize the biopolymer composition required to produce a solution with a particular microstructure and physicochemical properties.

A variety of different microstructures can be created in phase separated biopolymer systems by varying the preparation conditions or by shearing the system, *e.g.*, "water-in-water" emulsions can be formed (Fig. 2) or "oil-in-water-in-water" (Fig. 3). Once a particular microstructure has been formed by phase separation of a mixed biopolymer solution it is often possible to trap the system in a kinetically stable state, and thus create novel food microstructures and rheological properties (Norton and Frith, 2001). For example, kinetic trapping can be achieved by changing solution or environmental conditions so that one or both of the phases thickens or gels, *e.g.*, by changing temperature, pH, ionic composition or solvent quality. If this process is carried out in the presence of shear forces it is possible to produce a wide variety of different microstructures, *e.g.*, spheres, teardrops, fibers. Alternatively, it may be possible to adsorb another biopolymer around the water droplets that form the dispersed phase in a W/W emulsion, thereby stabilizing them.

Different types of gel microstructure can be created using biopolymer blends by varying the nature of the biopolymers involved, the solution composition and the prevailing environmental conditions, *e.g.*, interpenetrating networks comprised of different biopolymers, a single network that incorporates both types of biopolymer, or a "filled gel" consisting of regions rich in one biopolymer dispersed in regions rich in the other biopolymer. Each of these microstructures will have unique rheological and physicochemical properties, *e.g.*, gel strength, gelation rate, gelation temperature, water holding capacity and opacity. Many food scientists are currently attempting to understand the fundamental processes involved in the formation of structured biopolymer blends and in utilizing these systems to create foods with novel or improved physicochemical and sensory properties. In particular, mixed biopolymer systems appear to be an effective means of creating low-fat products with similar properties to high-fat products, *e.g.*, deserts, yogurts, dressings and spreads (Norton and Frith, 2001).

5. Creation of novel functionality based on biopolymer interactions at interfaces

It is also possible to use an understanding of protein–polysaccharide interactions at interfaces to create novel functional properties in colloidal systems, such as suspensions, emulsions or foams. The layer-by-layer (LbL) electrostatic deposition method has proved to be a particularly effective means of engineering novel or improved functional properties into colloidal systems (Decher, 2003). LbL begins with the electrostatically-induced adsorption of a charged polyelectrolyte (PE) onto an oppositely charged surface. Charge reversal of the surface occurs because the total number of charges on the adsorbed PE molecules is greater than the number

of charges on the surface. This charge over-compensation has two important consequences. (1) The adsorbing PEs tend to form mono-layers because additional PE's in solution are repelled. (2) Further layers can be formed by adsorbing oppositely charged PEs onto the first layer, *e.g.*, T–P_1–P_2, where T is the template surface, and P_1 and P_2 are two oppositely charged polyelectrolytes. Repetition of these adsorption steps leads to the formation of a polyelectrolyte multilayer (PEM) on the template, *e.g.*, T–$(P_1$–$P_2)_n$–P_1 or T–$(P_1$–$P_2)_n$–P_1–P_2. This procedure has been used widely in the medical, electronic, pharmaceutical and chemical industries to create materials with novel or improved properties.

Recently, it has been shown that the LbL technique can be used to improve the stability of food emulsions to environmental stresses, and to create novel encapsulation and delivery systems (Guzey and McClements, in press). The formation of a PEM shell around an oil droplet is demonstrated schematically in Fig. 4. First, a *primary* emulsion containing oil droplets charged by a layer of emulsifier is prepared by homogenization. Incorporating a PE of opposite charge into the primary emulsion yielded a secondary emulsion containing droplets stabilized by emulsifier–PE membranes. This procedure can be repeated to add more layers to the interfacial membrane.

Previous studies demonstrate formation of such multilayer emulsions with a variety of different proteins and polysaccharides, *e.g.*, β-lactoglobulin with pectin, carrageenan or alginate; and casein with pectin (Guzey and McClements, in press). Such multilayered interfacial membranes often provide oil droplets with more stability to environmental stresses such as thermal processing, ionic strength, pH, freezing and dehydration than single-layered membranes. In addition, the ability to systematically control the properties of the PEMs has led to the development of delivery systems with novel triggered release properties. The main challenge associated with preparing these multilayer emulsions is the selection of an appropriate PE to adsorb to the surfaces, and the avoidance of bridging flocculation during formation.

6. Conclusions

A fundamental understanding of the origin and nature of the various non-covalent molecular interactions operating between proteins and polysaccharides can be used to create novel nano-structures and microstructures in foods. In turn, these structures determine the bulk physicochemical properties (stability, texture, flavour, mouthfeel and appearance) and functionality (controlled or triggered release) of foods.

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