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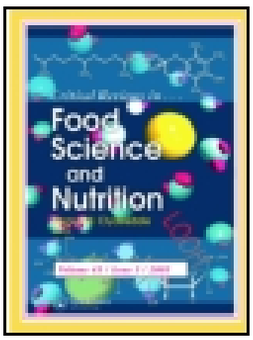

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REVIEW

Molecular forces governing protein-protein interaction: Structure-function relationship of complexes protein in the food industry

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ABSTRACT

The application of protein-protein interaction (PPI) has been widely used in various industries, such as food, nutraceutical, and pharmaceutical. A deeper understanding of PPI is needed, and the molecular forces governing proteins and their interaction must be explained. The design of new structures with improved functional properties, e.g., solubility, emulsion, and gelation, has been fueled by the development of structural and colloidal building blocks. In this review, the molecular forces of protein structures are discussed, followed by the relationship between molecular force and structure, ways of a bind of proteins together in solution or at the interface, and functional properties. A more detailed look is thus taken at the relationship between the various influencing factors on molecular forces involved in PPI. These factors include protein properties, such as types, concentration, and mixing ratio, and solvent conditions, such as ionic strength and pH. This review also summarizes methods tha1t are capable of identifying molecular forces in protein and PPI, as well as characterizing protein structure.

KEYWORDS

Molecular forces; proteins interaction; protein structure; functional properties

Introduction

In the last few decades, the growing world population has greatly increased the demands for high nutrient value-added food sources. Protein is an essential component of human nutrition because they are highly important to the recovery of damaged cells, immune response, cell signals, and the maintenance of muscle mass (Henley, Taylor, and Obukosia 2010). Amino acids are connected in a chain through peptide bonds, amino acid side chains or residues that protrude from a chain, thereby causing differences in form, size, and surface charge (Hedin 2010). All proteins can be identified according to their primary, secondary, tertiary, and quaternary structure (Rehman and Botelho 2020). The protein structure is a feature of the interaction of protein components (Mezzenga et al. 2005; Ubbink, Burbidge, and Mezzenga 2008). Molecular forces that are involved in protein-protein interaction (PPI) include non-covalent ones, such as electrostatic, hydrogen and hydrophobic forces (He, Hu, et al. 2020; Nicolai 2019; Sun, Wang, and Guo 2018; Wang, Xu et al. 2019), van der Waals forces (Karplus and Šali 1995), steric and hydration repulsive forces (Howell 1992), and covalent disulfide linkages (Lin et al. 2019). These forces have a major role in modifying the protein microstructure (Wang et al. 2020) and functional properties, such as solubility (Wang, Xu et al. 2019), emulsion (Liang et al. 2016; Wang et al. 2020), and gelation (Lin et al. 2019; Sun, Wang, and Guo 2018). As shown in Table 1, animals, plants, and fungi are the three main dietary sources of protein. Efforts have focused on replacing expensive animal proteins with cheap, nutritious plant proteins (Elmalimadi et al. 2017). The interaction between animal and plant proteins is being comprehensively investigated to develop balanced mixtures of animal and vegetable proteins. These studies involve determining and understanding protein structure-function relations, optimizing the use of the components of the product, improving the quality, reducing costs, and finding new protein applications (Graça, Raymundo, and de Sousa 2016; Ma et al. 2017).

PPI is considered one of the best techniques to enhance the functional properties, such as solubility, emulsion, and foaming, of plant protein (Ma et al. 2017; He, Wang, et al. 2020; Sun, Wang, and Guo 2018; Wang, Xu et al. 2019; Wang, Tue et al. 2018). The functional properties of a protein are influenced by surface hydrophobicity more than hydrophobicity because of the polymeric aspect of protein (Chandrapala et al., 2011). The hydrophobicity of the protein surface plays a major role in solubility, physical stability, adsorption behavior, and the aggregating tendency of the protein, whereby the PPI effect on the surface charge and surface hydrophobicity of protein via electrostatic and hydrophobic forces and hydrogen bonds (He, Hu, et al. 2020).

Recently, the utilization of plant protein in the food industry has substantially increased because of their low cost and environmental impact (Shigemitsu et al. 2018). Many studies have indicated that plant-based proteins have

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Table 1. Summary of food proteins sources commonly used in the food industry.

Sources of protein			Types and ratio of proteins	References
Animal		Milk	Whey 15–20% [β-lactoglobulin (35–65%), α-lactalbumin (12–25%); immunoglobulins (8%], albumin (5%), and lactoferrin (1%)][a]	(Ma et al. 2017; Camargo et al. 2018)
			Casein [80%) (α_{S1}-casein (40%), α_{S2}-casein (10%), κ-casein (15%), and β-casein (35%)]	(Beliciu and Moraru 2013)
		Egg	Ovoglobulin (G2 and G3, 8%), ovotransferrin (12%), lysozyme (3.5%), ovomucin (3.5%), ovalbumin (54%) and ovomucoid (11%),	(Abeyrathne, Huang, and Ahn 2018)
		Blood	BSA; hemoglobin; ferritin	(Alimam, Pendry, and Murphy 2018)
		Meat	Elastin; myosin; actin; collagen.	(Hurrell et al. 2006)
		Insect	Fibroin, resilin, chemosensory, Sf caspase-1, major royal jelly protein, melittin, myoblast city, doublesex and tra genes	(Angeli et al. 1999; Ardell and Andersen 2001)
Plant	Cereals	Wheat	Albumin; globulin; glutelin (80%); prolamin/gliadin	(Shewry et al. 2009)
		Corn	Albumin; globulin; glutelin (18%); prolamin/zein (60%) (α-zein, β-zein, γ-zein, δ-zein)	(Hojilla-Evangelista 2012; 2014; Zhuang, Tang, and Yuan 2013; Muthukumarappan and Swamy 2018)
		Barley	Albumin (22%); globulin; glutelin (47–27%); prolamin (35–55%)	(Kirkman, Shewry, and Miflin 1982)
		Oats	Albumin; globulin (75%); glutelin ($4-14$%); prolamin (10%)	(Webster 2016; Friesen 2017)
		Rice	Albumin (5–10%), globulin (7–17%), glutelin (75–81%) and prolamin (3–6%)	(Amagliani et al. 2017)
	Legumes and pulses	Soybeans	2S globulin (17%), 7S globulin (33%), 11S globulin (45%); 15S globulin (5%)	(Amagliani et al. 2017)
		Peas	2S globulin (25%), 7S globulin (43%), 11S globulin (28%); 15S globulin (4%)	(Amagliani et al. 2017)
		Mung bean	2S globulin (15%), 7S (79%) globulin; 11S globulin (6%)	(Amagliani et al. 2017)
		Lima bean	2S globulin (52%), 7S globulin (21%); 15S globulin (27%)	(El Fiel, El Tinay, and Elsheikh 2002)
		Chickpea	Albumin (8.39–12.31%), globulin (53.44–60.29%) prolamin (3.12–6.89%); glutelin (19.38–24.40%).	(Portari et al. 2005)
		Lentil	Albumins (16.8%), Globulins (11S/legumin (44.8%), 7S/vicilin (4.2%)), glutelins (11.2%); prolamins (3.5%).	(Boye, Zare, and Pletch 2010)
	Tubers	Potato	Sporamin + A N-termina (80%), glycoprotein, arabinogalactan	(Wang, Nie, and Zhu 2016)
	Oilseeds	Rapeseed, peanut, sunflower, hemp seed	Albumin + globulin (60%), gluten	(Bur et al. 2004)
		Cottonseed	Globulins (2S, 7S/congossypin, 12S)	
	Edible seeds	Quinoa, buckwheat	albumin (35%), globulin (37%), prolamins ($0.7-7$%)	(Steffolani et al. 2016)
	Pseudocereals	Chia	Albumin (22.8%), globulin (22.4%), glutelin (48.9%) prolamin (5.8%)	(Attalla and El-Hussieny 2017)
	Algae	Micromonas		(Simon et al., 2017)
Fungi		Mycoprotein		(Stoffel et al. 2019)

significant health benefits (Bianchi et al. 1993), including providing nutrients for patients with cirrhosis and reducing metabolic disorders caused by obesity (Wanezaki et al. 2015). Interactions between plant proteins with other types of protein undoubtedly occur in different ways. These interactions could have significant impacts on the structural and functional properties of plant proteins due to the molecular forces, e.g., hydrogen bonds and hydrophobic and electrostatic forces (He, Wang, et al. 2020). However, very limited information is available about the molecular forces that govern protein interaction between plant proteins with other proteins. Therefore, this review is the first to summarize the status of information about molecular forces governing PPI.

Molecular forces involved in PPI

Knowing what molecular forces regulate PPI enables us to understand the relationship between covalent and non-

Table 2. Summary of interactions types between proteins and their driving molecular forces.

Types of protein	Conditions	Structures	Main driving forces	Structural classification	Main influence	References
WPIs – RPs	1% (w/v) at different ratios of WPIs to PRs (1:1, 1.2:1, 1.5:1, and 2:1)	Synergistic	Hydrogen bonding and hydrophobic.	Secondary structure > Tertiary structure > Quaternary structure	Formation of novel structure. Increase in water solubility of rice protein from 1.5 to 50%.	(Wang, Xu et al. 2019)
CPs – RPs	1% (w/v) and different ratios of CPs to PRs (0.01:1, 0.1:1, 1:1, 1.5:1, and 2:1)	Synergistic	Hydrophobic interaction	Tertiary structure > Secondary structure	New backbone hybridization. Increase in water solubility of rice protein from 1.5 to 90%.	(Wang, Yue et al. 2018)
SPIs – WGPs	Different concentrations (40, 35, 30, 25, and 20%, w/w) and ratio of SPIs to WGPs (5:0, 4:1, 3:2, 2:3, 1:4, and 0:5)	Phase Separation	Not mentioned	Not mentioned	Formation of a new structure.	(Grabowska et al. 2014)
Collagen – SPIs Collagen – WPIs Collagen – BPPs Collagen – Gluten	4% (w/w) and different ratios of collagen to SPIs, WPIs, BPPs, and gluten (2.8 to 1.25%)	Synergistic Interaction	Hydrophobic Interactions	Not mentioned	Increased elasticity. Weakened gel matrix. Protein interaction no effect on collagen structure.	(Oechsle et al. 2015)
CPHs – SPIs	1% (w/v) of CPHs to SPIs	Aggregation	Hydrophobic interactions and electrostatic repulsion	Not mentioned	Increasing of interface protein coverage.	(Wang et al. 2020)
RPs – SPIs	1% (w/v) and different ratios of RPs to SPIs (1:0.1, 1:0.5, and 1:1)	Synergistic	Hydrogen bonding	Secondary structure	Formation of complex proteins. Enhance the solubility of RPs to over 80%.	(Wang, Xu et al. 2018)
SF – WPIs	The concentration of SF to WPIs was 2:1	Phase separation	Not mentioned	Not mentioned	Increase gel strength. Increase water holding capacity.	(Alu'datt, Alli, and Nagadi 2012)
SGs(7S/11S)–β-lg	Concentration of SGs to β-lg was 1:1 at 0.1% (w/w) ratio at pH 3.0 and 7.0	Synergistic	Electrostatic and hydrophobic interactions	Quaternary structures	Formation of new interfacial films. Improve foams properties. Enhance elasticity.	(Ruiz-Henestrosa et al. 2014)
SL–WPIs	10% (w/v) at different concentration of SL to WPIs (0.5 to 3%, w/v)	Aggregation	Hydrophobic and electrostatic forces	Secondary structure (decrease α-helix, increase β-sheet)	Enhance in physicochemical and functional properties. Change in structural properties.	(Sun, Wang, and Guo 2018)
PPs–RPs PPs–SPIs RPs–SPIs	14% (w/w) at different concentration of PPs–RPs, PPs–SPIs, and RPs–SPIs	Phase separation	Ionic and hydrogen bonds, hydrophobic interactions, and disulfide bonds.	Secondary structure	Unique network structures. Increase gel power of RPIs gel after complexation.	(Lin et al. 2019)
WGPs – SPIs	1% (w/v) and different ratios of SPIs – WGPs (0.1:1, 0.5:1, 1:1, 1.5:1, and 2:1)	Synergistic	Hydrogen bonding, hydrophobic and electrostatic interactions	Change on tertiary structures, no change on the primary structure	Formation of complex proteins. Enhance the solubility of RPs to over 72%.	(He, Wang, et al. 2020)
CPs – LFs	Different concentration of CPs and LFs (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0%)	Phase separation	Ionic strength	Not mentioned	Complex-coacervate of CPs and LFs.	(Anema and de Kruif 2016)
Pea – Whey protein	14.5% at ratio 1:1 of pea and whey protein at 30 °C for 20 h	Aggregation	Electrostatic interactions, hydrogen bonds and disulfide bonds.	Tertiary structure and Quaternary structure	Protein complexes have mixed gels less stiff than pure proteins of pea and whey protein.	(Ben-Harb et al. 2018)
LFs – SPIs	1% at ration SPIs – LFs (1:1, 1:2, 1:3, 1:4, and 1:5) at different pH range	Aggregation	Electrostatic forces	Secondary structure	Protein complexes of SPIs – LFs (1:3 at pH 6.25) have extra resistance of heat-sensitive.	(Zheng et al. 2020)

(*continued*)

Table 2. Continued.

Types of protein	Conditions	Structures	Main driving forces	Structural classification	Main influence	References
CPs – RGs	Range ration (RGs – CP = 0 – 3.2%) at pH 7.0 for 6 h	Aggregation	Electrostatic and van der Waals forces and hydrogen bonds	No change on secondary structure	No significant difference in the interfacial properties compared pure proteins.	(He, Hu, et al. 2020)
CPs – RPs	1% (w/v) and different ratios of CPs – PRs	Synergistic	Hydrophobic attraction	Primary and tertiary structure	Formation of nano-protein complexes. Increase in water solubility.	(Wang, Chen et al. 2019)
CFPs – SPIs	10% (w/v) and different ration of CFPs – SPIs (7S/11S ratio) at 100 °C for 30 min.	Aggregation	Hydrophobic interaction	Not mentioned	Increase the size and surface hydrophobicity of protein complexes with increase the amount 11S ratio.	(Wu et al. 2020)

β-lg = *β*-lactoglobulin; BPPs = blood plasma proteins; CFPs = cod fish protein; CPs = casein proteins, CPHs = corn protein hydrolysates; LFs = lactoferrins; PPs = peanut proteins; RGs = rice glutelin; RPs = rice proteins; SF = soy flour; SGs = Soy globulins; SL = soybean lecithin; SPIs = soy protein isolates; WGPs = wheat gluten proteins; WPIs = whey protein isolates.

covalent molecular forces, structure proteins, and the association of molecular forces that bind on PPI in solutions and at the interface. As shown in Table 2, the electrostatic, hydrogen, and hydrophobic interactions contribute to PPI in solution. This is in contrast with PPI at the interface, whereby the hydrophobic interaction plays the main role in the stability of foam or emulsion of protein complexes.

Molecular forces involved in protein structure

The amino acid sequences connect and hold the polypeptide chain, thus forming the primary protein structure (Rehman and Botelho 2020). The peptide bond is part of the covalent bonds that has an energy of formation around 100 kcal/mol (Whitaker 1977). The secondary structure consists of regions that are stabilized in the polypeptide backbone by the hydrogen bonds between the atoms (Thakur et al. 2019). Polypeptide backbone folding occurs to form α-helix and *β*-sheet (Jiang et al. 2019). In α-helix, the hydrogen bond linked carbonyl (C = O) with amino H (N – H) of amino acid, whilst in *β*-sheet, the hydrogen bond linked polypeptide backbone amino groups with the carbonyl of the backbone (Berg, Tymoczko, and Stryer 2002). The α-helix contributes to protein folding and performing functions, such as PPI, and α-helices are capable of mediating some PPI (Apurva and Mazumdar 2020). The α-helices and *β*-sheets are also good templates for modification of secondary protein structure. Molecular forces influence these shapes since these forces influence the length and strength of the chemical bond between two atoms, e.g., C-C (Howell 1992). Certain interactions contribute to the folding of proteins, including covalent bonds, such as peptide and disulfide bonds, and non-covalent bonds, such as electrostatic and hydrophobic interaction and hydrogen bonds (Apurva and Mazumdar 2020). As shown in Figure 1, the covalent disulfide bond is created with two cysteine residues. The sites are determined by the conformation of the polypeptide link, while hydrogen bonds, Van der Waals forces, and hydrophobic and ionic interactions play significant roles in the folding and bonding of secondary proteins' structure (Jiang et al. 2019; Howell 1992). Electrostatic forces exist between the two charged particles, which have either a net negative or a net positive charge, are based on their pH and the isoelectric point of the environment. On the contrary, Van der Waals forces between polar or non-polar molecules are weak attractive interactive forces because they happen through short distance between two proteins, which have a positive and negative charge.

The tertiary structure is a three-dimensional protein shape defined by regions stabilized by side-chain interactions (Jiang et al. 2019). This structure formed mainly due to interactions among R groups of the amino acid via non-covalent forces (e.g., electrostatic and London dispersion forces and hydrogen bond) and covalent forces (e.g., disulfide bond) (Berg, Tymoczko, and Stryer 2002). More specifically, in the tertiary structure, covalent disulfide bonds are involved and thus are impaired by denaturation of protein through heat gelation or foaming.

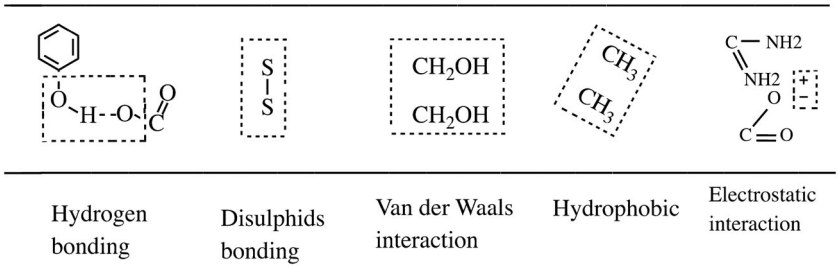

Figure 1. Types of molecular forces involved protein structure and protein-protein interaction.

The quaternary structure is a combination of two or more polypeptides. However, not all proteins have a quaternary structure (Aprahamian and Lindert 2019; Banach, Konieczny, and Roterman 2019; Bleiholder and Liu 2019). Ionic interaction, temperature, and pH have significant effects on the formation of the quaternary protein structure according to oligomers or dimers also even monomers. The dimer is considered to be the common shape under the physiological conditions depending on the accurate balance between the contribution of the hydrogen bonding, electrostatic and hydrophobic forces of protein (Edwards, Creamer, and Jameson 2009). For instance, β-lactoglobulin belongs to the protein family of lipocalins, all of which contain a β-barrel made up of dimer β-sheets. There are one hydrophobic side and one hydrophilic side of each β-sheet in β-lactoglobulin (Allen 2010). The free thiol group and hydrophobic residues were revealed by protein unfolding, indicating to involve the molecular forces, e.g., hydrophobic forces and disulfide bonds in form of the quaternary protein structure of whey protein isolate (Edwards, Creamer, and Jameson 2009).

The association of PPI is mainly due to the unique binding of peptides and proteins through non-covalent (Kuipers, Alting, and Gruppen 2007; Wang, Xu et al. 2019; Wang, Yue et al. 2018) and covalent (Lin et al. 2019) interactions. Consequently, protein structure and PPI were affected by molecular forces, including intramolecular as disulfide linkages and intermolecular forces, such as hydrophobic, electrostatic, hydrogen and Van der Waals forces (Figure 1).

Molecular force binding based on protein interaction in solutions

The three main pathways in the formation of PPI in protein solutions are aggregation, phase separation, and synergistic interaction (Firoozmand and Rousseau 2015; Ramirez-Suarez, Addo, and Xiong 2005; Sarbon, Badii, and Howell 2015). The characteristics of PPI are related to the net forces of protein in the solution (Gentile 2020; Neal, Asthagiri, and Lenhoff 1998). The synergistic interaction means that the effect of combination between two proteins is greater than the sum of the results separately caused by the individual protein. The contribution of long- and short-range forces, e.g., Van der Waals, hydrophobic, and steric forces, was also reported to play a significant role (Ferreira et al. 2019). The combination of two or more different proteins, e.g., coagulation and segregation, contributes in most cases to the independence of the phase separation network (Chronakis and Kasapis 1993; Sarbon, Badii, and Howell 2015). Anema and de Kruif (2016) found that a casein protein and lactoferrin mixture contributes to the formation of a uniform network structure based on phase separation, whereas a mixture of two opposite loaded proteins may contribute to aggregation through electrostatic attraction (Sarbon, Badii, and Howell 2015). Even if the attractive interactions between native proteins are not enough to cause phase separation, they may occur during heating because the mixing entropy effect decreases as growing aggregates (Nicolai 2019). Phase separation is more likely to occur when the concentration of reactive proteins is low. Synergistic interaction may lead to the alteration of protein complexes with a uniform structure formed according to each protein individually (Wang, Xu et al. 2019). Lin et al. (2019) reported that the microstructure of protein complexes derived from soy protein and peanut protein isolates altered after mixing, forming a continuous network, which is an ordered accumulation structure bound by non-covalent (hydrophobic interactions and hydrogen bonds) and covalent (disulfide) bonds. Wang, Xu et al. (2019) found that a mixture of 1% rice proteins with whey protein isolates at a ratio of 1:1 produced a novel structure of rice proteins and the unfolding effect occurred through a synergistic interaction of certain intermolecular forces (electrostatic, hydrophobic, and ion strength forces) between whey protein isolates and rice proteins. These results show that concentration, type and subunit composition of protein and molecular force (electrostatic, hydrophobic, and ion strength forces, and hydrogen and disulfide bonds) affect the change in the structure of protein complexes in the protein solution. When soy protein isolates, whey protein isolates, gluten, and blood plasma proteins are added to collagen, the collagen is strengthened and its microstructure is modified. However, the gel matrix is not the strongest after being formed, although its elasticity increases, thus enabling it to shrink and extend without permanent deformation (Oechsle et al. 2015).

In general, the surface charge of protein and protein complexes formed by PPI depends on the pH. The structure of complexes is neutrally charged at the isoelectric point, thereby leading to aggregation and causing the protein structure to have a fine network (Bengoechea et al. 2010). Whey protein isolates may shape the aggregated network at pH close to the isoelectric point or near the value (Alu'datt, Alli, and Nagadi 2012). Wang, Xu et al. (2018) showed that when rice proteins and soy protein isolates at pH 12 were adjusted to pH 7, this led to the formation of protein complexes through structural interaction via hydrogen bonds. Various protein binding mechanisms exist for hydrophobic

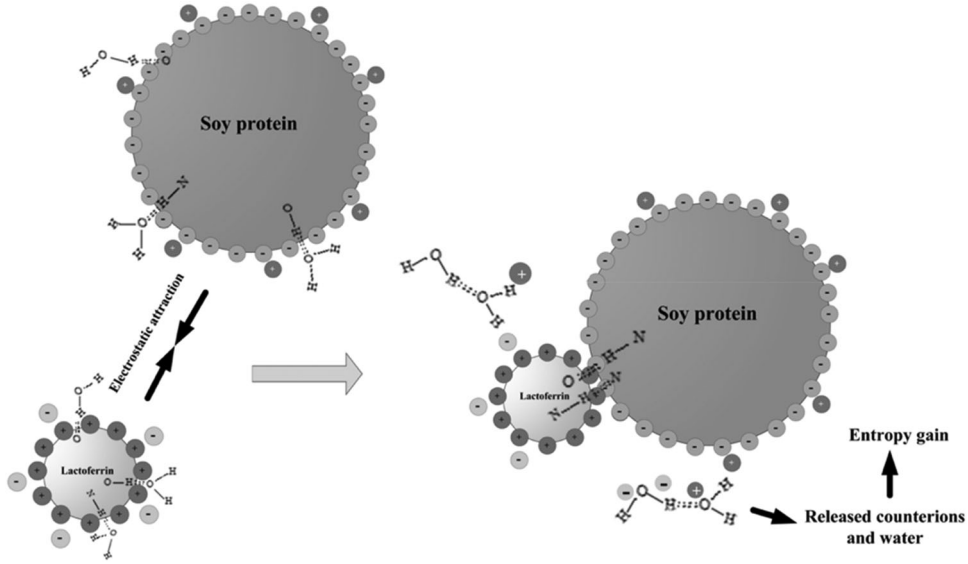

Figure 2. Schematic illustration of the interactions of hetero-proteins. When macroions of lactoferrins and soy protein isolates were close enough by electrostatic interaction, water molecules will be expelled.

molecules, such as Van der Waals attraction, hydrophobic interaction, and hydrogen bond (Kimpel and Schmitt 2015). Recent studies indicated that whey protein isolates could bind and deliver some proteins through the above mechanisms. These proteins include rice protein isolates (Wang, Xu et al. 2019), collagen (Oechsle et al. 2015), soybean flour (Alu'datt, Alli, and Nagadi 2012), soybean lecithin (Sun, Wang, and Guo 2018), 7S soy globulins and 11S soy globulins (Ruiz-Henestrosa et al. 2014), casein with rice protein (Wang, Yue et al. 2018), and peanuts protein isolates with rice protein isolates, peanuts protein isolates with soy protein isolates, and rice protein isolates with soy protein isolates (Lin et al. 2019) (Figure 2). Complexation of soy protein isolates and lactoferrins is an example of protein complexes generated via electrostatic interaction. When electrostatic interaction between soy protein isolates and lactoferrins became sufficiently close, a portion of water and counterions were released, upon which the peptide groups and polar groups (side chains) of protein complexes interacted to build intra- or intermolecular linkages via hydrogen bonding, thereby increasing the entropy (Zheng et al. 2020). PPI in solution has been a success in the preparation of protein composites and novel protein structure formation by molecular forces, especially electrostatic, hydrogen, and hydrophobic interactions.

Molecular forces binding based on interaction at the interface

Food proteins can prevent emulsion droplets from flocculating since they are more likely to adsorb the surface layer at a range of ~2 to 6 nm on hydrophobic–hydrophilic interfaces. A change or partial unfolding in the α-helix could lead to steric electrostatic stabilization (Dickinson 2010). The stability of the emulsion of single proteins can be affected by various factors, including pH, temperature, and salt (McClements 2004). The use of emulsifiers produced from

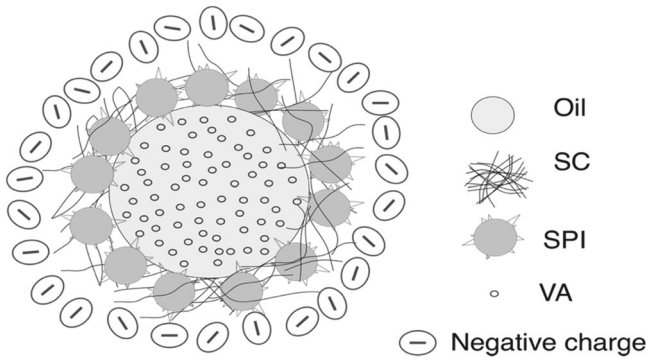

Figure 3. Schematic illustration of the soy protein isolates (SPIs) and sodium caseinate (SC) as stabilized droplet with the emulsion at pH 6.8 laden with vitamin A (VA).

different structures for two proteins is shown to be able to improve the stability of an emulsion when compared with a single protein (Liang et al. 2016; Ventureira, Martinez, and Añon 2012). In general, proteins can be charged either positively or negatively depending on the pH (Gentile 2020). Electrostatic interactions occur between two charged particles. These interactions are quantified by zeta potential measurements and depend on the particle charge (Purkait et al. 2018). Wang et al. (2020) clarified that combining corn protein isolates and soy protein isolates could improve the binding to the oil/water interface, thereby adjusting the microstructure and increasing the thixotropic properties of soy protein isolates introduced by the addition of corn protein isolates and increased interface of protein coverage. Besides, at pH 6.8, soy protein isolates and sodium caseinate were to bind interfaces between oil and water to form negatively charged and compact protein interface structures (Figure 3). Moreover, the particles size and charge of complex-stabilized protein emulsions may be altered by pH and ionic strength (Ruiz-Henestrosa et al. 2014). Further investigations on the influence of mixture ratio, concentration, and structure of PPI at oil/water interfaces are required to study the effect of molecular forces. Additional investigations are

needed to investigate the effect of molecular forces on mixture ratio, concentration and structure of proteins on PPI at oil/water interfaces.

Structural alterations of protein complex formation as a result of molecular forces

Protein complex molecular forces depend on interaction conditions, such as the unfolding degree (partial unfolding) of protein, agitation, pH, salt, protein concentration, ratio, and temperature (Hoyer et al. 2002; Jimenez et al. 2002; Pedersen et al. 2006; Pedersen, Andersen, and Otzen 2010). Covalent and non-covalent bonds play a major role in forming the unique network structure of a protein (Table 3). The molecular forces involved in the stabilization of a protein complex are non-covalent bonds, such as hydrophobic and hydrogen bonding and electrostatic attractions, and covalent bonds, such as disulfide bonds. The characteristics of protein include molecular weight and amino acid composition (Denavi et al. 2009). Rice proteins and whey protein isolates structures may interact with either secondary structures or peptide chains, but not with tertiary or other structures (Wang, Xu et al. 2019). By contrast, the structural interaction of rice proteins and soy protein isolates may occur at pH 12. Interchain hydrogen bonding can also play significant roles in the structural interaction of protein complexes produced by rice proteins and soy protein isolates, thereby indicating the charge occurs in the α-helix of the secondary structure (Wang, Xu et al. 2018) because the natural structure of α-helix is polar and is more flexible than β-sheet (Pelay-Gimeno et al. 2015). Consequently, a large effect is imposed on the secondary structure by pH based on hydrogen bonding (Figure 4). The literature shows that using a pH recycling process, in which rice proteins and caseins are combined in a solution and then the pH is adjusted to 7, increases the surface area and composition of protein complexes (Wang, Yue et al. 2018). Also, a study on the interaction and solubility of casein micelles and rice protein-based on nanomaterial pH recycling by Wang, Chen et al. (2019) resulted in the formation of nanonet derived from casein micelle with rice proteins of phosphorylated peptides through Ca^{2+} bridges and their molecular binding. Hence, pH could affect the unique protein structure formation and their synergistic interaction in aggregation.

Interfacial properties of protein complexes as a result of an interaction

As polymers, proteins have immense structural stability depending on their surroundings. The molecular protein structure is formed by different amino acids. Surface charge and hypomobility play major roles in the interfacial structure during PPI. This structure confers amphiphilicity in most proteins, therefore introduces surface action at the interfaces between air and oil and water (Noskov and Krycki 2017). In many PPI, proteins have net charges of the same sign and bind to complementary electrostatic interfaces. When the electrostatic interactions of protein occur completely at the interface, this leads to changes on the protein surface due to the pH of the surrounding and the occurrence of protein complexation on the interface (McCoy, Epa, and Colman 1997). In an alkaline environment, the negatively charged groups in proteins interact electrostatically and completely remove protons on the protein, resulting in limiting the hydrophobic interactions at pH 12 (Artigues, Iriarte, and Martinez-Carrion 1994). The net charge of proteins is not as significant as the distribution of their charge due to the long-distance character of electrostatic interactions and the influence of the heterogeneous dielectric constant (Klapetek 2018). Several studies have shown that rice proteins mixed with whey protein isolates and casein protein in a solution result in an interaction that increases surface charge, as indicated by hydrogen and electrostatic interaction obtained through pH recycling (Wang, Xu et al. 2019; Wang, Yue et al. 2018). A combination of bovine serum albumin and β-lactoglobulin proteins at the oil/water interface has weak electrostatic interactions because the net charge of the two proteins is practically null at the isoelectric point, hence resulting in a low desorption ability (Gálvez-Ruiz 2017). Interpreting potential interactions at the oil/water interface between two proteins requires a knowledge of the mechanism of desorption. Wang et al. (2020) showed that the combination of soy protein isolates and corn protein hydrolysates (under heat) has good emulsifying properties and is more stable. Soy protein isolates flow characteristics are adjusted and the interface protein complexes coverage of soy protein isolates are increased based on the emulsion. The altered structure of soy protein isolates indicates that corn protein hydrolysates helped soy protein isolates increase adsorption at the oil/water interface. These conditions could be due to the hydrophobic interactions between corn protein hydrolysates and soy protein isolates under heat. The increased ionic strength of the protein solution resulted in a condition that prevented the electrostatic interaction of loaded amino acid residues and to improve the hydrophobic interactions of the whole complex protein (Noskov and Krycki 2017).

The concentration and ratio of protein could affect the emulsion stability, and the increased ratio of protein in the solution may decrease the hydrophobicity of protein. For example, the surface charge increased with the reduction in the hydrophobicity of protein complexes of wheat gluten proteins and soy protein isolates after PPI. The hydrophobicity of protein complexes continues to reduce with the increase of soy protein isolates-wheat gluten proteins ratio (He, Wang, et al. 2020). The migration of additional protein in the oil/water interface of protein complexes resulted from hydrophobic interaction between proteins (Wang et al. 2020). Lin et al. (2019) proved that a protein new protein structure formed with enhanced surface charge as a result of disulfide bonds after PPI in the mixture of two different plant proteins (a combination of any two: soy protein isolate, peanut proteins, and rice proteins). This formation occurred due to a decrease in electrostatic adsorption

Table 3. Methods to identify and characterize the protein structure.

Methods	Detection principle	Observations	References
X-ray diffraction (XRD)	Secondary structure β-sheet	Measure the amount of α-helix and β-sheet	(Azevedo et al. 2015; Zhao et al. 2019)
X-ray photoelectron spectroscopy (XPS)	C, O, N, P, S, N/C, O/C, C–H, C = O, C–O/C–N, and C-C/C-H.[a]	Measure the compound of structure on the surface corresponding particle size, average around spheroidicity with average 45.9 and 29.0 nm in length and width, respectively.	(Zhao et al. 2019)
Fourier-transform infrared spectroscopy (FTIR)[b]	Secondary structure α-helix, β-sheet β-turn, and random coil	Measure the fraction of each secondary structure component (1,600 to 1,700 1/cm)	(Rumińska et al. 2020; Zheng et al. 2020)
Circular dichroism (CD)[c]	Secondary structure α-helix, β-sheet,	Measure the fraction of each secondary structure and observed the folding of the protein at 190–350 and 250–320 nm for near-UV CD and far-UV CD are, respectively.	(Bourbon et al. 2016)
Microscopy methods (SEM, AFM[e], TEM and SRM)[f]	Morphological characterization	Structural information, interleaving and/or exfoliation, flat ribbons, shape particle sizes between granules, polygons, and observed aggregation of complexes protein	(Adamcik et al. 2010; Pinotsi et al. 2014; Wang, Xu et al. 2019; Wang, Yue et al. 2018; Zheng et al. 2020)
Dynamic light scattering (DLS)	Determined the angle and particle size of protein	Hydrodynamic size and Correlation function of the protein at a range of 80–130 nm.	(Arnaudov et al. 2003; Bolisetty, Adamcik, and Mezzenga 2011)
Quartz crystal microbalance (QCM)	Measuring the change in frequency of a quartz crystal resonator and determining the quartz crystal resonator and nano/microgram in mass per unit.	Suitable for large molecular weight, range of (50–1,000 ng/mL) of protein surface	(Knowles et al. 2007; Kotarek, Johnson, and Moss 2008)
Fluorescence spectrum	Structure (a network structure, dispersed within the protein matrix)	Length of excitation (390 nm) and emission (400 to 750 nm) width of excitation and emission (5 nm)	(Muthukumarappan and Swamy 2018; Sun, Wang, and Guo 2018)
UV spectrophotometry	UV spectra are sensitive to protein conformations of protein solution. for instance, confirmational of protein folding, have a mutual relationship associated with an increase the absorbance at a wavelength of 230 nm	Spectra are acquired between 190 and 350 nm	(Liu, Avramova, and Park 2009)
Static Light Scattering (SLS)	Describe weak associations of proteins	Wavelength: 658.9 nm	(Ferreira et al. 2019)

[a]N/C is the ratio of nitrogen to a carbon atom and O/C is the ratio of oxygen to a carbon atom.
[b]Better with concentrated solutions, solid sample, or films.
[c]Suitable to diluted solutions with low content of protein solution.
[d]SEM = scanning electron microscopy, AFM = atomic force microscopy, TEM = transmission electron microscopy, and SRM = super-resolution microscopy.
[e]Average length and width is 45.9 and 29.0 nm.
[f]The thickness of protein sample at nm and length up μm.

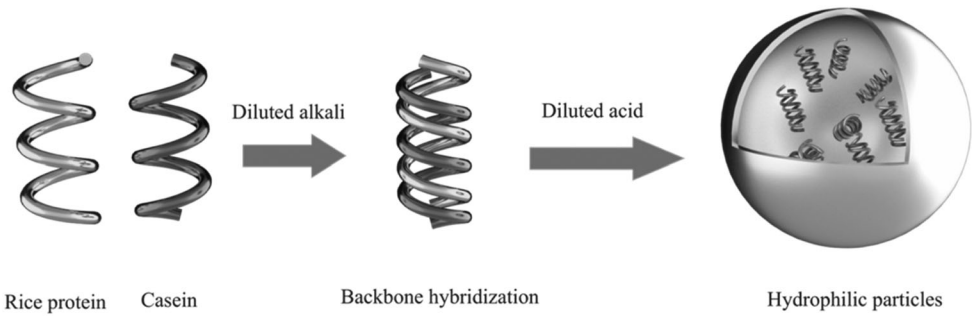

Figure 4. Schematic illustration of the protein-protein interaction mechanism between rice proteins and casein proteins to form protein hybrids.

resulting in binding on the oppositely charged protein's surface (Noskov and Krycki 2017). Also, low pH could increase the hydrophobicity of protein complexes. The hydrophobicity of complexed proteins increased as the pH reduced, hence serves as evidence of the occurrence of protein refolding. The decreased hydrophobicity refers to the formation of hydrophobic pockets, which lead to protein refolding (Hawe, Sutter, and Jiskoot 2008).

Inhibition of structure folding of protein through protein interaction

The heating condition can alter the folding of a protein, resulting in partial unfolding and thus a hydrophobic locus in a protein molecule. In many protein products, α-helices are a common motif among several commonly occurring secondary structures (Qin et al. 2020). The soy protein isolates and corn protein hydrolysates under heat showed that the hydrophobic interaction between proteins in solution is increased, thus indicating the aggregation of soy protein isolates (Wang et al. 2020). He, Wang, et al. (2020) clarified that the soy protein isolates merged into wheat gluten proteins, resulting in strong backbones of protein complexes due to less self-aggregation of soy protein isolates and weaker folding. The pH cycle is the best method of forming protein complexes and reducing folding (partial unfolding). When rice proteins and whey protein isolates were mixed at pH 12, their interiors were connected because the steric impediment had been removed and the development at pH 7 had decreased (Wang, Xu et al. 2019). This result can be attributed to increased interactions in protein complexes with a higher whey protein isolates in the wrapping of the unfolded structures in the protein bodies. Proteins may be unfolded and disulfide bonds may be broken with no hydrolysis of the peptide (Day and Golding 2016).

Characterization of protein structure

The identification and characterization methodologies of proteins are based on their distinctive structures. Several methods are used to check protein structures that are rich in α-helix and β-sheet, including circular dichroism (CD), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR). Table 3 describes these approaches along with relevant references. Transmission electron microscopy (TEM), electron microscopy scanning (SEM), atomic force microscopy (AFM), light-neutron X-ray scattering, X-ray photoelectron spectroscopy (XPS), and super-resolution microscopy (SRM), are commonly used to check for protein structure. Single-crystal XRD and cryo-electron magnetic resonance are used for solid-state cases. The molecular weight of the constituents is measured using mass spectrometry, such as LC-MS/MS, and gel electrophoresis. Protein fibrillation kinetics and scattering are measured by using intrinsic fluorophore and rheology, extrinsic dyes, fluorescence/spectrophotometer probe, dynamic light scattering (DLS), and quartz crystal microbalance (QCM). The fraction of protein can be measured using plasmonic chirality and AFM peak force.

XRD

XRD is used to measure β-sheet-rich and three-dimensional structures. It helps in observing the changes in the structures of protein complexes and single proteins. In most cases, the reflection index is around 4.8 Å. The interstrand distance of protein particles and the β-sheets that can be used to measure the refraction index is between 6 and 12 Å (Azevedo et al. 2015; Riek and Eisenberg 2016).

FTIR and CD

FTIR and CD are fast and straightforward methods for evaluating the secondary structure of proteins. FTIR spectra are also capable of measuring the fraction of secondary structure proteins, such as amide I (1,600–1,700 1/cm), α-helix (1,650–1,660 1/cm), β-sheet (1,600–1,639 1/cm), β-turn (1,661–1,699 1/cm), and random coil (1,640–1,649 1/cm), through quantitative analysis. Consequently, we can observe the change in the amount of α-helix and β-sheet, thus allowing us to measure the folding of protein structures. CD is suited for dilute solutions with a low content of aromatic residues, whereas FTIR works better with concentrated solutions, powders and films (Hammarström, Lindgren, and Nilsson 2013). FTIR and CD spectra show a clear peak in the range from 1,611 to 1,630 1/cm and ∼218 nm, respectively, in their evaluation of β-sheets. In some cases, β-sheet-rich proteins, such as β-lactoglobulin and transthyretin, occur and thus need to be further analyzed. The differences between simple proteins and proteins complexes can also be studied by using an algorithm based on CD, which can estimate the α-helix and β-sheet of the secondary structure of proteins by orienting the β-sheets either parallel or antiparallel and by twisting the antiparallel β-sheets in different ways, such as right-hand twisted, left-hand twisted and relaxed, along the portion of each protein structure (Micsonai et al. 2015).

Microscopy

Microscopy methods, such as SEM, TEM, and AFM, can provide structural information on protein complexes. TEM is used to show the interleaving and/or exfoliation of protein complexes and whey protein isolates (Azevedo et al. 2015). TEM provides a vastly improved resolution of atomic dimensions less than 1 nm, approximately one to two times better than SEM. However, the sample preparation process for TEM is tedious and the analysis time is long. Nonetheless, TEM was widely utilized to analyze particle morphology of protein (Gibson, Khanal, and Zubarev 2007). TEM analysis applications extend further than clear morphology of particles. It can produce a 3 D reconstruction of intracellular frameworks at resolutions of $3-8$ nm (Robinson, Sali, and Baumeister 2007; Volkmann 2010), hence providing knowledge regarding the topology, morphology, and composition of proteins.

Recently, many researchers are investigating the microstructure of protein complexes using SEM (Wang, Xu et al. 2018, Wang, Xu et al. 2019; He, Hu, et al. 2020). SEM shows that amyloid fibrils have a diameter ranging from 2 to 7 nm with approximately 2 to 8 protofilaments that often twist into flat ribbons (Chiti and Dobson 2017; Lara et al. 2011). Wang, Xu et al. (2019) demonstrated that whey protein isolates and rice proteins after structural interaction led to the formation of particle sizes between granules, polygons, and the reduced aggregation of protein complexes between individual protein structures was observed by using SEM.

Table 4. Methods to identify molecular forces of protein.

Methods	Detection principle	Comment	References
Fluorescence spectrum	Hydrogen bindings, hydrophobic reactions, electrostatic interactions	Thiourea[a], SDS[a], NaCl[a]	(Shigemitsu et al. 2018)
Dynamic light scattering (DLS)	Disulfide bonds	SH content mixing with proteins[b]	(Wu et al. 2020)
Static light scattering (SLS)	Hydrophobic, electrostatic, and Van der Waals forces and hydrogen bonds	ΔS and $\Delta H > 0$, hydrophobic forces are dominant ΔS and $\Delta H < 0$, Van der Waals forces and hydrogen bonds are dominant $\Delta S > 0$ and $\Delta H < 0$, electrostatic forces are dominant.[c]	(Mogridge 2015)
Isothermal titration calorimetry (ITC)	Electrostatic interaction Hydrogen bond	Determination of the binding affinity, stoichiometry, entropy, and enthalpy of the binding reaction in a protein solution	(Zheng et al. 2020)
Fourier-transform infrared spectroscopy (FTIR)	Hydrogen bond	Fourier deconvolution range from 3,000 to 3,700 1/cm. Hydrogen bonds at a range between 1,618 and 1,624 1/cm.	(Kubo and Kadla 2005; Yang et al. 2016; Bunsell 2018)
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	Disulfide bonds	Determination cysteine residues S-rich prolamins, such as a-type and gamma gliadins and low molecular weight (LMW), form intra-inter chain disulfide with cysteines in high molecular weight subunits and other LMW subunits.[d]	(MacRitchie 1987; Shewry and Tatham 1997; Shewry et al. 2003)
Size-exclusion chromatography-high performance liquid chromatography (SEC-HPLC)	Disulfide bonds	Sulfhydryl group mixing with proteins.[b]	(Wu et al. 2020)

[a]Material as representing each force (10 mM).
[b]Sample has a high amount of sulfhydryl group.
[c]ΔH is the enthalpy change and ΔS is the entropy change.
[d]Break disulfide bonds into N- and C-terminal regions.

AFM was created to overcome the restrictions of scanning probe microscopy methodology. Initially, this technique has been used to image surfaces and atomic resolution of protein. In AFM, atomic sample resolution is achieved by measuring small forces produced throughout a surface using a sharp probe placed on an elastic cantilever (Whited and Park 2014). This technology can show the protein complexes produced from PPI. Wang, Yue et al. (2018) explained the addition of casein to rice proteins can result in protein aggregation, forming a large (ranging between 116 and 207 nm) hybridized protein complexes that were observed using AFM.

Determination the molecular forces governing in PPI

Several methods are widely used to measure the contribution of molecular forces, including fluorescence spectrum, DLS, static light scattering (SLS), isothermal titration calorimetry (ITC), FTIR, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and size-exclusion chromatography-high performance liquid chromatography (SEC-HPLC). The identification of these molecular forces using the aforementioned methods is summarized in Table 4.

Fluorescence spectrum is suitable to study molecular forces in protein solutions. The principle of the fluorescence spectrophotometer probe is based on the measurement of the level of emission and excitation are 300–450 and 280 nm, respectively. Non-covalent bonds, such as electrostatic interactions, hydrogen bonding and hydrophobic interaction, in protein solutions during PPI are determined using 10 mM NaCl, thiourea, and sodium dodecyl sulfate, respectively. The fluorescence sintering result based on the excitation of the interactions increasing the strength of fluorescence refers to the governing the protein interaction. Meanwhile, the contribution from disulfide bonds in protein can be measured from the total free sulfhydryl group based on the Ellman's reagent solution at the absorbance at 412 nm using the following equation:

$$\text{SH group } (\mu\text{mol/g protein}) = \left[(A_1 - A_2 - A_3)/(13,600 \times b \times c)\right] \times 10^6$$

where, A_1 is the absorbance protein sample, A_2 is the absorbance of the Ellman's reagent blank, A_3 is the absorbance of the blank sample, c is the protein concentration of protein solution (g/L), b is the length of the light path (cm), and 13,600 is the molar extinction coefficient of Ellman's reagent. The FTIR spectrum can also be used to measure the hydrogen bonds at amide I (1,618 − 1,624 1/cm).

SLS can be used to study molecular forces by measuring the thermodynamics value of protein according to the Van't Hoff equation. The thermodynamics value of PPI can be determined based on the enthalpy change and entropy change of proteins using the following equations:

$$\Delta G = \Delta H - T\Delta S$$
$$\Delta G = -RTInKa$$

where, ΔG is the free energy change, ΔH is the enthalpy change, ΔS is the entropy change, R is the universal gas constant, T is the absolute temperature, and Ka is the acid dissociation constant. In cases where both ΔS and ΔH are more than zero, these indicate that hydrophobic forces are

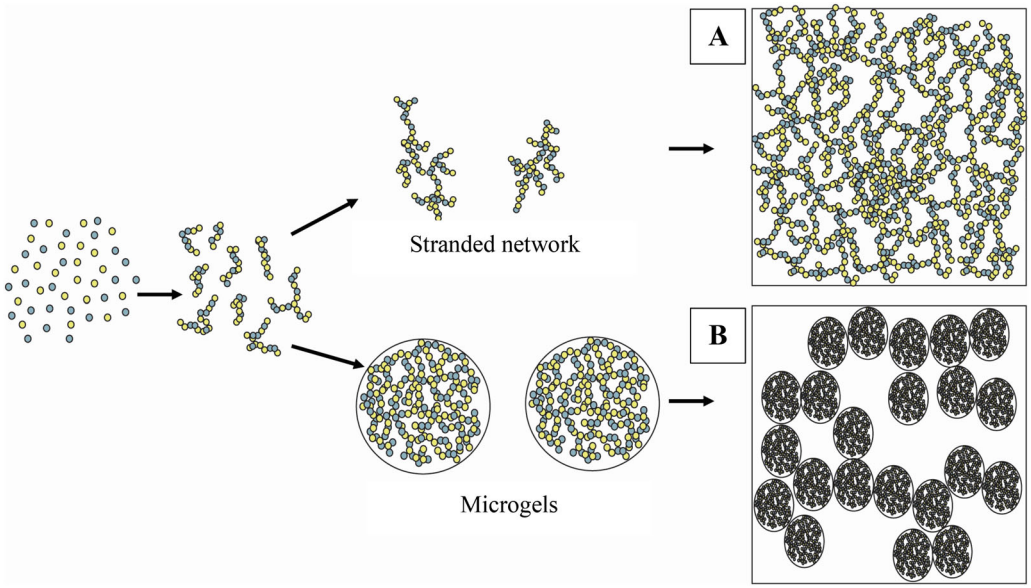

Figure 5. Schematic illustration of the gelation mechanism of binary mixture of globular protein formed as a result of protein-protein interaction. Gelation could be derived from crosslinking of (A) finely stranded networks or (B) microgels.

the dominant forces in the protein complexes. In contrast, when both ΔS and ΔH are less than zero, the dominant forces are Van der Waals forces and hydrogen bonds. Meanwhile, for cases with ΔS more than zero and ΔH less than zero, electrostatic forces are the predominant in the PPI (Mogridge 2015).

Effect of PPI on functional properties of protein

Functional properties of proteins are based mainly on their structure and ability to interact with other food materials and rely primarily on their source (Cao and Mezzenga 2019). In general, animal proteins have higher functional properties, such as solubility, foam and emulsion, when compared with plant protein. Hence, plant proteins have limited use in the food industry (Jansens et al. 2019), even though they have more types of amino acids than animal protein (Young and Pellett 1994). Some studies have shown that PPI could be used to enhance the functionalities of protein, such as gelation, emulsification, and solubility.

Gelation

Protein gelation is considered a type of decentralization of the processes, whereby formation of a network structure occurs under specific conditions depending on factors, such as temperature, pH, ionic interaction, protein concentration, and interaction with other food components (Lin et al. 2017). Gels of globular proteins, such as soy and whey proteins, can be formed by disulfide bonds between neighboring molecules and covalent bonds through various mechanisms, such as solvent condition pressure and temperature (Day and Golding 2016).

Figure 5 shows the illustration of the effect of different types of globular proteins mixed under heat. This phenomenon leads to the growth and formation of fine-stranded networks or microgels, which subsequently may lead to crosslinking. The types and concentration of protein may affect gelation during PPI via disulfide, ionic, and hydrogen bonds and hydrophobic interaction. Disulfide bonds and hydrophobic interactions have a greater effect on gelation through PPI compared with a single protein structure (Lin et al. 2019). Therefore, in an aqueous medium, a single protein is normally stabilized by electrostatic repulsion between the charged protein, which limits short-range attractive interaction. Hence, PPI can increase the contribution of non-covalent forces (e.g., hydrophobic and hydrogen bond) and steric repulsion forces to stabilize the protein complexes. A study conducted by Nicolai (2019) showed that when the pH is set around the isoionic point or when salts are added, the contribution from electrostatic forces is ceased due to the presence of charges on the proteins at isoionic points. These proteins continue to possess the very same amount of positive and negative charges, hence the electrostatic attraction between the positive and negative charges could lead to the increase in the contribution of disulfide, hydrogen bonds, and hydrophobic interaction.

The molecular driving forces (hydrophobic interactions and disulfide bonds) through PPI are greater than other molecular forces under specific heating, thereby affecting the unfolding (partial unfolding) of the tail myosin molecule, exposing hydrophobic amino acids and sulfhydryl groups to the polar environment and leading to intermolecular aggregation through hydrophobic forces and disulfide bonds (Gill and Conway 1989; Ko, Yu, and Hsu 2007; Zhang et al. 2013). This characteristic could be the reason for the reduced ability to create tense network structures relative to plant and animal proteins in mixed plant protein gels.

Protein gels may also be formed through non-covalent bonds, such as van der Waals forces, hydrogen bonds and electrostatic interactions (Sun, Wang, and Guo 2018). Researchers have studied the interactions between polymerized whey protein and soybean lecithin depending on the effect of non-covalent bonds and found that the main factor

in protein gel formation is hydrogen bonds (Nicolai, Britten, and Schmitt 2011; Sun, Wang, and Guo 2018), which also contribute to the increased size of protein particles. Electrostatic attractions also play a role in the formation of large particles through the binding of protein interactions (Sun, Wang, and Guo 2018). In thermoreversible cases, hydrogen bonds play the main roles in gelation (Lin et al. 2019).

Emulsification

Emulsifying properties of proteins derive from their interface structure (Gálvez-Ruiz 2017). Emulsion science and technology have been extremely important in the making of food products, such as milk, milk tea, walnut (or peanut) milk, cream, nutritional drinks, and sauces (Wang et al. 2020). Interest in combining plants with animal proteins or in the emulsification of plant proteins instead of animal proteins is growing (Karaca, Low, and Nickerson 2011; Wan, Guo, and Yang 2015). However, mixed-protein stable emulsions tend to be sensitive to extreme conditions. For instance, pea protein with casein protein-stabilized emulsions formed solid gels after being heated at 90 °C for 15 min (Liang et al. 2016). Some peptides are capable of changing the conformation of proteins, leading to changes in gel properties and aggregation (Kosters, Wierenga, and Gruppen 2010). A previous study by Ye, Lo, and Singh (2012) showed that the structure of a lactoferrin-stabilized emulsion can be modified with the aid of milk protein (caseinate or whey protein isolates) due to the electrostatic interaction under pH 7. The pH plays significant roles in altering non-covalent electrostatic interactions in PPI and the formation of stabilized emulsion protein than single proteins.

Ji et al. (2015) studied the structure and function relationship between sodium caseinate and SPI at oil-water interfaces based on the PPI. It was proven that electrostatic forces governing protein interaction between sodium caseinate and SPI, leading to its connection at the oil-water interfaces to form negatively charged compact interface structures at pH 6.8 (Figure 3). During complexation at alkaline condition, the amino groups of proteins were neutral (NH_2), while the carboxyl groups were negatively charged (COO^-). Thus, resulting in limiting the hydrophobic forces and hydrogen bonds. Meanwhile, at pH less than the isoelectric point, the amino groups became positively charged ($NH3^+$) and carboxyl groups became neutral (COOH), which led to the increased surface charge and hydrophobicity of protein complexes. Therefore, the negative strength of surface charge decreased. These protein complexes provide a more stable emulsion than those stabilized by a single protein. Hence, the stabilization of the emulsion by protein complexes is not due to electrostatic interaction, but rather it was due to steric stabilization.

There could be a strong electrostatic attraction in the emulsion between positive packs on one droplet and negative patches on another droplet, resulting in emulsion gels stabilized by PPI. This phenomenon occurred because the predominant non-covalent forces on the protein-coated droplets in the emulsion stabilized by PPI reduced the electrostatic repulsion between the droplets (Teo et al. 2016). Recently, Yan et al. (2019) showed that complexation of whey protein isolates with lactoferrins can control the rheological properties of the emulsion gels of protein complexes by varying the pH through non-covalent bonds such as electrostatic interactions between droplets.

Solubility

In many cases, solubility plays a key role in food processing and food product development (Wang et al. 2015). The pH can contribute to the change in the solubility and surface charge of proteins and thus their interactions, with hydrophobic and electrostatic interactions being the main contributors. Several physical methods have been applied to improve the solubility of rice protein (Wang, Yue et al. 2018). However, the effects were not satisfactory, and it consumed excessive energy. Hydrogen bonds play a more significant role than hydrophobic and ionic interactions in enhancing the solubility of rice proteins after complexation with whey protein isolates (Wang, Xu et al. 2019). Wang, Xu et al. (2019) found that a mixture of 1% rice proteins and whey protein isolates at a ratio of 1:1 increased the solubility to over 50% through synergistic interaction among intermolecular forces (hydrogen bonding, electrostatic interaction, hydrophobic interaction, and ion strength forces) on protein complexes, with the contribution of hydrogen bonding being greater than that of other molecular forces. The mechanisms of structural interaction of the protein molecules in solution depend on pH-recycling. For instance, in an alkaline environment of pH 12, the negatively charged groups in proteins interact electrostatically and subsequently remove protons from the proteins. This phenomenon limits ionic and hydrophobic interactions (Artigues, Iriarte, and Martinez-Carrion 1994). Meanwhile, during the neutralization process, ionic interaction, hydrogen bonds, and hydrophobic interaction increased during the PPI. These forces play an important role in the creation of protein complexes.

The solubility of wheat gluten proteins was increased to over 72.4% with the addition of soy protein isolates based on the pH cycle, whereby hydrogen bonds contributed more than non-covalent bonds, such as electrostatic and hydrogen interactions (He, Wang, et al. 2020). Wang, Xu et al. (2018) reported that a mixture of 1% rice proteins and soy protein isolates in an alkaline environment for 1 h at room temperature, followed by neutralizing the protein solution to pH 7.0, increased the solubility to over 80% through synergistic interaction among certain intermolecular forces (hydrogen bonding) between protein complexes, as indicated by pH recycling. The structural interaction causes the backbone to be resistant to refolding. As in alkaline conditions, the protein composites maintained considerable surface characteristics. Wang, Yue et al. (2018) showed that when rice proteins were mixed with casein proteins in a solution, followed by pH adjustment to 12 and then to 7, the solubility of the rice proteins increased to over 90%. Consequently, the rice proteins with other proteins, such as whey protein isolates, soy

protein isolates, and casein, can interact under alkaline conditions to form new structures and enhance solubility.

Conclusions

Both plant proteins, such as soy protein isolates, rice proteins, wheat gluten proteins, corn protein hydrolysates, and peanut protein isolates, and animal proteins, such as lactoferrins, caseins, whey protein isolates, and collagen, are widely used in food industries. Concentration, temperature, ratio, ionic strength, and pH can contribute to the binding of molecular forces on proteins and in the folding of proteins. A strong relationship exists between the molecular forces and the structure of a protein and their interaction based on the interface of protein and a solution via polypeptide backbone through non-covalent hydrogen bonds, hydrophobic interaction, and electrostatic interaction, in addition to covalent disulfide bonds. In most cases, the structural interactions of PPI lead to a novel structure of protein complexes, thus enhancing the functional properties of proteins, such as solubility, emulsion, and gelation. Hydrogen bonding plays an important role in enhancing the solubility in PPI based on pH cycle. Several simple methods, such as FTIR and CD, are used to identify the protein structure (secondary structure rich in α-helices and β-sheets) and molecular forces of protein complexes. These methods can measure the components of protein structures and the change degrees of α-helices and β-sheets of secondary proteins, which indicates the degree of folding of protein structures.

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Conflicts of interest

There are no conflicts of interest.

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