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Protein-Water Interactions and Functional Properties

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ABSTRACT

Hydration or rehydration is the first and perhaps most critical step in imparting desired functional properties to proteins in a food system. Water that interacts with the protein molecule exhibits different properties from those of "free" water. The types of water in protein-food systems are described in terms of structural, monolayers, unfreezable, hydrophobic hydration, imbibition or capillary condensation, and hydrodynamic hydration water. Protein functional properties such as swelling, solubility, gelation, water holding capcity, etc., are directly related to the manner in which the protein interacts with water. Methods for studying the protein-water interaction are discussed. The primary protein-water interaction is believed to take place at various water binding sites on the protein molecule. Theories that explain the mechanism of action of these different water binding sites are reviewed. Factors which affect the protein-water interactions include the number and nature of the binding sites on the protein molecule, protein conformation, plus environmental factors such as pH, salt, temperature and others. Finally, the protein-water interaction phenomenon and the physico-chemical and functional properties of proteins in protein isolate systems (dehydrated, solution, and gels) and in protein food systems are briefly examined.

INTRODUCTION

A number of protein isolates and concentrates have been developed from plant, animal and microbial sources to serve as functional ingredients in a wide and ever growing range of food applications. The successful use of these protein ingredients depends upon their abilities to fulfill one or more specific functional requirements, e.g., emulsion stabilizer, foam stabilizer, gel structure, etc.

Many of these protein ingredients are in the dehydrated form. However, since they are not generally functional in the absence of a liquid water phase, the first and perhaps most critical step in imparting their desired functional property to the food system is their interaction with water to rehydrate, swell and/or solubilize them. The chemical, physical and functional properties of the protein in a particular food system are dependent to a large degree upon rate and extent of hydration or rehydration. Further, the ability of the proteins to bind and immobilize water is itself one of its most important functional properties in most food applications. The nature of the protein-water and protein-protein interactions is critically important in determining whether the protein will function in the food system as a colloidal dispersion, gel or insoluble precipitate.

In this paper we will review methods for studying protein-water interactions, the basic nature of these interactions and their relation to certain functional properties.

EXPERIMENTAL TECHNIQUES FOR STUDYING PROTEIN-WATER INTERACTIONS

The interaction of water with proteins and polypeptides has long been the subject of investigation (1-7). The properties of the water "bound" to a macromolecule differ from those of the "free" or "bulk" water. Binding causes a decrease in vapor pressure and chemical potential of the water and may be accompanied by decreases in enthalpy, entropy and volume of the system. It also increases the hydrodynamic volume of the protein molecule and thus decreases the density of the hydrated protein, vis-a-vis, the unhydrated particles. Kinetic properties of bound water molecules also undergo change, as they rotate or translate more slowly than those in the bulk solvent. These changes in the thermodynamic and kinetic properties of water are reflected by shifts in infrared, Raman and magnetic resonance spectra.

The term, "bound," and the amount of water to be so designated are still subjects of controversy and dependent on the experimental measurement techniques employed (8). The total water associated with the protein also includes physically held water, i.e., capillary water or water held within a protein gel matrix. This water is not "bound" according to many of the definitions but is nevertheless restricted and difficult to remove from the material. Therefore, hydration of the protein molecule by interaction with water is still thought of in operational terms, and there appear to be many approaches to its operational definition (4). In this paper, we will use "bound" as a phenomenal term without implying that a specific quantity of water is

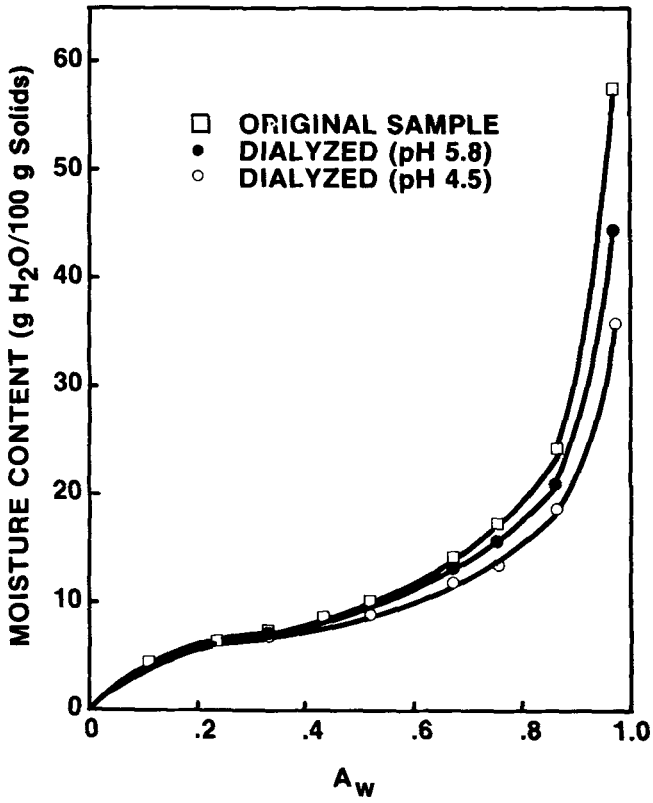

FIG. 1. Effect of dialzable solute and pH on water sorption of soy proteins.

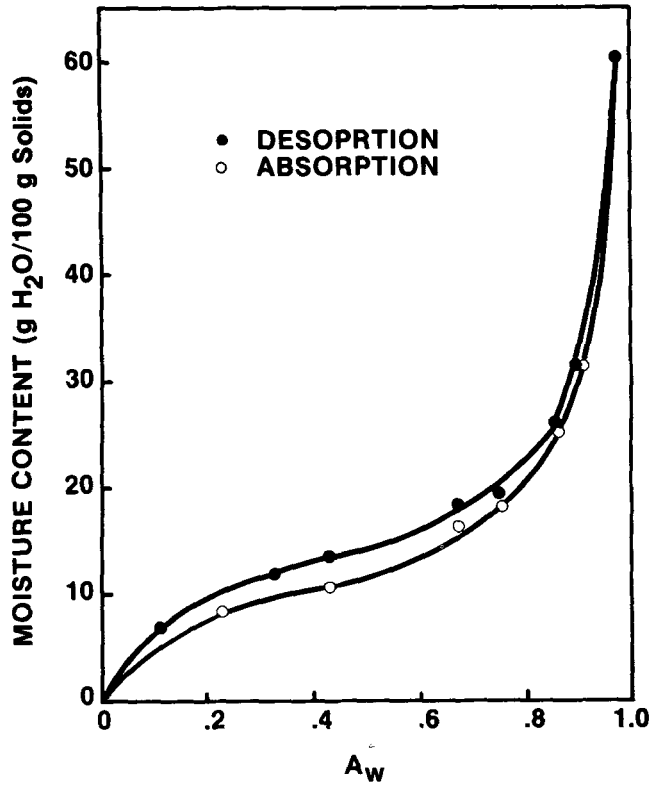

FIG. 2. Water sorption isotherms of soy protein isolates.

described.

Methods for studying protein-water interactions are numerous and may be classified as: thermodynamic, kinetic, spectroscopic, and diffraction (4).

Thermodynamic Methods

Changes in enthalpy, entropy, free energy, activity, freezing point and boiling point of water are useful in studying the protein-water interaction mechanism under equilibrium conditions. The water sorption-desorption isotherm technique (Fig. 1) is perhaps the simplest method in this group. It enables one to estimate degree of protein hydration as a function of A_W, and thermodynamic heat of sorption as a function of moisture content.

Experimental methods available for studying water vapor sorption of food substance have been reviewed by Gal (9). The most commonly used method is the gravimetric approach for determining weight changes of the protein system at equilibrium with different water vapor pressures. Data are normally plotted as grams of water per gram of protein or moles of water per mole of amino acid residue vs. water vapor pressure, or A_W. The water vapor sorption isotherm for proteins and most biological material is sigmoidal Type II, as classified by Brunauer et al. (10). It can be roughly separated into three regions (Fig. 2): 1) between 0-0.3 A_W, the moisture content increases rapidly with incremental increased in A_W, representing the adsorption of monolayer water; 2) between 0.3-0.9 A_W, there is a slower increase in water content with incremental increase in A_W, the water absorbed usually unfreezable; and 3) for A_W's above 0.90, water content again increases sharply with incremental A_W increases. The water in this region is imbibition and capillary water. Hysteresis usually takes place throughout the intermediate A_W range, e.g., the adsorption isotherm curve is positioned below the desorption (drying) curve.

Although there have been many mathematical equations developed to describe the isotherm, as well as the state of water in protein-water systems, these equations generally follow a common form. Regardless of the theoretical starting point, many of the isotherm equations can be rearranged to yield two constants. The first is strongly correlated with the number of water binding sites on the protein molecules, whereas the second constant is proportional to the strength of the binding (4).

The "isosteric" heat of absorption ($\Delta\bar{H}_A$) of protein is about -16 Kcal/mole at very low water coverage (11), but it changes rapidly in the vicinity of the monolayer water value and then slowly appraoches the values for the vaporization of pure water as moisture content further increases (Fig. 3). Similarly, the differential entropy ($\Delta\bar{S}_A$) values are in the range of -40 to -45 eu at low A_W but approach values of -28 eu at high A_W conditions (4,11). Enthalpy of sorption provides an indication of binding strength of water molecules to the protein and and provides useful information bearing on the energy balance for drying the protein. Calorimetric methods permit evaluation of changes in the melting and vaporation characteristics of ice or water in a protein-water system. This method enables us to establish phase changes, energy relationships and the fraction of unfreezable water (12).

Kinetic Methods

These methods determine the mobility of water in the immediate vicinity of proteins or the mobility of the protein molecule as affected by its interacting with water molecules. One well known method is based upon the principle that the association of water with a protein molecule alters its hydrodynamic properties, e.g., density, volume and shape. Thus, viscosity and frictional coefficient data can be used to determine the amount of water associated with the protein molecule (7). A variety of techniques, nuclear magnetic resonance (NMR), dielectrics, laser light scattering and intrinsic viscosity, have been used to investigate changes in relaxation rates of protein molecules as a function of hydration (5, 13, 14). Tracer diffusion

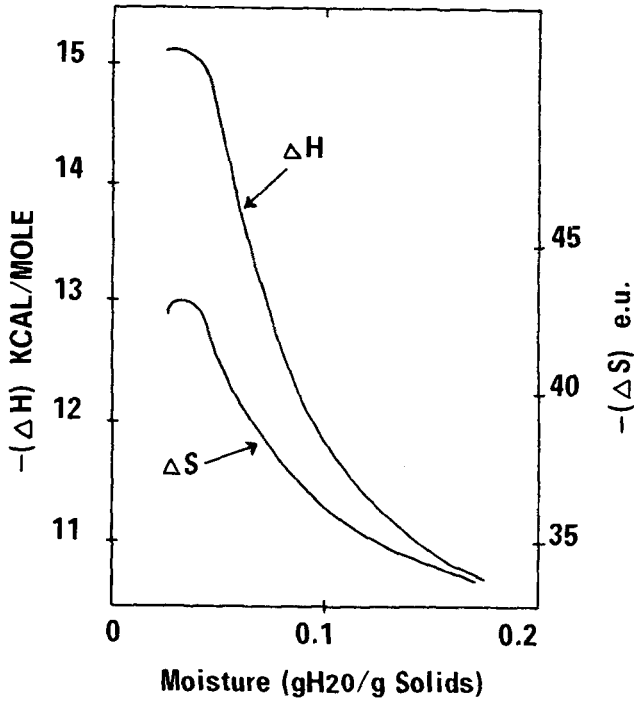

FIG. 3. Differential enthalpy and entropy functions at various water contents for soy protein concentrates (11).

methods have been used to determine changes in translational diffusion rates of water molecules in protein-water systems (15). A number of researchers have recently used NMR to follow changes in the mobility of water molecules and to define the state of water in protein-water systems (4). Information concerning the transfer of water between different states can also be obtained.

Spectroscopic Methods

Changes in the spectroscopic properties of water are used to reflect the hydrogen bonding environment of the water molecule. Infrared (IR) and RAMAN spectroscopy are useful in evaluating the nature and strength of the protein-water hydrogen bonding (16). NMR has been used to determine the amount of unfreezable water in protein systems (17).

Diffraction Methods

These techniques provide information on the average position and orientation of water molecules with respect to each other and to the protein molecule. Light scattering and small angle X-ray scattering techniques provide information about the density and concentration fluctuations of water molecules in solution and in the vicinity of the protein molecule. High resolution X-ray and neutron diffraction techniques are capable of locating regions of structural water in the vicinity of the protein molecule.

It is expected that each experimental method relates to different properties of the water or protein molecule and determines molecular processes that occur at different time scales (Fig. 4). For example, the approximate rotational correlation time of "bulk" water is in the range of 10^{-12} sec, and those of strucutral or monolayer water are about 10^{-6} sec (18). It should also be mentioned that each of these methods possesses inherent limitations and uncertainties. In many cases background contributions from protein and "bulk" water are difficult to separate from the reaction of interest, and, therefore, only qualitative information is obtainable. However, each of the experimental techniques provides mutually supporting information with respect to location, dynamics and energy of water molecules associated with proteins. By properly selecting data

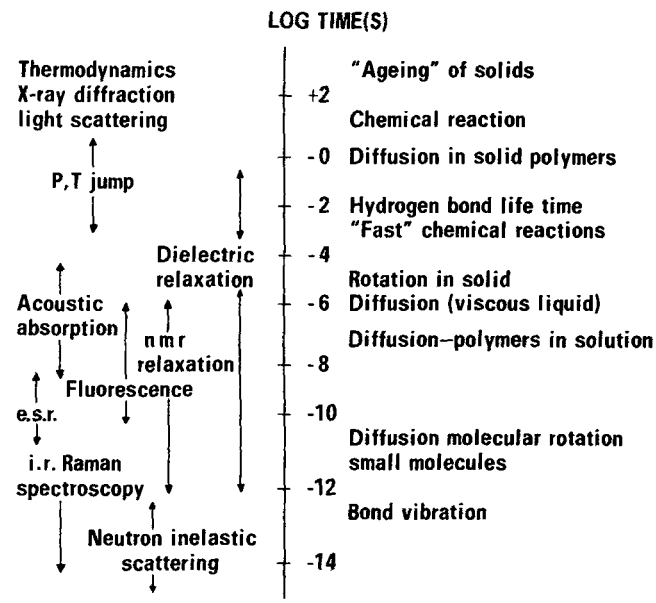

FIG. 4. Time scales of molecular processes and application ranges of experimental techniques (23).

from these different techniques, it is possible to gain a reasonably accurate description of the state of water in the protein-water system.

SITE AND STOICHIOMETRY OF PROTEIN-WATER INTERACTION

Based on theoretical considerations, researchers have attempted to determine details of the mechanism for protein-water interaction and the number of water molecules bound per protein molecule. In order to predict the water binding properties of a protein, one must ascertain the water binding properties of each binding site. Various appraoches have been used to investigate this latter relationship. For example, one may selectively "block" specific binding sites by appropriate chemical modification (19) or by selectively removing binding sites through deamination reactions (20). Problems associated with these approaches are the uncertainties of the roles of the new and modified binding sites which may also alter the protein's conformation and its ability to interact with water. Another approach is to determine the water-binding characteristics of various chemical groups on synthetic polymers (21). Problems here include uncertainties due to possible steric factors caused by conformational rearrangements on the polypeptide (synthetic and protein), including folding and aggregation which might alter their hydrophilic group's ability to bind water.

Despite these difficulties, the stoichiometry of protein-water interaction has been explored by several researchers. Pauling (22) found that each polar group on a protein molecule binds one water molecule with the exception of carboxyl groups that are hydrogen bonded to an imido group of glutamine or asparagine. A comparison of the number of polar groups and the "monolayer water" is presented in Table I. This generally covers A_w from 0 to about 0.3. Leeder and Watt (23) attempted to determine the number of water molecules bound per protein polar amino acid group by systematically removing known amounts of specific binding sites and empirically determining the water binding capacity of each group as a function of A_w (Table II). They further attempted to develop water-binding isotherms for several proteins by assigning binding capacity values to each binding site. Good agreement was obtained for these protein water-binding isotherms at low A_w, but actual water binding capacities were higher than theoretical values calcualted for a given protein at high A_w levels. It is

TABLE I

Comparison of the Number of Water Molecules Held by Proteins in Initial Adsorption and the Number of Polar Groups in the Proteins (22)

Protein	Water adsorbed in first layer (moles/10^5 g)	Number of polar groups (moles/10^5 g)	Total reported amino acids
Silk	226	219-228	107.0
Ovalbumin			
crystallized	329 342 344	277-313	75.7
lyophilized	314		
heat denatured	276		
Wool	366	303-341	71.4
Gelatin, collagen	485 529	328-609	108.8
C-zein, B-zein	210 228	305-390	106.0
Salmine	592	611-707	110.5
Serum albumin	374	424-424	86.8
β-Lactoglobulin			
crystallized	370	472-508	115.8
lyophilized	329		

TABLE II

Water Associated with Hydrophilic Groups in Proteins (23)

Sorption site	Moles H_2O per mole of sorption site at RH of: 10%	20%	50%	80%
-COOH	0.92	1.2	2.0	2.5
$-NH_2$	0.83	1.2	2.1	2.7
-OH (aliphatic)	0.09	0.17	0.34	0.60
-OH (phenolic)	0.25	0.5	1.0	1.8
-CO-NH-	0.06	0.11	0.25	0.56

TABLE III

Water Molecules Associated with Hydrophilic Groups (24)

Hydrophilic groups	Number of water molecules bound
H_2O	4
-OH	3
-COOH	4 - 5
$-NH_2$	3
N^2	1
= 0	2
NH	2

TABLE IV

Water Binding Capacity of Amino Acids in Synthetic Polypeptides by NMR (21)

Amino acid residues	Hydration[1] (mole H_2O/residue)
Ionized polar	
Asp−	6
Glu−	7
Tyr+	7
Arg+	3
His+	4
Lys+	4
Non-ionized polar	
Asn	2
Gln	2
ProPro	3
Ser, Thr	2
Trp	2
Asp	2
Glu	2
Tyr	3
Arg	3
Lys	4
Nonpolar	
Ala	1
Gly	1
Phe	0
Vla	1
Ile, Leu, Met	1

[1]The water count assigned to each amino acid residue includes water bound to one peptide bond.

postulated that the discrepancy in these values may be due to the formation of water multilayers and condensation of liquid water on to the protein molecule. Sponsler et al. (24) previously employed a similar approach to determine the water binding capacity of gelatin as a function of water content using the mximum water binding capacity of the various polar groups (Table III). They reported that at 15% water content levels each protein molecule binds approximately 260 water molecules and that the distance between amino acid side chain spacings increased from 10.4 to 11.3 Å due to this binding phenomenon. Also, these spacings increased from 11.3 to 13.0 Å as the water binding increased to 33%.

Kuntz (17, 21) used NMR to investigate the degree of hydration of protein molecules and synthetic polypeptides. The information was then used to assign a specific number of nonfreezing water molecules to each amino acid residue which were classified into three groups: 1) polar amino acids with ionized side chains which bind the greatest amount of water; 2) nonionized amino acids which bind an intermediate amount of water; and 3) hydrophobic groups which bind little or no water (Table IV). From these findings, he derived an approximate formula:

$$A = f_c + 0.4\ f_p + 0.2\ f_n,$$

where A is grams of bound water per gram of protein, f_c is the fraction of charged amino acid side chains, f_p is the fraction of polar amino acid side chains and f_n is the fraction of nonpolar amino acid side chains.

As to the sequence of water binding, Speakman (25) suggested that water binding occured at polar side chains under low A_w conditions (in the order of amino, carboxyl and hydroxyl groups), followed sequentially by binding by peptide linkages at intermediate A_w and finally by the formation of multilayers at higher A_w.

In conclusion, there is little doubt that the primary protein-water interaction occurs at polar amino acid sites on the protein molecule. Each of the different polar groups will have their own characteristic water binding affinity and capacity. The stoichiometry proposed by Pauling represents only the monolayer water. The value reported by Bull and Breese (six water molecules per polar groups at $A_w = 0.92$) is an empirical regression constant with no specific physical meaning (26). However, it is interesting to note that the water binding capacity at $A_w = 0.92$ roughly equals the quantity of unfreezable water as determined by NMR and calorimetry (Table V).

TABLE V

Protein Hydration of Different Sources (12,21,22,26,59)

	BET Monolayer	A_w=0.92	Unfreezable water	
			NMR	Calorimetry
	(g H_2O/100g protein)			
Collagen	9.52	45	50	35
Casein	5.47	40	39[a]	43
Lactoglobulin	6.67	32	---	55
Ovalbumin	5.65	30	33	32 (33)[b]
Serum albumin	6.73	32	40	32 (33)[b]
Hemoglobin	---	37	(42)[b]	32 (34)[b]
Myoglobin	---	42	42	---
Soy protein	5.80	33	35[a]	

[a]Calculated value using Kuntz's prediction.
[b]Denatured conformation.

FACTORS AFFECTING PROTEIN-WATER INTERACTION

Number and Nature of Hydration Sites

Polar amino acid groups are the primary sites for protein-water interactions. One must consider whether these polar sites are cationic, anionic or nonionic. According to Kuntz (21), cationic, anionic and nonionic binding sites bind different amounts of water molecules. An equally important consideration is whether the protein's conformation permits these binding sites to be sterically available for interaction with water. For example, they may be freely exposed to the water environment under certain protein conformational states, they may interact with other amino acid groups on the protein or they may be buried within the interior region of the protein or protein aggregate and thus may not be available for interaction with water. Any stoichiometic estimation work should probably be conducted on dissociated, unfolded and reduced protein forms (Table VI).

TABLE VI

Protein Hydration Prediction (unfreezable water) (21)

Protein	Calculated	Observed
	(g H_2O/100 g protein)	
myoglobin	45	42
hemoglobin (denatured)	42	42
ovalbumin	37	33
bovin serum albumin (BSA)	45	40
BSA + urea	45	44
BSA, pH 3	32	30

Physical-chemical Environment

pH: Kuntz (21) has shown that ionized amino acid groups bind considerably more water than nonionized groups (Table IV). Thus, lowering pH values below 4 converts carboxyl groups toward nonionized forms, thereby reducing the water binding properties of the protein (Table VI). In addition, changes in pH affect the magnitude of the net charge on the protein molecule which in turn alters its attractive and repulsive interaction forces. For example, at their isoelectric point, where the net charge is zero, protein molecules normally exhibit minimal hydration and swelling and a protein matrix becomes shrunken (Fig. 1 and Table VII).

Solutes: Ionic strength and species have a significant effect on solubility, viscosity, gelation, swelling and water-binding capacity of proteins (27-29). Salt-related changes in protein-water interactions may be produced by competitive binding of water and salt molecules by the amino acid side groups. The amount of salt bound by a protein molecule is a function of A_w in the system (9, 26), while the amount of water bound to proteins is a function of salt concentration (30). The salt dependence of protein solubility has been recently analyzed by Melander and Horvath (31) based on the dual effect of salt on the elctrostatic and hydrophobic interactions. The increase in solubility at low salt concentration is due to the "salting-in" effect of electrostatic interactions, while the precipitation of protein at higher salt concentration is due to the "salting-out" effect

TABLE VII

Effect of pH Protein-Water Interactions

Protein	pH	Water removed (g/100g)[a]
Raw beef muscle	5.0[b]	7.68
	6.5	2.42
		Water holding capacity (% of normal muscle)[c]
Beef muscle homogenate	3.5	101
	5.0[b]	42
	10	135
		Water absorbed (g/100g)[d]
Dry soy protein isolates	4.5[be]	168
	7.0[e]	253
	7.0[f]	720

[a]Water removed by centrifuging at 12,000 X g for 10 min. (61).
[b]Near isoelectric point of proteins.
[c]Normalized water holding capacity, normal muscle = 100 (60).
[d]Spontaneous water uptake (method described in ref. 27).
[e]Commercial soy protein isolate.
[f]Freeze-dried soy protein gel, 12% protein, 100 C for 30 min., cool then freeze-dried.

of hydrophobic interactions. Salt affects hydrophobic interactions by increasing the surface tension, which is found to correlate well with the lyotropic series (the order of the effectiveness in "salting-out" proteins).

The increase in water-binding capacity of meat proteins upon the addition of salt may be due to preferential anion binding (Cl^-) by the protein molecules. Such preferential binding of chloride ions by the protein molecule at pHs above the isoelectric point increases its net negative charge and its resulting repulsive forces, thus permitting additional water imbibition within the protein network, while at pH's below the isoelectric point, the positive charge of protein is neutralized by chloride ions, thus decreasing its net positive charge and its water holding capacity (32, 33). Finally, protein dehydration will occur at high salt (solute) concentrations due to the competition of solutes and proteins for the available water.

Protein conformation: Conformational changes of the protein molecule may affect the nature and availability of the hydration sites and hence the thermodynamic characteristics of their water-binding reaction. Changes in protein conformation and swelling during the course of water vapor sorption could be a problem in the interpretation of enthalpy and entropy values. The nature of such conformational changes may also depend upon the rate of water addition or removal from the system. Transition of the protein molecule from a compact, globular conformation to a random coil conformation results in exposure of previously buried peptide bonds and amino acid side chains so that they may now interact with the aqueous environment. Thus, a denatured, unfolded conformation should permit the protein molecule to bind more water than in its native, globular conformation (Table VI) (30). However, processes such as heating, concentrating, drying, texturization, etc., which alter the quaternary structure of the protein system by aggregation of individual molecules, may reduce the availability of polar amino acid groups for binding water. The aggregation of protein through hydrophobic interactions may also effectively reduce the total surface area of the protein or cause the collapse of the protein matrix network. On the other hand, the altered structural characteristics of the agggregated protein systems may in some case provide additional protein-water interaction by means of imbibition of water within the newly formed structural network, such as the gelation of globular proteins upon heating and cooling.

Temperature: Temperature is an important factor in all kinds of reactions including the protein-water interactions. At the same A_w, protein usually binds less water at higher temperature than at lower temperature (11). But with temperature changes, protein conformation may be also altered which would probably override the possible effect of the temperature on protein-water interaction.

Others: Factors such as surfactants, organic solvents (especially water miscible) and pressure are also expected to affect the protein-water interaction either at molecular or macroscopic levels.

PROTEIN-WATER INTERACTION IN RELATION TO FUNCTIONAL PROPERTIES

Many of the important functional properties of food proteins are related to their interaction with water. Following are those functional properties that are directly related to their mode and degree of interaction with water:

Wettability and Dispersibility

These are largely determined by the hydrophilic/hydrophobic properties of the protein macromolecular surface, solvent surface tension, and the relative rates of water absorption and protein swelling.

Swelling

This is usually denoted as being caused by a spontaneous uptake of water by protein matrix. The degree of swelling may be controlled by various intermolecular forces or bonds within the swollen matrix. Swelling will thus have a direct effect on the amount of water that a food system can absorb and upon the ultimate body and texture of the food product.

Solubility

The solubilization of a protein molecule is a process which simultaneously involves wetting, swelling, solvation, and dissolution. Other functional properties of proteins including foaming and emulsifying properties depend upon their ability to dissolve in the solvent. Protein solubility is dependent upon conformation, pH, ionic strength, temperature, mechanical disruption forces and a number of other factors.

Viscosity and Thickening

The thickening property of a protein as expressed by viscosity is also a function of protein-water interaction as affected by the size, shape, charge and concentration of the protein and other factors.

Gelation and Coagulation

Gelation of proteins results in the formation of a gel which may be thermally reversible. Coagulation is an irreversible protein aggregation reaction which normally inolves limited protein swelling due, in part, to the formation of covalent bonds such as disulfide. Aging or repeated heating may increase these protein-protein interactions resulting in a "tighter" gel structure which exudes solvent by syneresis.

Water-holding Capacity

This is a quantitative indication of the amount of water retained within a protein matrix under certain defined conditions. It usually includes entrapped water.

Protein-Water Interactions in the Dry State

Food protein ingredients such as caseinate and soy protein isolates are customarily produced in a dehydrated form. The importance of water vapor sorption and its concomitant effect upon related physical and chemical properties of dehydrated foods are well illustrated in the literature (34-39). Okamato and Matsuura (40) demonstrated the dramatic effect of water activity and moisture content on the insolubilization of protein during storage in the dehydrated form. Koury and Spinelli (41) also reported that the functional stability (as reflected by changes in emulsifying capacity) of fish protein isolates is closely related to the moisture content and water activity of the dried isolates.

It is likely that the protein insolubilization involves changes in the protein molecular conformation. These molecular conformation changes may be brought about by formation of new polar-polar or hydrophibic-hydrophobic interaction pairs.

PROTEIN-WATER INTERACTIONS IN SOLUTION

There has been an increasingly popular view during recent years that in an aqueous environment the polar groups of the protein molecule are directed outward in direct contact with water and that the nonpolar groups are directed inward away from the water phase. These factors result in a folded, "globular" conformation for water soluble proteins and their associated properties in solution (7, 42). The hydrophobic bond is the term used to describe the gain in free energy resulting from the transfer of nonpolar amino acid residues from an aqueous environment to

the interior of the protein molecule (43). It is believed that hydrophobic bonding plays a dominant role in stabilizing the helical, globular conformation of the protein molecule in an aqueous environment in preference to the ramdom coil form (30). Richards (44) has shown that globular protein molecule interiors are closely packed with individual amino acid residues occupying the same volume as they do in their crystalline state.

The majority of the polar side groups distributed on the surface and those buried in the interior of the molecule would be stabilized through hydrogen bonding. It also has been shown by X-ray diffraction that some water molecules are inside the macromolecule and entirely isolated from the surrounding solvent (45). Most of these water molecules engage in two or three hydrogen bonds either with the macromolecule or with other water molecules. These specifically bound water molecules must fulfill a structural role stabilizing the native protein conformation.

Besides this structural water, a great deal of evidence is available that proteins in aqueous solution are hydrated to a considerable degree. For example, their hydrodynamic volume (time average of molecular volume plus volume of immobilized solvent) is greater than that indicated by the partial specific volume (7). Thus, protein molecules must be in a solvation state surrounded by shells of water. The state of these water layers has been an issue of controversy. Klotz (3) theorized that the protein molecule is surrounded by layers of immobilized, "ice-like" water. However, from NMR results and from molecular mobility data this water is more "liquid-like" than "ice-like" (17, 46). Therefore, this water should be called "unfreezable" water. It covers the immediate surface of the macromolecules but clusters about the protein's ionic and polar side chains. Water layers beyond this unfreezable water layer can be expected to move with the protein molecules but would have physical and chemical properties similar to those of "bulk" water. This may be defined as the hydrodynamic hydration water.

It should be noted that the native conformation of a protein molecule possesses only marginal stability because it is highly constrained (30, 47). Small changes in conditions can cause "denaturation" and unfolding of the protein chain. Conformational changes which will have different degree of interaction with water are believed to be responsible for the observed differences in functionalities.

PROTEIN-WATER INTERACTIONS IN GELS

Although they consist mostly of water, protein gel structures are remarkable in that they behave essentially as rigid, semisolids. The water component retains many of its normal properties, e.g., vapor pressure, electrical conductivity, and solute dissolution properties. The theory on the interaction of macromolecules carrying reactive groups that form a three-dimensional network via junction linkages or zones has been considered an essential feature of polymer (gelatin and polysaccharide) gels (48-50). The gelation mechanism for the reversible formation of polysaccharide gels has been classified according to the nature of the junction zones, i.e., double-helical junctions, stacked junctions and micelle junctions (50). Stainsby (51) suggested that the junction sites on gelatin gels consist essentially of proline and hydroxyproline. Such junction zones are probably absent in gels produced from denatured globular proteins, and thus, a higher protein concentration is usually required to form a rigid gel structure ($\geqslant$ 10% protein to produce a rigid soy protein gel). The protein network may be stabilized by primary bonds (largely disulfide), by secondary forces localized on the protein, or by nonlocalized secondary attractive forces. In order to form a gel, it is necessary to provide a proper balance between attractive and repulsive forces on the respective polypeptide chains (48). If attractive forces predominate, the result will normally be an insoluble protein precipitate. If disulfide cross linkages become significant, an irreversible gel will result, e.g., heat-coagulated egg albumen.

The exact physical state of the majority of water within the three-dimensional gel network is still somewhat uncertain. Since the water is so strongly immobilized that it cannot be "squeezed out," it may be that certain long range forces are involved in holding it within the protein-gel system. Some investigators have cited evidence that NMR relaxation times (T_1 and T_2) are shorter for water in muscle tissue than in salt solution (52-55) as confirmation that water present near the protein macromolecule is in an ordered state. Ling (5) considered that water is present as polarized multilayers on the surface of the protein molecule with progressive loosening of the rotational motion of the water molecules as the distance from the protein surface is increased. He also suggested that this mechanism is favored where the protein molecule has alternating negative and positive charged sites on its surface. This hypothesis, if correct, offers an explanation for the protein sol-gel transition and the ability of gels to hold water. When the protein is widely dispersed, as in a dilute solution, the bulk of its CO and NH groups are probably involved in hydrogen bonding with adjacent polar groups of the same molecule (α-helical conformation) or of other neighboring protein molecules (β-pleated sheet conformation). The protein-water interaction is limited to that bound by the protein's polar groups as discussed in previous sections. However, disruption of hydrogen bonds between CO and NH groups of the polypeptide chain, as by heating, may result in alternating negatively and positively polarized centers along the polypeptide chain that may then interact with water to create water multilayers. Subsequent interaction of polypeptide chains with alternating negatively and positively polarized centers on opposing protein molecules may occur upon cooling via hydrogen bond reformation to provide the structure necessary to immobilize the free water.

Whether the water in the gel is present as a polarized multilayer or in another structured form, the interaction between protein and water should definitely play an important role in gel formation. First of all, protein denaturation, which appears to be an essential precursor to gel formation by globular proteins, occurs most readily in the presence of water. Tanford (47) suggested that the change of water structure is probably not as important in causing protein denaturation as changing the water to a state that will accommodate the denatured protein's hydrophobic groups. The denaturation phenomenon may represent a cooperative transition of the protein macromolecule. It one assumes that the water in the gel has an ordered structure, then the protein-water interaction could also be cooperative during the sol $\rightarrow$ gel phase transition. If this is the case, the protein molecule must be appropriately arranged and in the appropriate (unfolded) molecular conformation, and the water molecules must be in a suitable state to accept and propagate the structure imposed by the protein molecules. Thus, the protein must be "activated" in the presence of water to attain a proper, unfolded conformation. This represents an irreversible transition from sol to progel state (56). In the progel state, the properties of water may be modified by the exposure of hydrophobic and hydrophilic groups of the activated protein. At this point the protein would be in a fully hydrated state. When the activated proteins are properly arranged, protein-protein interactions take place and a three-dimensional gel network will result. Transition from progel to gel states are reversible simply by adjusting the temperature (56).

It should be noted that direct evidence of such ordered water sturctures in gel systems is still lacking. On the other hand, there are suggestions that the majority of the water in gels has properties similar to those of free water (57,58). The apparent difference in water mobility between sol and

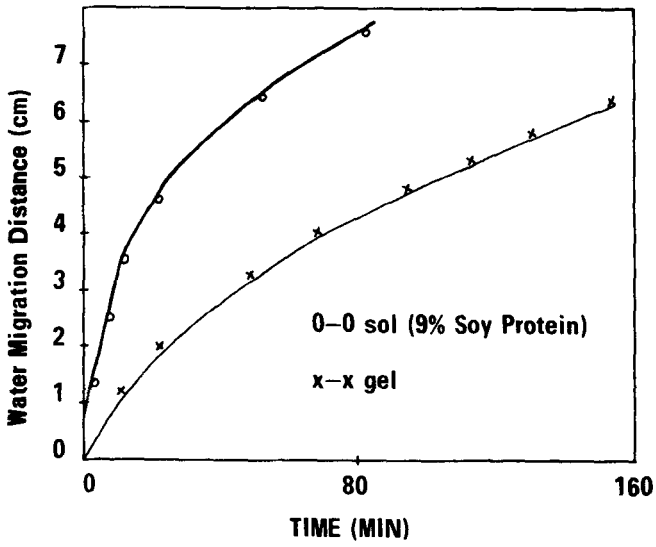

FIG. 5. Migration of water from samples to chromatography paper. The chromatography paper strip is about 1 cm wide and in direct contact with the sample.

TABLE VIII

Water Removed from Beef Skeletal Muscle by Centrifugation at Various Speeds for 10 minutes (62)

Centrifugal Force (g)	Water Removed (g H_2O/100g)
94	0.00
720	0.45
2400 2400	1.20
5200 5200	1.85
8800 8800	2.10
12000 12000	2.80
15000 15000	2.80

gel could also be due to viscosity and physical barrier effect as well as protein-water interactions (Fig. 5) (59).

WATER-HOLDING CAPACITY IN PROTEIN FOOD SYSTEMS

Empirical measurements of the amount of water held in model and experimental food systems has been used by various researchers (60-63). The following conditions have been used to define the amount of water held in protein-food systems: (a) it is not removed by a defined g-force of centrifugation or gravity (Table VIII) (b.), it is not expressed by a defined suction or compression force. In most cases it is difficult to establish which parameters are actually being investigated. Furthermore, expressions such as water absorption, water uptake, hydration, water-binding, bound water, water affinity, water-holding capacity and water retention are frequently used without specific definition.

If the water is already present in the food system, e.g., as in raw muscle tissue, one may consider water retention or water loss as the food is subjected to freeze-thaw, cooking, centrifugation or compression. On the other hand, if the protein is in a dehydrated form, such as for a spray dried protein isolate, one would be concerned first mainly with water absorption or water uptake as it is exposed to water vapor or liquid (Fig. 6). A diagram of the probable sequence for protein-water interaction starting with a dry powder is illustrated in Fig. 7. When a dry protein powder is exposed to water vapor, water molecules will be absorbed onto all available surface polar sites, thus forming a monolayer coverage. Upon further water absorption, additional layers of water are formed as multilayers followed by water-water interaction to result in liquid-water condensation. At this state, the protein particles become swollen and, if the protein is soluble, swelling continues until the individual protein molecules are surrounded by sufficient water molecules to solubilize them. This is a continuous, overlapping process with no clear-cut boundary between the individual stages. For example, protein swelling may occur at any point during the water absorption process.

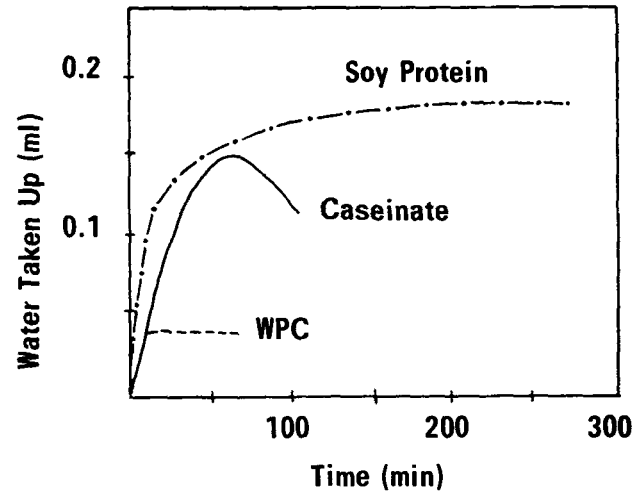

FIG. 6. Swelling (water uptake) as a function of time for 20 mg sample (27).

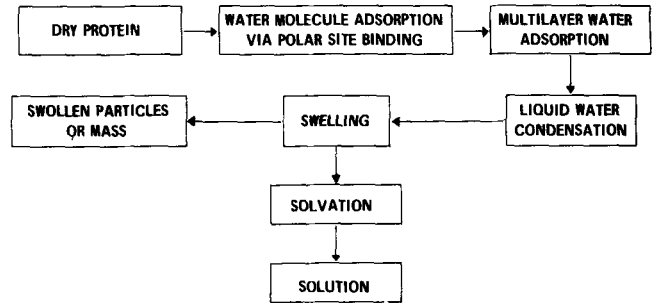

FIG. 7. Sequence of protein-water interaction for dry protein.

It should be expected that a portion of the changes in water-holding capacity of food systems may be attributed to structural changes of the protein matrix. Factors such as temperature (heating and freeze-thaw) and pressure may disrupt the existing structure and sometimes create a new one. Transmission and scanning electron microscopy are valuable techniques for the visualization of protein microstructures which possess potential sites for immobilization of water (49). Transmission electron microscopy can be used to examine internal microstructure, whereas scanning electron microscopy is useful for assessing the size, shape and surface characteristics of protein-containing food particles or gels. Useful information on the size, shape, locality and number of pores and cavities of the aggregates and spaces in three-dimensional biopolymer networks may be estimated by a combination of the two microscopic techniques.

Based on the current knowledge, we would like to offer the following definitions to describe the type of water which is "interacting" with or under the influence of proteins in a food system:

Structural water: This water engages in hydrogen bonding to the protein molecule and fulfills a role in stabilizing the native structure of protein. It may be inside the macromolecule and engaged in two or more hydrogen bonds per water molecule. This water, in most cases, is unavailable either for reaction or as a solvent.

Although the amount of water involved is small, the removal of this water will probably have profound effects on the structure and conformation of the protein molecule some of which may be irreversible.

Monolayer water: This water fills the first adsorbed monolayer around the protein molecule and is attached to specific water-binding sites through hydrogen bonding or dipole interaction. A typical protein would have a value of about 4-9% monolayer water. It is usually unavailable as a solvent, but may be available for certain reactions.

Unfreezable water: This water does not freeze at a sharp transition temperature, may represent the total water molecules clustered around each polar group of the protein molecule and thus includes structural and monolayer water. It may account for about 0.3 to 0.5 g H_2O/g protein which roughly includes water up to water activity (A_w) about 0.90. The amount of unfreezable water correlates with amino acid composition and polar side chains on various proteins. The reactivity of this water will usually be expected to be a function of water activity in the protein system.

Hydrophobic hydration water: This water in the vicinity of protein hydrophobic groups is believed to adopt a clathrate-type structure. The real nature of this type of water is not yet clear, though it may have important effects on protein properties since a considerable number of protein hydrophobic groups might remain in contact with water despite their involvement in hydrophobic protein-protein interactions.

Imbibition or capillary water: This water is either held physically or by surface forces on the protein molecule. It consists of a major portion of the water in gelled foods such as cheese curd, meat, meat emulsions, etc. It is freely available for chemical reactions as well as for solvent functions. However, considerable force is required to remove it from the protein mass.

Hydrodynamic hydration water: This water, which surrounds the protein macromolecule, is transported along with it during diffusion and other motion and is more or less independent of water activity over a reasonable range. There is no fundamental relation between hydrodynamic hydration and thermodynamic hydration, as the "entrapped" and "surrounding" water involved in hydrodynamic hydration has normal physical properties, whereas thermodynamically bound water does not. Hydrodynamic hydration is therefore equal or greater than hydration values determined by other techniques.

The above classifications of the type of water in protein-water interactions have the advantage of providing us with a series of quantitatively and physically definable definitions. Furthermore, they are consistent with and explain the basic structure and properties of the protein macro-molecules in model and food systems. In addition, they are more specific than the more generalized classifications such as "irrotationally bound," "bound" and "bulk" water (18), and it will be easier to describe their relationships to the physical, chemical, and functional properties of proteins in foods. Thus, measurement of different types of water in a given protein system can reveal more about the protein structure, the nature of protein-water interaction, and differences in protein functionality.

There is a need to better understand the basic interaction mechanisms and to develop improved methodologies to determine and predict how a protein will interact with water and other components in a complex food system. Only by such improved approaches will we aquire the ability to predict with any degree of accuracy how a given protein source is likely to function in future experimental food applications.

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