Protein Hydration

The contribution of water to protein structure

Protein hydration is very important for their three-dimensional structure and activity [472, 1093, 1345, 2005]. Indeed, proteins lack activity in the absence of hydrating water. The aqueous structuring around proteins is affected out to at least 1 - 1.5 nm from its surface or 2 - 3 nm between neighboring proteins, as shown by terahertz spectroscopy [1368, 2102],[e]with even small proteins (e.g. bovine serum albumin, 66,463 Da) affecting the whole of its unstirred (Nernst) layer of about 20,000 neighboring water molecules [2102]. Additionally, the presence of glycans attached to (glyco)proteins impose a long-range order on the water structure out to several nanometers, dependent on the orientation of the glycan [2104]. Some water molecules interact with the surface, reorienting both themselves and the surface groups whereas other water molecules link these to the bulk in an ordered manner whilst remaining in dynamically active [1695]. In solution proteins possess a conformational flexibility, which encompasses a wide range of hydration states, not seen in the crystal[a] or in non-aqueous environments. Equilibrium between these states will depend on the activity of the water within its microenvironment; that is, the freedom that the water has to hydrate the protein [434]. Thus, protein conformations demanding greater hydration are favored by more (re-)active water (for example, high density water containing many weak bent and/or broken hydrogen bonds) and 'drier' conformations are relatively favored by lower activity water (for example, low-density water containing many strong intra-molecular aqueous hydrogen bonds). Surface water molecules are held to each other most strongly by the positively-charged basic amino acids. The exchange of surface water (and hence the perseverance of the local clustering and the overall system flexibility) is controlled by the exposure of the groups to the bulk solvent (that is, greater exposure correlates to greater flexibility and freer protein chain movement) [975]. Hydration also affects the reactions and interactions of coenzymes and cofactors; thus, the various redox potentials (and hence whether they oxidize or reduce) of some iron-sulfur proteins are accounted for by differential hydration rather than direct protein binding effects [1344].

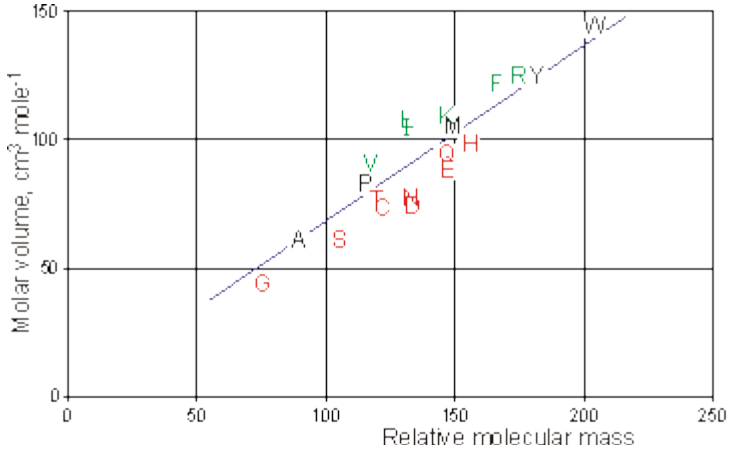
The folding of proteins depends on the same factors as control the junction zone formation in some polysaccharides; that is, the incompatibility between the low-density water (LDW) and the hydrophobic surface that drives such groups to form the hydrophobic core.[b] This drive for hydrophobic groups to mostly cluster away from the protein surface (in water soluble proteins) is controlled by the charged and polar group interactions with each other and water. Interestingly, in pure water and in the absence of screening dissolved ions, some buffer-insoluble proteins are quite soluble due to the weak (here unshielded) interactions between the protein's intrinsic charges [1375]. Non-ionic kosmotropes, which stabilize low-density water, consequentially stabilize the structure of proteins. In addition, water acts as a lubricant [822], so easing the necessary peptide amide-carbonyl hydrogen bonding changes. The biological activity of proteins appears to depend on the formation of a 2-D hydrogen-bonded network spanning most of the protein surface and connecting all the surface hydrogen-bonded water clusters [978]. Such a water network is able to transmit information around the protein and control the protein's dynamics, such as its domain motions [977]. Much life, however, functions optimally at about 37 °C where this spanning network is about to break down on heating, perhaps to help compensate for the biosystem's entropic changes [1541].

Intramolecular peptide (amide) hydrogen bonding makes a major contribution to protein structure and stability but is only effective in the absence of accessible competing water. Even just the presence of close-by water molecules causes peptide hydrogen bonds to lengthen [524], so loosening the structure. Water mediated hydrogen bonding between peptide links has been found to be particularly important in the structure of collagen where only a third of the peptide links directly hydrogen bond to other peptide links [1314] and the stability of the triple helix is sequence dependent [1438]. Water molecules can bridge between the carbonyl oxygen atoms and amide protons of different peptide links to catalyze the formation, and its reversal, of peptide hydrogen bonding as well as forming long-lived linkages stabilizing protein-ligand and protein-protein interfaces (for example, [688]). The internal molecular motions in proteins, necessary for biological activity, are very dependent on the degree of plasticizing, which is determined by the level of hydration [810]. Thus internal water enables the folding of proteins and is only expelled from the hydrophobic central core when finally squeezed out by cooperative protein chain interactions [352]. Many water molecules (similar in amount to individual amino acids) remain behind buried in the core of the proteins, so forming structurally-important hydrogen bonded linkages. The position of the$ES \rightleftharpoons CS$ equilibrium around enzymes has been shown to be important for their activity with the enzyme balanced between flexibility (CS environment) and rigidity (ES environment). Addition of non-ionic chaotropes only, or kosmotropes only, both may inhibit the activity of enzymes (by shifting the equilibrium to the right or left respectively) whereas an intermediate mixture of kosmotropes and chaotropes restores optimum activity [276]. Also, the ease of enzyme-substrate contact may be controlled by the level of water structuring in the protein environs [882]. The crystal structures of proteins suggest that the commonest water polygons surrounding proteins are $(H_2O)_3$ trimers (typical of CS environments) and $(H_2O)_5$ pentamers (typical of ES environments) with lower amounts of tetramers, hexamers and heptamers [1849].

The first hydration shell around proteins (~0.3 g/g) is ordered; with high proton transfer rates and well resolved time-averaged hydration sites; surface water showing coherent hydrogen-bond patterns with large net dipole fields [702]. The hydrogen bonds holding these water molecules to the protein are stronger with longer lifetimes than bulk water [1355] and this water is unavailable to colligative effects. As hydration sites may be positioned close together and therefore mutually exclusive, it has been argued that the solvation is better described as a water distribution density function rather than by specific water occupied sites. For example, no more than 164 of the 294 high-density hydration sites around myoglobin are occupied at any moment and there is no correlation between the maximum site density, occupancy and residence time [542]. The first hydration shell is also 10-20% denser than the bulk water and probably responsible for keeping the molecules sufficiently separated so that they remain in solution [315] (that is, solutions are kinetically stable but often thermodynamically unstable).[c] Although a significant amount of this density increase has been shown to be due to simple statistical factors dependent upon the way that the surface is defined in depressions [401], much is due to a protein's structure with the excess of polar hydration sites (tending to increase surface density)[d] easily counteracting the remaining non-polar surface groups (tending to produce low density surface water). This water is required for the protein to show its biological function as, without it, the necessary fast conformational fluctuations cannot occur.

Thus proteins have no activity (and enter a glassy state, at about 220 K) when the surrounding water becomes predominantly low-density [1197].

Using X-ray analysis, the hydration shell shows a wide range of non-random hydrogen-bonding environments and energies. Proteins are formed from a mixture of polar and non-polar groups. Water is most well-ordered round the polar groups where residence times are longer, but where they will interfere with water's natural hydrogen bonding, than around non-polar groups where aqueous clathrate structuring may form. Interestingly, the clathrate pentagonal structuring has been found to extensively surround the helices in the four-helix bundle winter flounder antifreeze protein, so retarding the formation of harmful ice crystals in fish living in near-freezing water [2084]. Both types of group create order in the water molecules surrounding them but their ability to do this and the types of ordering produced are very different. Polar groups are most capable of creating ordered hydration through hydrogen bonding and ionic interactions (a excellent guide toamino acid hydrogen bonding is given elsewhere). This is most energetically favorable where there is no pre-existing order in the water that requires destruction. The ordering created in the water surrounding proteins extends the proteins' electrostatic surfaces well away from their physical (that is, amino acid) surfaces giving them far greater electrostatic visibility to visiting ligands [1156]. This non-specific electrostatic effect of the water effect is additional to any specific directed hydrogen bonding that may extend away into the bulk from the surface

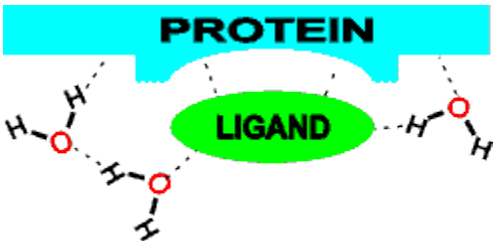


The line is the best straight line from these amino acid data but is expected to lie to the low density side of aqueous structuring.

The graph opposite shows the molar volumes of the amino acids [1063] (using the one-letter code). Leucine (L) has the largest molar volume relative to that expected from its molecular weight and forms low density clathrate water structures if exposed to the solution. It is followed by isoleucine (I), valine (V), phenylalanine (F), lysine (K) and arginine (R). Aspartic acid (D) has the smallest molar volume relative to that expected from its molecular weight and forms higher density water structuring. It is followed by asparagine (N), glutamic acid (E), serine (S), cysteine (C), glutamine (Q), histidine (H), threonine (T) and glycine (G). The hydration properties of alanine (A), proline (P), methionine (M), tyrosine (Y) and tryptophan (W) are slightly kosmotropic .

The water is slow to exchange, showing the dynamic behavior of bulk water 25 °C colder [147]. Low-density water (such as ES) is promoted [148, 276] surrounding this dense hydration and polyelectrolyte double layer (as described in the 'Polysaccharide hydration' section). Non-polar groups promote clathrate structures [153] (such as ES) surrounded by denser water. It is no surprise, therefore that the degree of hydrophobic hydration is correlated with the hydration of the polar groups. Clathrate shells contain loosely held water with greater rotational freedom than in the bulk [139]. However, under favorable conditions clathrate hydrophobic hydration may exert pressure on non-polar C-H bonds pushing them in, so contracting their bond length and increasing their vibrational frequency. This blue-shifting (that is, the vibration frequency increases and intensity reduces) 'push-ball' hydration [149] should not necessarily be thought of as 'typical' hydrogen bonding even if the CH···OH_2 distances are suitably close (see [1293]). They can be considered as part of a continuum of hydrogen bonding behavior, however, where sometimes the OH_2 behaves as a much more weakly interacting base than usual and the C-H behaves with reversed dipolar behavior compared with the more usual O-H hydrogen-bonding partners [625].

There are significant differences in the directional rates of water diffusion perpendicular and parallel to the protein surface that are maximal at about 6 Å but still determinable at 15 Å from the surface [542]. It is clear that evolutionary processes have made use of the organization in this water surrounding proteins to create preferred diffusive routes and orientation for metabolites and favored conformational changes and interactions. Such diffusive paths lead to binding sites with their own helpful hydration. It has been suggested that pressure waves formed from flickering water clusters (for example, as described elsewhere) may link protein molecular vibrations, so carrying information through the intracellular milieu [549] and powering product movement between enzymes in biochemical pathways [665].



The energetic optimization of mutual hydrogen bonded networks between protein, water and ligand is an intrinsic part of the molecular recognition process in enzymes, binding proteins and biological macromolecules generally [412].
Note that water bound and oriented in empty ligand binding sites will reduce the entropy of activation when replaced by the ligand [414].

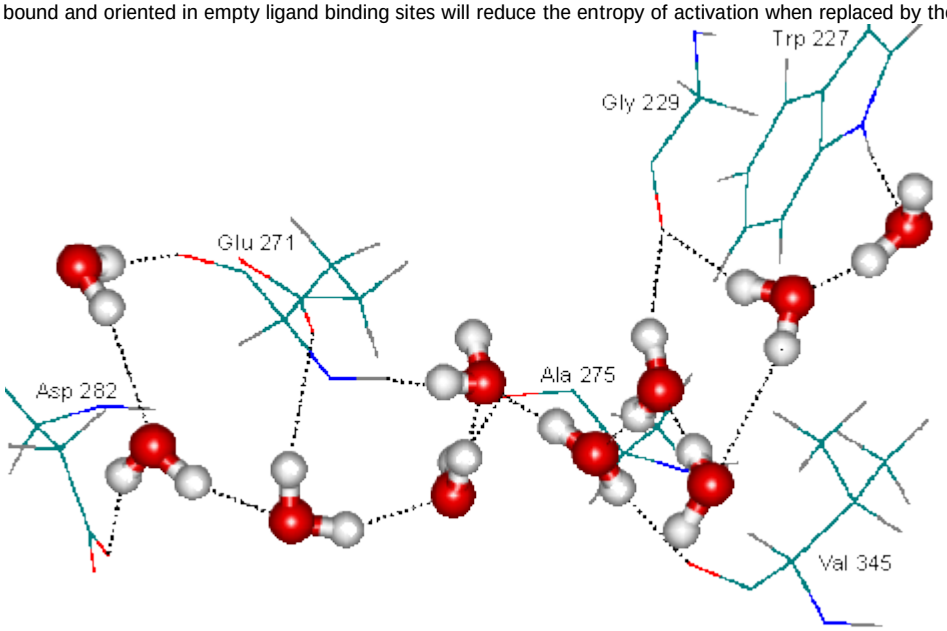


Figure 1. The water network links secondary structures within the protein and so determines not only the fine detail of the protein's structure but also how particular molecular vibrations may be preferred. The above chain of ten water molecules, linking the end of one α-helix (helix 9, 211-227) to the middle of another (helix 11, 272-285) is found from the X-ray diffraction data of glucoamylase-471, a natural proteolytic fragment of *Aspergillus awamori* glucoamylase [155].

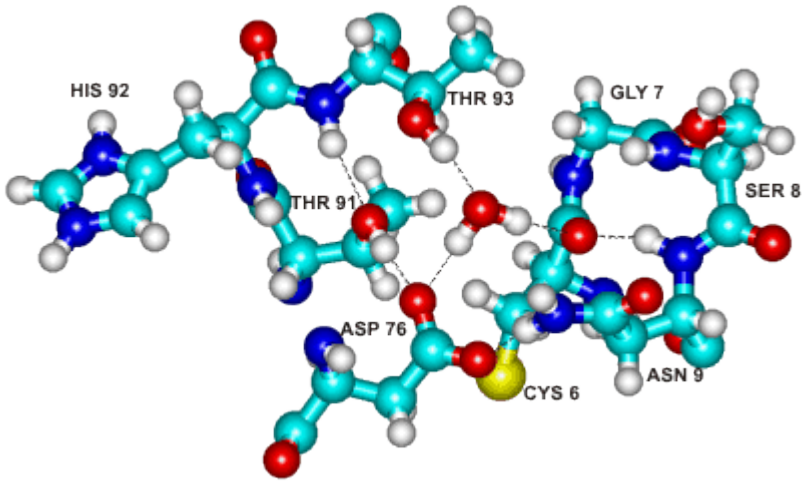


Figure 2. The above centrally-placed water molecule makes strong hydrogen bonds to residues in three separated parts of the ribonuclease molecule holding them together. This water molecule and its binding site are conserved across the entire family of microbial ribonucleases [345].

Water molecules have also proved integral to thestructure and biological function of a dimeric hemoglobin [377]. The internal water molecules in proteins have been surveyed [725].

Both proton-transfer processes [907, 160b] and electron-transfer reactions [908] may be facilitated by ordered water molecules connecting proton donor to acceptor sites or electron donor to electron acceptor sites respectively. In both cases, the transfer is faster where the linking water molecules possess stronger hydrogen bonding. Further, a network of hydrogen bonded water molecules plays a catalytic role in water oxidation in photosystem II [1941].

Local water structures and proton-conducting hydrogen-bonded water wires can form rapidly (~10 ns or so) in response to control by relatively small-scale rearrangements of the protein matrix [2201].

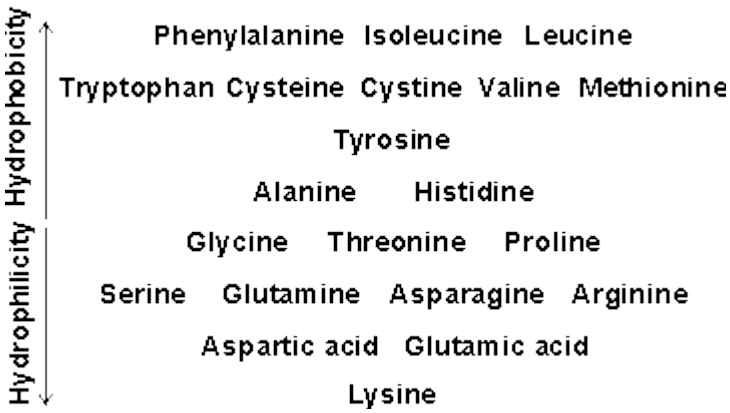
Water in protein recognition and binding

Water molecules form an integral part of most protein-protein [1339], protein-DNA [1340] and protein-ligand [1341] interactions, aiding the mutual recognition and both the binding thermodynamics and binding kinetics [1338]. The small size, polarity and conformational flexibility, together with the strength and directionality of the interactions, ensures good fits whilst retaining flexibility and ease of reversibility. The

driving force for binding depends not only on the interaction of the biological molecules with each other but the energetic cost for the necessary removal of hydration water and the energetic gain for the subsequent molecular rearrangement of the hydration water molecules [1793, 1805]. The use of water may also be useful in broadening the specificity of such links; for example, the peptide-binding protein OppA uses several flexibly adaptive water molecules to hydrogen bond and shield charges when binding to lysine-X-lysine tripeptides, where X is any one of the twenty common amino acids [1445].

Footnotes

To avoid such activity loss, proteins generally avoid crystal formation, perhaps by evolutionary design involving surfacekosmotropic lysine residues that minimize self-aggregation [771]. [Back]



A historical review of the 'hydrophobic effect' in proteins is available [1393].

An approximate comparison of the hydrophobicity of the amino acids is given in the table opposite. Further discussion of relative hydrophobicities is given elsewhere as is a classification of hydrophobicity scales [633]. It should be noted that such hydrophobic interactions are particularly important in stabilizing interdomain and quaternary interactions. [Back]
The effect of strongly-bound surface water molecules on preventing protein-protein interactions is described in [881]. [Back]

Alternatively to this molecular group approach, some of this increased density may be attributed to electrostriction pressure (that is, local pressure increase due to the localized electric field) [951]. [Back]

Other techniques, such as NMR, are incapable of showing this

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