"The Physics and Chemistry of Water"

11 – Water and hydration in biological systems

Proteins

• Hydration in the solid state and in solution
• Protein folding and stability
• Protein interactions
• Solubility - salt and cosolvent effects

Nucleic acids

• Structure and hydration in the solid state
• Structure and hydration in solution
• Double-helix denaturation

Simulations

• Quantitative information $\sim \frac{1}{\text{System complexity}}$
• Total force fields - complex descriptions
Proteins

\[ \text{N} \iff \text{D} \]

native state \hspace{1cm} \text{denatured state}
unique conformation \hspace{1cm} \text{deviant conformation}

Denaturation is typically associated with small free energy changes, due to the cancellation of (sometimes large) enthalpic and entropic contributions.

\[ \text{NH} - \text{CH} - \text{C} \]

\[ \text{O} \]

\[ \text{R} \]

\[ n \]

The amino acid residues \((R)\) are ionogenic, polar or non-polar.

A protein generally ceases to function if it is dry, or – in solution – if it unfolds.
Protein folding I – Energetics

Upon folding of a polypeptide chain into a 3D structure, the number of hydrophobically hydrated water molecules will be reduced by internal hydrophobic association; the total amount of water ordered by the protein decreases, increasing the entropy, while the enthalpy change is very small.

\[ \Delta H \approx -200 \text{ kJ/mol}; \text{ electrostatic and van der Waals interactions, hydrogen bonding.} \]

\[ \Delta S \approx -0.5 \text{ kJ/mol} \cdot \text{K}; \text{ decrease from polypeptide chain folding, increase from reduced hydrophobic hydration.} \]

\[ \Rightarrow \Delta G \approx -50 \text{ kJ/mol (} \sim 3 \text{ hydrogen bonds!)} \]

Thermal destabilization can be induced by heating or cooling (...or freezing!).

\begin{table}[h]
\centering
\begin{tabular}{lcc}
\hline
Interaction & \((\Delta G)_{N \rightarrow D}\) & \(\left(\frac{d(\Delta G)}{dT}\right)_{N \rightarrow D}\) \\
\hline
Hydrophobic interactions & +++ & + \\
Salt bridges & ++ & + \\
Configurational free energy & - - & - \\
Intrapeptide H-bonds & ++ & - \\
Water-peptide H-bonds & - - & - \\
vander Waals & + & - \\
\hline
\end{tabular}
\end{table}

Interactions for which \(\frac{d(\Delta G)}{dT} > 0\) weaken at low \(T\).
Protein folding II

- Of the nearly fully hydrated polypeptide chain in an unfolded protein, about 40-50% remain bound to water in the folded state, the remaining involved in internal H-bonds.

- About 50% of the surface area of globular proteins is occupied by apolar residues.

- Most amino-acid residues are accessible by water molecules also in the native state; about 80-90% of H atoms in hydrogen or amide bonds exchange with D upon immersion in D₂O.

- The mean fractional area loss upon folding (ranging from 60 to 90%) is greater for non-polar residues (see figure below).

![Graph showing average area, ΔA, buried upon folding vs. standard area, A°.](From Rose et al., Science 229, 834 (1985))
Hydration of proteins in the solid state

Progressive hydration of enzymes in the solid state can help us to understand the role of water in enzyme activity.

- Several studies have been carried out, particularly on hen egg white lysozyme for this purpose.
- Acidic groups are ionized first, and return to their normal pK at 10 wt% hydration.
- Onset of activity occurs at a hydration of about 20%, and increases further upon addition of water.
- At activity onset, hydration is almost complete for polar side-chains and amide NH groups, but not to peptide CO groups.
- About 30% is required to fully hydrate the polar and charged groups of the protein.
- The enzyme appears to recover its native solution structure before activity begins; the remaining structural variations are mostly small and localized.
- Enzyme flexibility increases with increasing hydration, allowing access of solvent water to many buried amide groups.
- Only at hydration levels corresponding to several 100% does the enzyme reach its turnover capacity.
Hydration of proteins in solution

The degree of hydration is a relative measure of the amount of water in the solution which differs from bulk water due to interaction with the solute (protein).

Many methods are used to estimate the degree of protein hydration in solutions, but the results are very much method-dependent, reflecting the different aspects of protein-solvent interactions measured by various techniques.

- Protein hydration is typically about $30 \pm 5$ wt%.
- The degree of hydration varies with pH and temperature.
- Addition of a third component (salt, cosolvent) may facilitate hydration studies via preferential hydration coefficients, again allowing total water hydration to be determined (at moderate concentrations, salt or cosolvents are excluded from the protein hydration water).
- Methods to study hydration includes e.g. calorimetry, x-ray and neutron diffraction, sedimentation, infrared and Raman spectroscopy, adsorption isotherms, diamagnetic susceptibility, NMR and UV absorption.
Salt effects on proteins

Proteins are polyampholytes, carrying patches of positive and negative charge on their surface.

At the isoelectric pH, the net charge is zero, and the solubility may be reduced due to increased aggregation, while for other pH values, where they have a net charge, there is a net electrostatic repulsion which will increase solubility.

Salts in protein solutions will

- Modulate electrostatic interactions, generally in a rather non-specific manner.
- Affect protein solubility, and
- Change protein stability

The actual effect of a salt on a protein results from a balance between specific ion binding (e.g. dispersion interaction), and hydration, which reflects non-specific interactions of the protein surface with the salt.
Protein solubility

Some salts increase protein solubility (salt-in), while others have the opposite effect (salt-out).

The contributions from anions and cations in this respect are approximately additive, and follow a "Hofmeister series".

Stabilizing, salting-out

Kosmotropes $\leftarrow$

$SO_4^{2-} \simeq HPO_4^{2-} > F^- > Cl^- > Br^- > I^- >$
$\simeq ClO_4^- > SCN^- > K^+ > Li^+ > Ca^{2+}$

$\rightarrow$ Chaotropes

Destabilizing, salting-in

Similar results have been obtained with hydrocarbons, and complexes of apolar residues with peptide groups. Experimental results can be represented by the empirical Setchenow equation;

$$\log \frac{S_0}{S} = K_s C_s$$

where $S_0$ is the compound solubility in pure water, $S$ the actual solubility in the presence of added salt, $C_s$ the salt molarity, and $K_s$ a salting-out coefficient, which is $>0$ for salting-out agents.

Irrespective of the details of these interactions, it has been inferred that almost all salts salt-out apolar groups, and salt-in amide groups, so the effectiveness of a salt to salt-in or -out will depend on the ratio of polar to apolar groups accessible to it.
Protein stability

Salting-out ions stabilize the peptide chain conformation, while salting-in ions are potent destabilizers (also cosolvents may (de)stabilize proteins).

Melting curves for collagen in various solutions (From Kunz).

General conclusion:

All solvent stabilizing additives are preferentially excluded from the vicinity of the protein, which therefore is preferentially hydrated.

Structure destabilizers (denaturants) preferentially bind to the unfolded polypeptide chain; guanidinium chloride and urea interact with peptide groups and hydrophobic side chains, denaturing alcohols and detergents bind to apolar groups.
Nucleic acids

- Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two nucleic acids.
- Heteropolymers consisting of nucleotides containing a cyclic sugar, a phosphate residue and a base.
- The backbone is formed by sugar moieties connected by phosphodiester links.
- Like for proteins, water and salts are responsible for structure (de)stabilization of nucleic acids, hydrophilic and hydrophobic effects play a role, and electrostatic repulsions between counter-ions may screen repulsions between phosphate groups.

Transition temperatures for RNA for different salts over a range of concentrations. (From Kunz).
Nucleic acid structure

Three main helical structures of DNA have been identified (see figure on next page):

- **A-DNA** with bases strongly tilted about the helix and well off it. Diameter ca. 2.3 nm.

- **B-DNA**, diameter about 1.9 nm. Bases nearly perpendicular to helical axis.

- **Z-DNA**, left-hand helix with backbone following a zig-zag path.

The occurrence of these types depends on preparation conditions and environment. In fibers, A-DNA is observed at a low relative humidity, while B-DNA is favoured at higher humidity. (Double-helical RNA is always in the A-form.)

- Above 80% humidity the DNA helix is fully hydrated in the B-form with about 20 H$_2$O molecules per nucleotide.

- At Rh < 65%, the bases are no longer hydrated, and 11-12 H$_2$O molecules are bound to each nucleotide.

- At even lower humidities, only the phosphate O’s are hydrated, ca 3-4 H$_2$O per nucleotide.

This decrease in hydration induces the B → A transition. (See Franks p. 134 for details!)
Helical structures of DNA

(Arnott & Chandrasekaran, Proc. of the 2nd SUNYA
Conversation in the Discipline Biomolecular stereodynamics, Sarna
(ed.), Adenine Press 1981.)

DNA structures.

A-form  B-form  Z-form
DNA in solution

- Usually in B-form in aqueous solution.
- Hydration water forms two domains; a) bound molecules detected by crystallography, and b) the usual hydration shell which differs from bulk water.
- Total hydration ~20-25%.

Potential binding sites for metal ions are O and N atoms. Cations binding to phosphate groups tend to stabilize the structure, while those binding to bases promote destabilization (e.g. K\(^+\)). In general alkali metal ions increase the melting temperature of the double-helix, unlike transition metal ions.

![Denaturation temperature for DNA vs. ionic strength (from Kunz).](image)

The melting temperature increases with ionic strength due to (non-specific) screening of electrostatic interactions.
A flashback – Water structure in coenzyme (Vitamin) B$_{12}$

(From Savage)
Gramicidin - a "simple" ion channel
(Jordan, J. Phys. Chem. 91, 6582 (1987))

Simulation of a $\sim$2000 residue ion channel.

Water structure at 300K for three situations, Top: No ions, Middle: Cs$^+$ in the channel interior, Bottom: 2 Na$^+$ ions, one near each end.
Modelling biosystems...

(Clementi et al., J. Am. Chem. Soc. 99, 5531 (1977))

Interaction potentials for water-amino acid interactions – to facilitate hydration studies.

\[ I(M, W) = \sum_i \sum_{i \neq j} (-A_{ij}^{ab}/r_{ij}^6 + B_{ij}^{ab}/r_{ij}^{12} + C_{ij}^{ab}q_i q_j/r_{ij}) \]

Interaction energy contours for a water molecule interacting with amino acids, 1 kcal/mol intervals.
General references


