Chemistry 2A (Biological Sciences)

14 lectures:

a) Biopolymers and Biocolloids (3)
   • Biopolymer structure and bonding
   • Colloids, colloidal stability
   • Membranes, osmotic pressure, membrane transport

b) The Physical Basis of Spectroscopic Methods (6)
   • Wave nature of matter, electron microscopy
   • Molecular orbital theory, vision
   • Rotational, vibrational, electronic energy levels
   • UV/vis, IR and microwave spectroscopy
   • Fluorescence and light scattering

b) Principles of Modern Biochemical Analysis (5)
   • Spectrophotometry, instrumentation
   • Atomic absorption spectroscopy
   • Mass spectrometry, gas chromatography
   • Radioimmunoassays, enzyme-linked assays
   • Electrochemical techniques
Suggested References

   Chapters 5, 9, 10, 12

   Chapters 2, 5, 7-9, 25

   Chapters 1, 3, 8, 9, 11, 12, 13

   Chapters 9-15

   Chapters 1-9

   Chapters 6-11, 13-20, 22-23, 26-28
1. Secondary Structure of Proteins

Conformation of the segments of the polypeptide chain.
Polypeptide chains often adopt helical conformations with hydrogen bonds between carbonyl oxygens and amino hydrogens along the chain stabilizing the structure.

<table>
<thead>
<tr>
<th>Residues/helix turn</th>
<th>Atoms between O and H</th>
</tr>
</thead>
<tbody>
<tr>
<td>2\textsubscript{7} ribbon</td>
<td>2</td>
</tr>
<tr>
<td>3\textsubscript{10} helix</td>
<td>3</td>
</tr>
<tr>
<td>α helix</td>
<td>3.6</td>
</tr>
<tr>
<td>π helix</td>
<td>4.4</td>
</tr>
</tbody>
</table>
$\alpha$-Helix (Pauling and Corey, 1951)
Antiparallel β-Pleated Sheet  (Pauling and Corey, 1951)
How did Pauling and Corey arrive at the $\alpha$-helix and $\beta$-pleated sheet conformations?

Structural principles:

1. The peptide linkage (O-C-N) must be planar, because of resonance.

![Diagram of peptide linkage]

C-N is approx. 30% double bond. High barrier to rotation (torsion).

2. The *trans* configuration is more stable than the *cis* because of steric interactions around the $C_\alpha$s.

3. All C=O and N-H groups should be involved in hydrogen bonds.
   The N-H···O atoms should be linear.
Fixing the peptide linkage in a trans planar geometry leaves only two free bonds along the backbone which can rotate:

\[ \psi \] torsion angle (about the \( C_\alpha \)-carboxyl C bond)
\[ \phi \] torsion angle (about the amino N-\( C_\alpha \) bond)
**Ramachandran Plot**

Ramachandran et al. (1963) *J. Mol. Biol.* 7:95. Steric contour diagram of an amino acid residue as a function of the values of the two torsion angles.

Defines the sterically allowed conformations of the polypeptide chain.

E.g. for an L-alanyl residue,

<table>
<thead>
<tr>
<th>Structure</th>
<th>$\phi$</th>
<th>$\psi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right-handed $\alpha$ helix ($\alpha_R$)</td>
<td>$-57^\circ$</td>
<td>$-47^\circ$</td>
</tr>
<tr>
<td>Left-handed $\alpha$ helix ($\alpha_L$)</td>
<td>$+57^\circ$</td>
<td>$+47^\circ$</td>
</tr>
<tr>
<td>Parallel chain $\beta$-sheet ($\uparrow\uparrow$)</td>
<td>$-119^\circ$</td>
<td>$+113^\circ$</td>
</tr>
<tr>
<td>Antiparallel $\beta$-sheet ($\uparrow\downarrow$)</td>
<td>$-139^\circ$</td>
<td>$+135^\circ$</td>
</tr>
</tbody>
</table>
Which conformation is preferred?

The most favourable structure has the lowest potential energy, $V$.

$$V(\phi_i, \psi_i) = \sum V(\text{all intermolecular interactions})_{\text{all atoms}}$$

**Intermolecular Forces**

1. *Hydrogen Bonding*
   Integral to the three-dimensional structure of the protein.
   $V \approx -10$ to $-20 \text{ kJ mol}^{-1}$
   Between amide N and carbonyl O in $\alpha$-helix and $\beta$-pleated sheet.

   However, on the surface of a water-soluble protein:
   $$\text{H}_2\text{O} \cdots \text{NH} + \text{OH}_2 \cdots \text{O}=\text{C} \Leftrightarrow \text{NH} \cdots \text{O}=\text{C} + 2\text{H}_2\text{O}$$

   Thus, whether or not the formation of a H bond is favourable depends on the solvent.
   In the protein interior H bond formation will be more favourable than on the surface.
2. Hydrophobic Interactions

Some amino acids are non-polar and therefore do not dissolve readily in water, e.g. valine, leucine, isoleucine, tryptophan, phenylalanine. These amino acids prefer to be on the interior of a protein rather than on the surface.

Example:

Benzene in benzene → Benzene in water

\[ \Delta H \approx 0, \Delta S \approx -58.6 \text{ J K}^{-1}\text{mol}^{-1} \]

At 20°C (293K),

\[ \Delta G = \Delta H - T\Delta S = +17.2 \text{ kJ mol}^{-1} \]

Thus, the interaction of phenylalanine with water is unfavourable. Transfer of phenylalanine into a non-polar environment (protein interior) is favourable (\( \Delta G < 0 \))

Note: \( V (\Delta G) \approx -20 \text{ kJ mol}^{-1} \) is comparable to H bonding energy.
3. van der Waals potential

The van der Waals potential is the sum of two contributions:
1) short range repulsion
   (repulsion of electron clouds, i.e. Pauli exclusion principle, and nucleus-nucleus repulsion)
2) London dispersion force (induced dipole-induced-dipole attraction)

These two contributions can be described together mathematically by the 6-12 or Lennard-Jones potential function:

\[
V_{vdW} = \frac{A}{r^{12}} - \frac{B}{r^6}
\]

where \(r\) is the distance between the centres of the two atoms, and A and B are coefficients specific to the atoms involved.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>A (kJ nm(^{12})/mol)</th>
<th>B (kJ nm(^6)/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H···H</td>
<td>1.84 x 10(^{-8})</td>
<td>1.92 x 10(^{-4})</td>
</tr>
<tr>
<td>H···C</td>
<td>1.57 x 10(^{-7})</td>
<td>5.27 x 10(^{-4})</td>
</tr>
<tr>
<td>H···N</td>
<td>1.11 x 10(^{-7})</td>
<td>5.15 x 10(^{-4})</td>
</tr>
</tbody>
</table>
Equilibrium distance, $r_0 = \text{sum of the van der Waals radii of the two atoms}$

At $r_0$, $V \approx 0 \text{ to } -40 \text{ kJ mol}^{-1}$, but falling off rapidly at longer distances.
Therefore, only becomes important when atoms are closely packed.
4. Other electrostatic interactions

<table>
<thead>
<tr>
<th>Type of Interaction</th>
<th>Equation</th>
<th>Order of Magnitude (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion-ion</td>
<td>$E = \frac{Z_1Z_2e^2}{Dr}$</td>
<td>60</td>
</tr>
<tr>
<td>Ion-dipole</td>
<td>$E = \frac{Ze\mu_1\mu_2}{Dr^2}$</td>
<td>-8 to +8</td>
</tr>
<tr>
<td>Dipole-dipole</td>
<td>$E = \frac{\mu_1\mu_2\theta'}{Dr^3} - \frac{3(\mu_1r\theta')(\mu_2r\theta'')}{Dr^5}$</td>
<td>-2 to +2</td>
</tr>
<tr>
<td>Ion-induced dipole</td>
<td>$E = \frac{Ze^2\alpha_3}{2Dr^4}$</td>
<td>0.2</td>
</tr>
<tr>
<td>Dispersion</td>
<td>$E = \frac{3h\pi\alpha^2}{4Dr^6}$</td>
<td>0 to 40</td>
</tr>
</tbody>
</table>

Note the dependence of $V$ on $1/r^n$

$n$ small $\rightarrow$ long range interaction
$n$ large $\rightarrow$ short range interaction
What ions are present?

\[
-\text{NH}_3^+ \cdots -\text{OOC-}
\]

salt bridges, e.g. between glutamic acid and lysine.

What dipoles are present?

\[
\text{H}_2\text{O}
\]

In order to maximize the interaction with water, charged amino acid residues, i.e. glutamic acid, aspartic acid (negative), and histidine, lysine, arginine (positive), are generally located on the surface of proteins.

5. Torsional potential

There is a small barrier to rotation about single bonds. Thus, the \( \phi \) and \( \psi \) rotations have intrinsic torsional hindrance potentials, with three minima (60°, 180° and 300°). The barrier heights between minima are quite small, \( \approx 4 \text{ kJ mol}^{-1} \).
Energy Contour Diagram

L-alanyl residue

Region I: right-handed $\alpha$-helix
Region II: left-handed $\alpha$-helix
Region III: $\beta$-pleated sheet

$X =$ energy minimum

$\rightarrow$ poly-L-alanine prefers an extended $\beta$-pleated sheet conformation
Intramolecular Forces

Disulphide bridges, -S-S-

Bond energy S-S = 268 kJ mol\(^{-1}\)

Occurs between cysteine residues:

```
  |      
O=C-CH-CH\(_2\)-SH 
  |      
NH\(_2\) 
```

Allows cross-linking between polypeptide chains and the stabilisation of the protein folding (i.e. 3D structure).

Examples

*Collagen* (skin, cartilage, tendon, bone)
3 intertwined type II trans-helices → strong fibrous protein

*Keratin* (wool, hair)
α-helical linked by disulphide bridges,

*Silk*
Antiparallel β-pleated sheet
Collagen

*Amino acid composition*

- Unusually high glycine content, \( \sim 33\% \) (cf. for a typical globular protein, such as haemoglobin, \( \sim 5\% \))
- High proline content, \( \sim 25\% \)
- Unusual amino acids, 4-hydroxylysine and 5-hydroxyproline
- Very regular sequence, i.e. nearly every 3\(^{rd}\) residue is glycine and the sequence, gly-pro-hydroxypro, occurs frequently (regularities rarely occur in globular proteins)

Note: Hydroxyproline and hydroxylysine are formed by hydroxylation after protein synthesis.
Secondary Structure

The ring structure of proline does not allow collagen to form an \(\alpha\)-helix. Instead it forms a more open type II trans helix, stabilized by steric repulsion of the proline rings (no H-bonding within a chain).

The small glycine residues allow 3 helices to intertwine to form triple helix, with glycine (red) in the core and the proline rings at the exterior.

The triple helix is stabilized by H bonds between the peptide C=O and N-H residues of glycines from neighbouring chains and by H bonds between neighbouring O-H groups of hydroxyproline and C=O groups.
Melting Curve of Collagen

Above the melting temperature, $T_m$, collagen converts from a rod-like shape to a random coil (gelatin).

$T_m$ of collagen lacking hydroxyproline is $\sim 15^\circ$C. $T_m$ of normal collagen is $\sim 50^\circ$C. Thus, at a body temperature of 37°C, hydroxylation of proline is essential for collagen to maintain its rigid structure.
Molecular basis of Scurvy

1536: Jacques Cartier, explorer of the St. Lawrence River, North America:
"Their mouths became stinking, their gums so rotten that all the flesh did fall off, even to the roots of the teeth, which did also almost fall out."

1753: Scottish physician, James Lind, urges the inclusion of lemon juice in the diet of sailors (adopted 40 years later by the Royal Navy).

Scurvy is caused by a dietary deficiency of vitamin C (ascorbic acid)
Ascorbic Acid

Ascorbic acid is an effective reducing agent, which maintains the enzyme *prolyl hydroxylase* in its active reduced form and thus ensures the hydroxylation of proline residues of collagen, necessary for its thermal stability.

Oxidation of ascorbate:

\[
\text{Ascorbate} \rightarrow \text{Dehydroascorbic Acid}
\]

Etymology:

\[
a + \text{scorb(ut)ic acid}
\]

*scorbut* (French) = scurvy
Tertiary Structure

Individual *tropocollagen* triple helices undergo intermolecular crosslinking (covalent bonds) into mature collagen fibres by the reaction of lysine side chains.

The reaction is catalyzed by the enzyme *lysyl oxidase*, which causes an aldol condensation reaction between the aldehyde derivatives of two lysine sidechains on adjacent triple helices.