Role of Hydrogen Bonding on Protein Secondary Structure

Introduction

The function and chemical properties of proteins are determined by its three-dimensional structure. The final “architecture” of the protein begins with the linear assortment of amino acids (its primary structure) and progresses through three other modifications (secondary -tertiary to quaternary structures) that create the final shape of the protein. These modifications are the result of various intermolecular forces that exists as a result of the protein’s primary structure, but are manifested in the proteins secondary structure. The secondary structure of proteins is defined as the local conformations of the primary backbone, which is characterized by regular repeating structures such as $\alpha$ (alpha) helixes and $\beta$ (beta) sheets. The structural stability of these repeating structures is the result of hydrogen bonding between the amide proton (the hydrogen atom bonded to the nitrogen of the peptide bond) and the carbonyl oxygen (the oxygen atom bonded to the carbon atom in the amide plane).

Alpha helixes and Beta sheets can function as independent peptides, (alpha keratin, collagen, and blood vessels). Beta sheets can be found in silk, and beta keratin, however they function predominantly as structural components of globular proteins. Globular proteins have varying proportions of helixes, sheets, and other structural elements known as turns, twists and crossovers. Hydrogen bonding is the primary stabilizing force in all of these structures. This lesson plan will introduce students to the chemistry of hydrogen bonding and its affect on the biology of the secondary structure of proteins.
Outline

I. Chemistry of Hydrogen Bonding
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      3. Calculation of electronegativity difference: related to bond polarity
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II. Secondary Structure of polypeptides
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   C. The Amide Plane and its affect on conformations (Figure 4)
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      2. Allowed Conformations and Steric Hindrance (Figures 5 and 6)
      3. Ramachandran Plot (Figure 7)
      4. Ramachandran Conformational angle distribution (Figure 7a)
   D. Helixes: Secondary structure defined
      1. \( p, n \) and \( n_m \) notation system (Figure 8)
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      a. Parallel and anti parallel constructions
Discussion

I. Chemistry of Hydrogen Bonding

Hydrogen bonds belong to the class of intermolecular forces that arise as a result of a molecule’s dipolar characteristic. Hydrogen bonds exist between the hydrogen atom in a polar molecule such as (NH) and an unshared pair of electrons in a nearby (highly electronegative atom) such as Nitrogen, Oxygen or Fluorine. The polarity of a covalent bond is the result of the difference in electronegativity between the atoms that are bonded together. Electronegativity is defined as the ability of an atom (in a molecule) to attract shared electrons to itself. Electronegativity values of the elements can be found in figure 1. To determine the relative polarity of a covalent bond, one uses the values in the table of to calculate the difference in relative strength between bonded atoms: A molecule such as NH has an electronegativity difference of 0.8; which suggests a polar covalent bond. (Typically values between 0 and 0.5 suggest a non-polar covalent bond, 0.5 to 2.0 a polar covalent bond and 2.0 and above represent ionic compounds). The charge separation in these bonds is such that the electropositive hydrogen atom carries a partial positive charge ($\sigma^+$) and the more electronegative atom such as Nitrogen carries a partial negative charge ($\sigma^-$). (See figure 2). A hydrogen bond results when the partially positive hydrogen approaches the lone pair of a Nitrogen, Oxygen or Fluorine atom. Hydrogen bonds exist preferentially between these atoms because of two reasons. The hydrogen atom is small and has but one electron in its electron cloud, when the more electronegative atom distorts the cloud, the nucleus is not shielded by other electrons (as in other atoms), thus the lone pair of the approaching atom can approach the nucleus fairly directly. Secondly, nitrogen, oxygen and fluorine make exceptionally strong hydrogen bonds because of their high electronegativity and relatively small radius. Their small size means that their electron cloud is confined to a small region of space about the atom, this contributes to the strong directionality characteristic of hydrogen bonds. The combination of these factors makes for exceptionally strong interactions between these sets of atoms. The effect of hydrogen bonding can be seen in the graph (Boiling points of group 4, 5, 6 and 7). The increased boiling point of H$_2$O, NH$_3$ and HF is the result of their hydrogen bonds.

Although hydrogen bonds energies may seem small on the order of 4 to 25 kJ/mole (Brown & LeMay, p 414), it should be remembered that they normally exist as a system of interconnections: thus they are additive and contribute greatly to the overall stability of systems such as the polypeptide polymer.

A typical hydrogen bond is shown in Figure 2: Hydrogen bonding in water and between the Carbonyl atom and the Amide Hydrogen of a polypeptide.) In the figure, the delta positive $\sigma^+$ represents the positive pole and the $\sigma^-$ is the negative pole. Typically in the Polypeptide chain, the hydrogen bonded to the Nitrogen atom is termed (D-H) hydrogen donor and the Carbonyl oxygen is the (D-A); hydrogen acceptor. This notation will be used in the subsequent discussion of hydrogen bonding in the secondary structure of proteins.

II. Secondary Structure of Polypeptides:

The primary structure of proteins is the linear sequence of amino acid residues. These residues are linked together by amide bonds between the amine and carboxylic acid ends of respective amino acids. The resulting amide bonds are planar and exist on either side of the alpha carbons of the residues: they are referred to as amide planes (see figure 4).
These alpha carbons have a tetrahedral geometry, thus the amide planes have free rotation about this bond (See Figure 4a: Rotations about the Alpha Carbon Bond), however they cannot rotate freely because of the steric interactions between the atoms of each amide plane. Figure 5 shows the least hindered conformation of the amide planes: note the trans orientation of the R groups and carbonyl carbons: this is the most energetically favorable conformation of the amide planes. Figure 6 shows a “forbidden conformation” between the carbonyl oxygen and amide hydrogen of two amide planes. The arrangement is forbidden because the atoms are “closer” than the normally allowed van der Waals radius, thus this combination of angles for $\phi$ & $\psi$ is not allowed.

G.N Ramachandran used this logic and the normally allowed interatomic distances for the atoms in the peptide polymer to determine the sterically allowed $\phi$ & $\psi$ values for the dihedral angles: (See figure 6a: The dihedral angle). The plot of $\phi$ vs. $\psi$: (a Ramachandran plot: figure 7) shows the allowed combinations of $\phi$ & $\psi$. The areas in the second quadrant show the allowed dihedral angle combinations for beta sheets, and those in the third quadrant are values for alpha helixes. Ramachandran’s diagram was later confirmed by the X-Ray crystallography of ~1000 non-Glycine residues: see figure 7a). (Glycine’s structure allows for a larger area of allowed values.)

The secondary structure of a polypeptide is created by the local interaction (hydrogen bonds) between the carbonyl oxygen and amide hydrogens within the alpha helix and between beta sheets. The alpha helix and the beta sheet are the only conformations whose dihedral angles fall within the allowed values of $\phi$ & $\psi$ as determined by the Ramachandran diagram and allow for favorable hydrogen bond formation. The alpha helix has torsion angles of $\phi = -57^0$ and $\psi = -47^0$: parallel beta sheets have angles of $\phi = -119^0$ and $\psi = +113$: anti parallel sheets have angles of $\phi = -139^0$ and $\psi = +135^0$ (Voet & Voet pg 147.)

**Helixes**

As mentioned the alpha helix is energetically favorable arrangement. The twisting pattern creates hydrogen bonds within the same polypeptide chain. Helixes are described by their pitch (p): distance per turn, (n) the number of peptide residues per turn and (m) the number of (figure 8 explains the p, n system of nomenclature), and figure 8a shows the most favorable angles for the alpha helix. While there are various p and n helical arrangements (figure 9 tells us that the right handed alpha helix provides the optimal number of residues per turn, a “nearly optimum” hydrogen bond distance and the helix is packed tightly enough so as to enhance Van der Waals associations between neighboring atoms. (Voet & Voet pg. 149). This then is the better secondary helical arrangement. Refer to figure 3a: The alpha helix: note the intra-peptide hydrogen bonding, that is characteristic of an alpha helix.

**Beta Sheets:**

Beta sheets are the second type of secondary structures with optimal dihedral angles and close hydrogen bonding distances. The bonding in these sheets is between neighboring sheets, which can be oriented in a parallel or anti parallel orientation. Figure 10 shows these arrangements. The direction of the peptide chain in the upper polypeptide chain has the amide nitrogen to the left of the carbonyl oxygen, while in the lower chain the amide is to the right of the carbonyl oxygen. The hydrogen bonding in ant parallel sheets “connect” head on creating a stable bonding environment. Anti parallel sheets are typically from 6 – 25 residues long and can consist of from 2 to 15 chains. Parallel beta sheets have the same direction of N to C in their chains, however the hydrogen bonding occurs at a slant, creating a less favorable interactions.
Figure 1: Table of Electronegativity

→ **Atomic radius** decreases → **Ionization energy** increases → **Electronegativity** increases →

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Periodic table of electronegativity using the [Pauling scale](http://www.mywiseowl.com/articles/Pauling_scale)

See also [Periodic table](http://www.mywiseowl.com/articles/Periodic_table)

Source: [http://www.mywiseowl.com/articles/Pauling_scale](http://www.mywiseowl.com/articles/Pauling_scale)
Figure 2: Affects of Polar Bond

Two water molecules  Two parts of one large molecule

Source: http://bio.winona.edu/bates/Bio241/images/figure-02-09.jpg
Figure 2a: Affect of Hydrogen Bonding on Boiling Point of Group 5A, 6A, & 7A Halides

Source: http://www.chemguide.co.uk/atoms/bonding/hbond.html
Figure 3: Hydrogen Bonding in the Beta Sheets: Parallel and Anti Parallel

β Sheets
Chains are nearly completely extended
Hydrogen bonds are non-local
Antiparallel sheets more stable
Twisted in 3D

http://www.olemiss.edu/courses/chem471/Lecture10.pdf
Figure 3a. Hydrogen Bonding if the Alpha Helix

Source: http://www.olemiss.edu/courses/chem471/Lecture10.pdf
Figure 4: Peptide Bonds are Planar

Figure 7-1. The trans-peptide group.

Figure 7-2. The cis-peptide group.

Source: http://www.nd.edu/~aseriann/ctpep.html
Figure 4a: Rotations about the Alpha Carbon

Figure 5: Energetically Favorable Conformations of Amide Planes

**Torsion angles**

Rotation or dihedral angles

\[
\begin{align*}
C_{\alpha-N} & \quad \phi \quad \text{phi} \\
C_{\alpha-C} & \quad \psi \quad \text{psi}
\end{align*}
\]

When a peptide chain is fully extended the angles are defined as 180° or -180°.

At 180° one gets a staggered conformation. (all trans) i.e. ethane

Note: alternating C=O pointing in opposite directions.

Figure 6: Sterically Hindered conformation about the Amide Plan.

Steric hindrance between the amide nitrogen and the carbonyl

$\Phi = -60^\circ$ and $\Psi = 30^\circ$

Ramachandran plot of sterically accessible conformations

- Only certain combinations of phi and psi are allowed (others bump)
- Regular secondary structures have defined locations on the $\Phi \Psi$ map

Source: [http://www.olemiss.edu/courses/chem471/Lecture10.pdf](http://www.olemiss.edu/courses/chem471/Lecture10.pdf)
Secondary structure can be defined by $\phi$ and $\psi$ angles

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<td>$\beta$ sheet</td>
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<tr>
<td>$\downarrow\uparrow$ $\beta$ sheet</td>
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<td>$3_{10}$ helix</td>
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<td>-26</td>
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<tr>
<td>collagen</td>
<td>-51</td>
<td>153</td>
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</table>

Repeating local protein structure determined by hydrogen bonding helices and pleated sheets.

12 proteins except for Gly and Pro

The $N_m$ nomenclature for helices

$N =$ the number of repeating units per turn

$M =$ the number of atoms that complete the cyclic system that is enclosed by the hydrogen bond.
The most favorable $\Phi$ and $\Psi$ angles with little steric hindrance.
Forms repeated hydrogen bonds.
$N = 3.6$ residues per turn
$P = 5.4 \, \text{Å}$ (What is the $d$ for an $\alpha$ helix?)
The $C=O$ of the $n^{th}$ residue points towards the $N-H$ of the $(N+4)^{th}$ residue.

The $N \cdots H \cdots O$ hydrogen bond is $2.8 \, \text{Å}$ and the atoms are $180^\circ$ in plane. This is almost optimal with favorable Van der Waals interactions within the helix.

Figure 9: Examples of Helices

Examples of helices

Figure 10: Parallel and Anti Parallel

(b) Parallel

http://cmgm.stanford.edu/biochem201/Slides/Protein%20Structure/Parallel%20Beta-strands.JPG

(a) Antiparallel

http://cmgm.stanford.edu/biochem201/Slides/Protein%20Structure/Anti_parallel%20Beta-Strands.JPG
References:
