D-ribose (natural enantiomer)

Mirror Images

L-ribose (not present in any natural nucleic acids)
• Double-stranded DNA is *antiparallel*
Purines

Adenine

Guanine

Pyrimidines

Thymine (DNA only)

Cytosine
Problem: The GC base pairs are more stable than AT base pairs. Why?
5. Triple-stranded DNA was first observed in 1957. Scientists later discovered that the formation of triple-stranded DNA involves a type of base pairing called Hoogsteen pairing, which differs from the Watson-Crick base pairing observed in normal double-stranded DNA. Below is an example of a Hoogsteen base pair.

\begin{itemize}
  \item[a.] Identify each of these bases by name.
  \item[b.] Redraw these two base pairs showing all lone pair electrons.
  \item[c.] Draw the hydrogen bond interactions expected for this Hoogsteen base pair.
\end{itemize}
a. Formation of a triple helix requires three bases to interact with each other to form a “base trio” rather than a “base pair”. One way in which this three-way interaction can take place is for the three bases to engage in both Watson-Crick base pairing and Hoogsteen base pairing. Show how two thymines and an adenine could interact with each other to form a “base trio” by using both Watson-Crick and Hoogsteen base pairing.
(D)-Ribose found in RNA

No methyl group

Uracil (RNA only)

(D)-2'-Deoxyribose found in DNA

Thymidina (DNA only)
The DNA molecules have T instead of U (which is found in RNA).

Can you hypothesize why that is, knowing that it was established that the amino group in the cytosine is spontaneously hydrolyzed about 120 times in a day in a normal human cell.
Problem: deaminated C is identical to U (and pairs with A)
Base Pairing Determines Selectivity During DNA Polymerization

- The nucleotide capable of forming a base pair with the template is added to the primer

Extended primer with new 3’ OH group
DNA Polymerization in Action II

ATG

CTP

AG

GT

CA

G

T

C

5'

3'

template

primer

dCTP
dGTP
dTTP
dATP

(dCTP)
Problem:

The nucleotide sequence of one DNA strand of a DNA double helix is:

5’-GGATTCTTTTGCGATCGGG-3’

What is the sequence of the complementary strand?
Lamivudine is phosphorylated in a cell and is a substrate for HIV RT.

Lamivudine is a nucleotide analogue. Which one?

Based on your knowledge of DNA polymerization can you predict the effect of this molecule on RT and DNA polymerization.
The PCR Cycle

1. Melt template (heat)
2. Extend primers
3. Hybridize primers (cool)
4. Repeat
Problem

To amplify the DNA fragments Taq polymerase is used during the PCR reaction. Can you name some of the qualities of Taq polymerase which make it the enzyme of choice?

Can you name several differences between Taq polymerase and human polymerase?

The incorporation of the non-complementary nucleotide within the daughter chain of DNA is a relatively rare event. Why?
Problem:

Two strands of DNA double helix can be separated by heating. If you raise the temperature in a solution that has the following three molecules, in what order do you suppose they would melt?

GCGGGGGCTCCCCCGATTTTCGGGCGCCCGGGGTTTTTAAAA
CGCCCCCGAGGGGCTAAAGCCCGCGGGCCCCCAAATTTT

ATTTTTTCCTTTTAACGCTCAGTGCATGCCCCATATCGATGC
TAAAAAGGAAAATTGCGAGTGTACGTTAGCTACG

AGATCGCTCAGGCTGCAATG
TCTAGCGAGTCCGACGTAC
DNA polymerase

dATP, dTTP, dGTP, dCTP

3'  5'
|    |    |
A    T
T    A
T    A
G    C
C    G
T    T
C    A
T    A

3'  5'
|    |    |
A    T
T    A
T    A
G    C
C    G
A    T
A    T
G    C
T    A

DNA polymerase

dATP, dTTP, dGTP, dCTP

72°
Reverse transcriptase incorporates 10 to 100 times more DNA errors when generating the daughter DNA molecules, unlike mammalian polymerases. Explain how this affects the use of drugs that target viral protein-dependent functions?

You need to diagnose HIV infection. Briefly describe how you could go about detecting the target?
LS1a Review Session

• Peptide bond
• Structures and properties of the 20 key amino acids
• Amino acid interactions

Alison Stewart
Sunday, October 15th, 2006
Peptide bond

- Amide or peptide bond
- What is unique about this bond?
Resonance of peptide bond

- Double bond character
- Does not rotate
- Polar due to resonance, strong dipole
Amino acid structure

- All amino acids have the same general formula, but differ in their side chains or R groups
Aspartate or Aspartic acid

- Acidic
- Aspartic acid or Aspartate = Asp = D
- pKa = 4 (Do not need to know!)
Glutamate or Glutamic acid

- Acidic
- Glutamic acid or GlutamatE = Glu = E
- pKa = 4.5 (Do not need to know!)
- Basic
- Lysine = Lys = K
- pKa = 10.5 – 11 (Do not need to know!)
• Basic
• Arginine = Arg = R
• pKa = 12 (Do not need to know)
Histidiné

- Basic
- Histidiné = His = H
- pKa = 6-7 (Do not need to know!)
• Polar
• Serine = Ser = S
Threonine

- Polar
- Threonine = Thr = T
Cysteine

- Polar, not very polar so can be called nonpolar
- Cysteine = Cys = C
- Makes disulfide bonds!!!
Proline

- Important for peptide shape
- Conformationally constrained
- Can be cis OR trans
- Proline = Pro = P
Asparagine

- Polar
- Asparagine = Asn = N
Glutamine

- Polar
- Glutamine = Gln = Q
Phenylalanine

- Cyclic, nonpolar
- Phenylalanine = Phe = F
- Cyclic, polar
- Tyrosine = Tyr = Y
Tryptophan

- Cyclic
- Tryptophan = Trp = W
Glycine

- Important for peptide shape
- Conformationally flexible
- Glycine = Gly = G
Alanine

- Nonpolar
- Alanine = Ala = A
Valine

- Nonpolar
- Valine = Val = V
Leucine

- Nonpolar
- Leucine = Leu = L
Methionine

- Nonpolar
- Methionine = Met = M
Isoleucine

- Nonpolar
- Isoleucine = Ile = I
Amino Acid Interaction Question 1

• Which amino acids can form ionic bonds (at pH 7)?

• Two categories:

  • Acidic: Aspartic acid and Glutamic acid

  • Basic: Histidine, Lysine, Arginine
Amino Acid Interaction Question 2a

• Which amino acids can participate in hydrogen bonding (via their side chains)?

• Acidic: Aspartic acid and Glutamic acid

• Basic: Histidine, Lysine, Arginine

• Polar: Asparagine, Glutamine, Threonine, Serine, Tyrosine, Tryptophan
  (Tyr and Trp both are also nonpolar and cyclic)
Amino Acid Interaction Question 2b

• What is unusual about hydrogen bonding for proline?

• It is conformationally constrained so it can be inaccessible to make linear H bonds
• The nitrogen is positively charged so it can only be a donor not an acceptor
Amino Acid Interaction Question 3

- What is the strongest interaction between amino acids?

- Two good answers:
  - The peptide or amide bond in the backbone
  - Disulfide bonds between cysteines
Amino Acid Interaction Question 4

• Which amino acids interact most strongly via Van der Waals forces?

• Nonpolar: Alanine, Leucine, Isoleucine, Methionine, Valine

• Cyclic: Phenylalanine, (Tryptophan & Tyrosine can potentially H bond or have dipole interactions)

• Other: Glycine, Proline (possible for the amine in Pro to be an H bond donor if it aligns properly)
Amino Acid Interaction Question 5

• Which amino acids are important for peptide shape?
  • Glycine, Why?
    – conformationally flexible
  • Proline, Why?
    – cis OR trans, conformationally constrained bends backbone
  • Cysteine, Why?
    – makes disulfide bonds, covalently links sections of peptide together
Peptide bond, Amino acid structure or interaction questions?
At last!
Protein folding & thermodynamics!
Anfinsen’s protein folding experiment

Conclusion: The information required to fold a protein is contained within its sequence
Four levels of protein structure

1) Primary Structure: The linear sequence of amino acids
3) Tertiary Structure: The fold of a single polypeptide chain.
4) Quaternary Structure: The interaction of individually folded polypeptide chains to form a higher order complex.
Protein Folding

What stabilizes a given fold?
Thermodynamic interactions that stabilize the folded state of a protein

Ionic bonding (salt bridges)
Thermodynamic interactions that stabilize the folded state of a protein

Hydrogen bonds in a protein molecule
Thermodynamic interactions that stabilize the folded state of a protein

The hydrophobic effect
Details about α-helices
Secondary Structure: $\beta$-sheets
How do we understand this equilibrium?

\[ U \rightleftharpoons F \]

\[ K_{eq} = \frac{[F]}{[U]} \]

\[ \Delta G^\circ = -RT \ln K_{eq} \]

\[ G = \text{Gibbs Free Energy} \]

\[ \Delta = \text{Change} \]

**Recall 3 Cases:**
- \( K_{eq} > 1 \): rxn favors folded state
- \( K_{eq} < 1 \): rxn favors unfolded state
- \( K_{eq} = 1 \): equal mixture of U and F

**3 Cases:**
- \( \Delta G^\circ < 0 \): rxn favors folded state
- \( \Delta G^\circ > 0 \): rxn favors unfolded state
- \( \Delta G^\circ = 0 \): equal mixture of U and F
Protein Folding

U → ΔS → F

Water plays a large role in protein folding.
Shown below is a picture of a protein. The major interactions that hold the protein in its properly folded conformation are highlighted in yellow.

Briefly describe each interaction a-e