Lehninger

SIXTH EDITION

Principles of Biochemistry

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8| Nucleotides and Nucleic Acids

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Week 8 Nucleotides and Nucleic Acids

8.1 Some Basics

8.2 Nucleic Acid Structure

8.3 Nucleic Acid Chemistry

8.4 Other Functions of Nucleotides

Functions of Nucleotides

- Energy for metabolism (ATP)
- Enzyme cofactors (NAD^+, FAD)
- Signal transduction (cAMP, cGMP)

Nicotinamide adenine dinucleotide (NAD^+)

Adenosine 3′,5′-cyclic monophosphate (cyclic AMP; cAMP)

Functions of Nucleic Acids

- Storage of genetic info (DNA)
- Transmission of genetic info (messenger RNA)
- Protein synthesis (transfer RNA and ribosomal RNA)

Functions of DNA

- Deoxyribonucleic acid (DNA)
- **Gene**
  - A segment of DNA that contains information required to synthesize a functional biological product (protein or RNA).
- Storage and transmission of biological information are the only known functions of DNA.

Functions of RNA

- Ribonucleic acid (RNA)
- A broad range of functions
  - Ribosomal RNA: catalyze protein synthesis.
  - Messenger RNA: carry genetic information from genes to ribosome.
  - **Transfer RNA**: Translate information in mRNA into sequence of amino acids.

Building Blocks of Nucleic Acids

- Nucleotides are building blocks of nucleic acids.
- Nucleotide:
  - Nitrogenous (nitrogen-containing) base
  - Pentose (five-carbon ribose sugar)
  - Phosphate
- Nucleoside:
  - Nitrogenous base (nucleobase)
  - Pentose

Nucleobases

- Nitrogen-containing heteroaromatic molecules
  - Derivatives of **purine** or **pyrimidine**
- Planar or almost planar structures
- Absorb UV light around 260 nm

Purine

Pyrimidine

Nitrogenous Bases

Purine

Adenine

Guanine

Purines

Pyrimidine

Cytosine

Thymine (DNA)

Uracil (RNA)

Pyrimidines

Purine Bases

- Both are good H-bond donors and acceptors.
  - Adenine and guanine are found in both **RNA** and **DNA**.

Pyrimidine Bases

- All are good H-bond donors and acceptors.
  - Cytosine is found in both **DNA** and **RNA**
  - Thymine is found only in **DNA**
  - Uracil is found only in **RNA**

Pentose

- Ribose in RNA
  - β-D-ribofuranose ring structure
- Deoxyribose in DNA
  - β-D-**2'-deoxy**ribofuranose ring structure

Aldehyde β-Furanose

DNA RNA

N-Glycosidic Bond

- Pentose ring attached to nucleobase via ***N*-glycosidic bond**.
- Glycosidic bond formed to **anomeric carbon** of sugar in β configuration
- Glycosidic bond formed with:
  - Atom **N9 in purines**
  - Atom **N1 in pyrimidines**

Purine

Pyrimidine

Conformation of Glycosidic Bond

- Relatively free rotation can occur around the *N*-glycosidic bond in free nucleotides.
  - Purines (A and G) occur in **anti** or **syn** conformation.
  - Pyrimidines (C, T and U) only occur in anti conformation.
- Anti conformation is found in natural DNA.

Numbering of Base and Sugar

- Carbon and nitrogen atoms in base are numbered from 1 to 6 (or 9).
- Carbon and oxygen atoms in sugar ring are given a prime (’) designation.

Purine

3’-hydroxyl group

5-methyl group

Pyrimidine

Phosphate Group

- Phosphate group bridge
  - 3'-hydroxyl group of previous unit
  - 5'-hydroxyl group of following unit
- **Phosphodiester** linkage
  - Alternating phosphate and pentose residues in backbone
  - Bases may be viewed as side chains
- Nucleic acid is soluble in water
  - Negative charge of phosphate group
  - H-bond of sugar ring

Nucleic Acid vs. Polypeptide

- Linkage
  - Nucleic Acid: phosphodiester bond
  - Polypeptide: peptide (amide) bond
- Backbone
  - Nucleic acid: alternating phosphate and pentose residues
  - Polypeptide: alternating amide group and α-carbon atom
- Side chain
  - Nucleic acid: 4 nucleobases
  - Polypeptide: 20 various groups
- Sequence orientation
  - Nucleic acid: 5’ -> 3’
  - Polypeptide: N terminus -> C terminus

Hydrolysis of Nucleic Acid

- RNA is hydrolyzed rapidly under **alkaline** conditions.
  - DNA is stable under alkaline conditions.
- Deprotonated 2' hydroxyl group acts as a nucleophile.
  - DNA lacks 2' hydroxyl group.
- Phosphorus atom is target of nucleophilic attack.

Hydrolysis Product of RNA

- Cyclic 2’,3’-monophosphate nucleotide.
  - Further hydrolyzed to a mixture of 2’- and 3’-nucleoside monophosphate.
- Shortened RNA segment.

Nomenclature: Base and Nucleoside

Base	Nucleoside
Purines	
Adenine	Adenosine
	Deoxyadenosine
Guanine	Guanosine
	Deoxyguanosine
Pyrimidines	
Cytosine	Cytidine
	Deoxycytidine
Thymine	Thymidine or deoxythymidine
Uracil	Uridine

Adenine

Adenosine

Nomenclature: Ribonucleotide

Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

Ribonucleotides

ATP: adenosine 5’-triphosphate

Nomenclature: Deoxyribonucleotide

Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

Deoxyribonucleotides

dAMP: deoxyadenosine 5’-monophosphate

Summary 8.1 Some Basics

- A nucleotide consists of a nitrogenous base (purine or pyrimidine), a pentose sugar, and one or more phosphate groups.
- Nucleic acids are polymers of nucleotides, joined together by phosphodiester linkages between 5’-OH group of one pentose and 3’-OH group of the next.
- In RNA, the pentose is ribose, and the bases include A, G, C and U. In DNA, the pentose is deoxyribose, and the bases include A, G, C and T.

Week 8 Nucleotides and Nucleic Acids

8.1 Some Basics

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Polynucleotides

- Covalent bonds formed via phosphodiester linkages
  - Negatively charged backbone.
- Linear polymers
  - No branching or cross-links.
- Directionality
  - 5’ end is different from 3’ end.
  - We write/read the sequence from 5’ to 3’.

Natural DNA Forms a Double Helix

- Two helical DNA strands.
- Right-handed double helix.
  - Diameter 20 Å
  - 3.4 Å per base
  - 10.5 bases per helical turn.
  - 36 Å per turn
- Hydrophilic and hydrophobic parts.
  - Hydrophilic backbone on outside.
  - Bases stacked inside.

Complementarity of DNA Strands

- Two chains run antiparallel.
- Two chains are complementary.

Hydrogen Bonding Interactions

- Two bases can **hydrogen bond** to form a base pair.
- Purine pairs with pyrimidine.
  - A pairs with T
  - G pairs with C

Replication of DNA

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

— Watson and Crick, *Nature,* 1953

1. Strand separation occurs first.
2. Each strand serves as a template for the synthesis of a new strand.
   - Catalyzed by enzymes (DNA polymerases)
3. Newly made DNA molecule has one **daughter strand** and one **parent strand**.

Semi-conservative

DNA Replication: Structural Basics

DNA Replication: Enzymes

Unusual DNA Structures: Palindrome

- Palindromes: words or phrases that are the same when read backward or forward.
  - Level, noon, Anna, Bob.
  - Was it a car or a cat I saw?
- In molecular biology, a palindrome refers to a region of DNA with **inverted repeats** of sequence having twofold symmetry over two strands of DNA.
  - Self-complementary.
  - Form a hairpin.

Palindrome vs. Mirror Repeat

Palindrome can form hairpin.

Mirror repeat cannot form hairpin.

Messenger RNA

- DNA in nucleus, and protein synthesis in cytoplasm.
  - Messenger RNA carries genetic message from nucleus to cytoplasm.
- **Messenger RNA** is synthesized using **DNA template**.
  - Contains ribose instead of deoxyribose.
  - Contains uracil instead of thymine.

Monocistronic vs. Polycistronic

- One mRNA may code for more than one protein.
  - Monocistronic: code for only one polypeptide.
  - Polycistronic: code for two or more different polypeptides.
    - “Cistron” means gene.

Introns vs. Exons

- Polypeptide-encoding sequence may not be contiguous.
  - Introns: noncoding segments of mRNA.
  - Exons: coding segments of mRNA.
- RNA splicing.
  - Introns are removed and exons are joined to form a continuous sequence.

From DNA to Protein

Summary 8.2 Nucleic Acid Structure

- Native DNA consists of two antiparallel chains in a right-handed double-helical arrangement. Complementary base pairs, A=T and G≡C, are formed by hydrogen bonding within the helix.
- DNA strands with appropriate sequences can form hairpin structures.
- Messenger RNA transfers genetic information from DNA to ribosome for protein synthesis. Transfer RNA and ribosomal RNA are also involved in protein synthesis.

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Double Helical DNA Can Be Denatured

- Hydrogen bonds broken.
  - Two strands separate.
  - Reversible process (annealing).
- Covalent bonds remain intact.
  - Genetic information remain intact.
- Denaturation can be induced by high temperature, or change in pH.

Thermal DNA Denaturation (Melting)

- DNA exists as double helix at normal temperatures.
- Reversible thermal denaturation and annealing process.
  - Two strands dissociate at elevated temperatures.
  - Two strands re-anneal when temperature is lowered.

Factors Affecting DNA Denaturation

- Denaturation temperature, or melting point (T_m)
  - Half DNA is present as separated single strands.
- T_m depends on base composition.
  - High GC content increases T_m.
  - G≡C base pair is stronger than A=T.
- T_m depends on DNA length.
  - Longer DNA has higher T_m.
  - More H-bonds to break.

Partially Denatured DNA

- Large DNA molecules are not denatured uniformly.
- AT-rich regions melt at a lower temperature than GC-rich regions.

Spontaneous Mutagenesis: Deamination

- Deamination: loss of exocyclic amino group.
  - Very slow reactions (1 in 10^7 cytidine residues in 24 hours).
  - Net effect is significant: 100 C → U events/day/mammalian-cell.
  - Most are corrected by repair system (base excision repair).
- Why does DNA contain thymine rather than uracil?

Spontaneous Mutagenesis: Depurination

- Depurination
  - *N*-glycosidic bond is hydrolyzed.
  - 10,000 purines/day/mammalian-cell.
  - Most are corrected by repair system (base excision repair).
- Loss of pyrimidine bases occurs at a lower rate.

UV-Induced Pyrimidine Dimer Formation

- Cyclobutane Pyrimidine Dimer (CPD)
  - Condensation of 2 ethylene groups.
  - Form a cyclobutane ring.
- 6-4 photoproduct.
  - Linkage between C-6 and C-4.
- Corrected by repair system (nucleotide excision repair).

What Happens When DNA is Damaged

DNA Sequence Can Be Determined

- Sequence by synthesis
  - DNA polymerase.
  - Primer and **template** strands.
  - dNTPs and **ddNTP (dideoxynucleoside triphosphate) analog**.

No 3’-OH group.
Chain terminator.

Sequencing by Sanger's Method

- Polymerase reaction
  - Radioactively labeled primer.
  - Template to be sequenced.
  - All 4 dNTPs.
- Four parallel reactions
  - A small amount of a single ddNTP in each reaction.
  - Appropriate dNTP/ddNTP ratio.
  - Premature chain termination.

Sequencing Results

- Sequence read from gel electrophoresis (from bottom to top):
  - 5'-GATTCGAGCTGA-3'
  - **Is this the sequence we want?**
  - This is the sequence of newly-synthesized strand.
- Sequence we actually want:
  - Complementary to the newly-synthesized strand.
    - 3'-**CTAAGCTCGACT**-5'
    - 5'-**GATTCGAGCTGA**-3'

Automated DNA Sequencing

- Modified polymerase reaction.
  - ddNTPs fluorescently labeled.
  - One single reaction instead of 4.
  - Primer not labeled.
- Gel electrophoresis.
  - In a capillary tube (fast separation).
  - Color detected using laser.
  - Color peak “translated” to DNA sequence.

DNA Sequencing

Automated DNA Synthesis

1. 1st monomer attached to silica support.
   - 5'-OH protected.
2. Deprotection.
   - 5'-OH exposed.
3. 2nd monomer comes in.
   - Phosphate group protected and activated.
4. 2nd monomer joined to 1st monomer.
   - Phosphate group protected.
   - Repeat step 2 - 4.
5. Base and phosphate group deprotected.
6. Cleaved from silica support.

DNA Synthesis vs. Peptide Synthesis

- Direction of synthesis.
  - DNA: 3’ -> 5’ (written from 5’ to 3’).
  - Peptide: C terminus -> N terminus (written from N to C).
- Length of product.
  - DNA: 100 nucleotides.
  - Peptide: 100 amino acid residues.

4 synthesis columns, silica support, where synthesis occurs

4 phosphoramidites, monomer building blocks

Summary 8.3 Nucleic Acid Chemistry

- Native DNA undergoes reversible unwinding and separation of strands on heating or pH change. DNA rich in G≡C have higher melting points than DNA rich in A=T.
- DNA is a relatively stable polymer. Spontaneous reactions such as deamination and depurination occur at low rates.
- DNA sequence can be determined, and oligonucleotides can be synthesized chemically.

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Carrier of Chemical Energy

- ATP is by far the most widely used chemical energy carrier.
  - GTP, UTP and CTP are also used in some reactions.
- Hydrolysis of **phosphoanhydride linkage** yields 30 kJ/mol energy.
  - α,β and β,γ linkages.
- Hydrolysis of **ester linkage** yields 14 kJ/mol energy.
  - bond between ribose and α phosphate.

Abbreviations of ribonucleoside 5′-phosphates			
Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

Abbreviations of deoxyribonucleoside 5′-phosphates			
Base	Mono-	Di-	Tri-
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

Components of Enzyme Cofactors

- Adenosine is present in a number of enzyme cofactor structures.
  - Not participate directly in reaction.
  - Likely involved in binding.
  - CoA, NAD^+, and FAD
- β-ketoacyl-CoA transferase.
  - Removal of 3'-P-ADP reduces reactivity by a factor of 10^6

Coenzyme A

Nicotinamide adenine dinucleotide (NAD^+)

Flavin adenine dinucleotide (FAD)

Regulatory Molecules

- Cells respond to environment.
  - External signal (1st messenger).
  - Interact with cell surface receptor.
  - 2nd messenger produced inside cell.
- cAMP, or cyclic AMP.
  - Phosphate group connects 3'-OH and 5'-OH groups.

Adenosine 3',5'-cyclic monophosphate (cyclic AMP; cAMP)

Leu and Ile

Amino acids for 8th week

- Leucine and Isoleucine are a pair of **constitutional isomers**.
  - Same chemical formula ($C_6H_{13}NO_2$).
  - Different bond connectivity.

Summary 8.4 Other Functions

- ATP is the central carrier of chemical energy in cell.
- The presence of adenosine in enzyme cofactors may be related to binding energy.
- Cyclic AMP is a common second messenger produced in response to external chemical signals.

Example Question

The compound that consists of ribose linked by an *N*-glycosidic bond to N-9 of adenine is:

A) a deoxyribonucleoside.
B) a purine nucleotide.
C) a pyrimidine nucleotide.
D) adenosine monophosphate.
E) adenosine.

Example Question

Phosphodiester bonds that link adjacent nucleotides in DNA:

A) always link A with T and G with C.

B) are hydrolyzed under alkaline conditions.

C) are uncharged at neutral pH.

D) form between the amino group and carbonyl carbon of adjacent bases.

E) join the 3'-OH of one nucleotide to the 5'-OH of the next.

Example Question

The alkaline hydrolysis of RNA does *not* produce:

A) 2′-AMP.
B) 2′,3′-cGMP.
C) 2′-CMP.
D) 3′,5′-cAMP.
E) 3′-UMP.

Example Question

For the oligoribonucleotide pACGUAC:

A) the nucleotide at the 3′ end has a phosphate at its 3′ hydroxyl.

B) the nucleotide at the 3′ end is a purine.

C) the nucleotide at the 5′ end has a 5′ hydroxyl.

D) the nucleotide at the 5′ end has a phosphate on its 5′ hydroxyl.

E) the nucleotide at the 5′ end is a pyrimidine.

Example Question

In a typical double-stranded DNA:

A) A = G.
B) A = C.
C) A = U.
D) A + T = G + C.
E) A + G = T + C.

Example Question

Natural double-stranded DNA is a _____-handed helix, _____ Å in diameter, with a rise of ____ Å per base pair.

A) left; 20; 3.4
B) right; 2; 3.4
C) right; 2; 34
D) right; 20; 3.4
E) right; 2; 34

Example Question

In the Watson-Crick model of DNA structure:

A) both strands run in the same direction, 3' -> 5'; they are parallel.
B) phosphate groups project toward the middle of the helix, where they are protected from interaction with water.
C) T can form three hydrogen bonds with either G or C in the opposite strand.
D) the distance between the sugar backbone of the two strands is just large enough to accommodate either two purines or two pyrimidines.
E) the distance between two adjacent bases in one strand is about 3.4 Å.

Example Question

In nucleotides and nucleic acids, syn and anti conformations relate to:

A) base stereoisomers.
B) rotation around the phosphodiester bond.
C) rotation around the sugar-base bond.
D) rotation around sugar-phosphate bond.
E) sugar stereoisomers.

Example Question

Which of the following deoxyoligonucleotides will hybridize with a DNA containing the sequence (5′)AGACTGGTC(3′)?

A) (5′)CTCATTGAG(3′)

B) (5′)GACCAGTCT(3′)

C) (5′)GAGTCAACT(3′)

D) (5′)TCTGACCAG(3′)

E) (5′)TCTGGATCT(3′)

Example Question

In DNA sequencing by the Sanger (dideoxy) method:

A) radioactive dideoxy-ATP is included in each of four reaction mixtures before enzymatic synthesis of complementary strands.
B) specific enzymes are used to cut the newly synthesized DNA into small pieces, which are then separated by electrophoresis.
C) the dideoxynucleotides must be present at high levels to obtain long stretches of DNA sequence.
D) the role of the dideoxy-CTP is to occasionally terminate enzymatic synthesis of DNA where G occurs in the template strands.
E) the template DNA strand is radioactive.

Example Question

Which of the following statements about chemical synthesis of DNA is *false*?

A) the synthesis reaction is not catalyzed by DNA polymerase.

B) the direction of synthesis is from 3′ to 5′.

C) the maximum length of oligonucleotide that can be synthesized is 10 nucleotides.

D) the nucleotide initially attached to the silica gel support will become the 3′ end of the finished product.

E) the synthesis process involves protection and deprotection of phosphate and base functional groups.

Example Question

The composition (mole fraction) of *one* of the strands of a double-helical DNA is [A] = 30%, and [G] = 24%. Calculate the following, if possible. If impossible, write "I."

For the *same* strand:

[T] = **I**

[C] = **I**

[T] + [C] = **46%**

For the *other* strand:

[A] = **I**

[T] = **30%**

[A] + [T] = **I**

[G] = **I**

[C] = **24%**

[G] + [A] = **46%**

Example Question

Based on the spontaneous deamination of cytosine, explain why it is advantageous for DNA to contain thymine, not uracil.

Example Question

What happens in automated Sanger DNA sequencing reaction if you forget to add:

a) all four fluorescent ddNTPs?

b) fluorescent ddGTP?

Example Question

What happens in automated Sanger DNA sequencing reaction if you forget to add:

c) all four dNTPs?

d) dGTP?