Setif 1 University – Ferhat ABBAS

Structural Biochemistry Tutorial Handout

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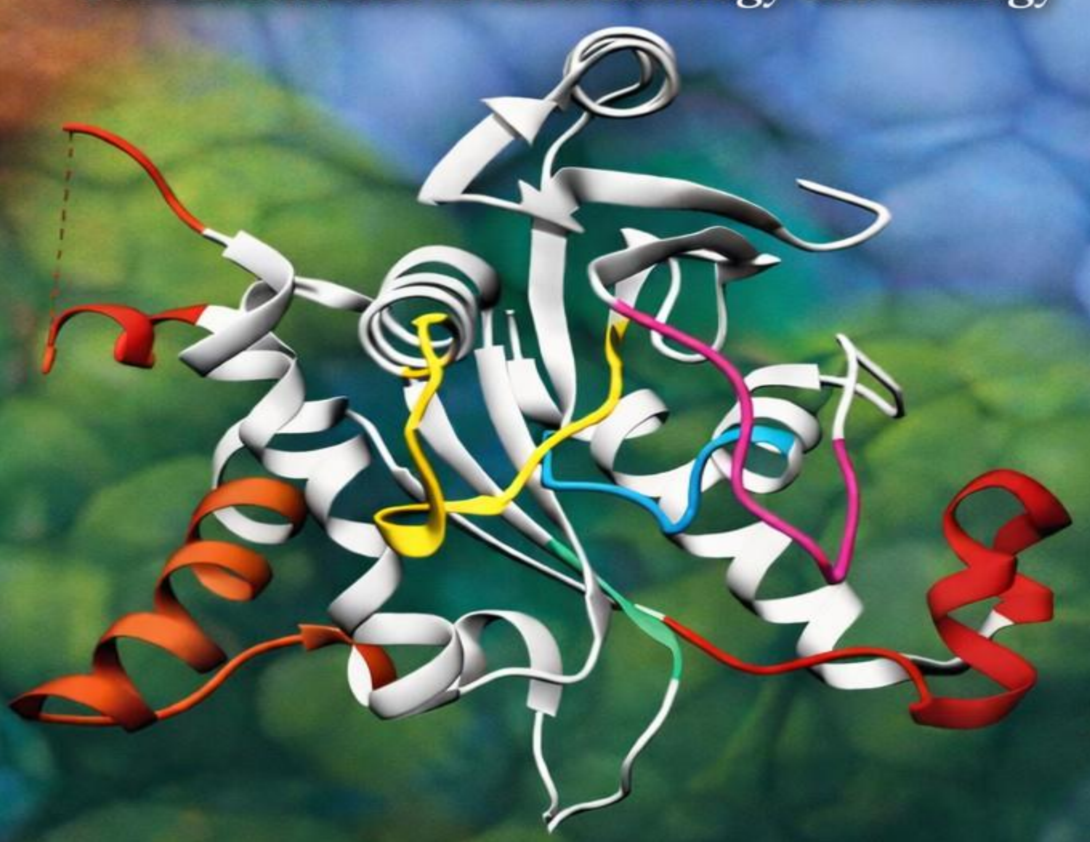
For L2 students in Biotechnology and Biology



Academic year 2025-2026

Foreword

This course manual is the result of my teaching experience with second-year undergraduate students (L2) at the Faculty of Natural and Life Sciences, University Ferhat Abbas Sétif 1. It is designed to provide a solid foundation in structural biochemistry for L2 students, as well as for learners from other disciplines interested in this field.

This manual is organized into four chapters:

The first chapter introduces the fundamental concepts of carbohydrates, followed by exercises with detailed solutions.

The second chapter is devoted to lipids, including their structure, classification, and physicochemical properties, with exercises provided at the end of the chapter.

The third chapter focuses on amino acids and peptides, covering their structures, classifications, and fundamental properties, along with exercises to reinforce understanding.

The fourth chapter addresses enzymes, including their classification, mechanisms of action, and basic principles of enzyme kinetics. Exercises with solutions are also included.

A bibliographic reference list is provided at the end of this manuscript for further reading.

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Introduction

Structural biochemistry focuses on the molecular structure of biological macromolecules, including carbohydrates, lipids, amino acids, peptides, and enzymes, and how their organization determines their biological functions. Understanding these structures is essential for explaining key processes such as metabolism, enzyme activity, and molecular interactions in living systems.

This tutorial workbook is designed for second-year undergraduate students (L2) to strengthen their understanding of these fundamental concepts through problem-solving and guided exercises. Each chapter combines theoretical reminders with practical exercises and detailed solutions, enabling students to develop analytical skills and apply biochemical principles effectively.

The study of structural biochemistry is essential in many fields, including medicine, nutrition, and biotechnology, as it provides the foundation for understanding diseases, drug action, and metabolic regulation.

Chapter I.
Structure and Physiochemical Properties of Carbohydrates

Chapter I. Structure and Physicochemical Properties of Carbohydrates

Learning objectives

-To acquire a comprehensive understanding of the classification and structural diversity of carbohydrates.

-To develop proficiency in the Fischer projection, enabling accurate representation of the stereochemistry of monosaccharides.

-To understand and differentiate the various forms of isomerism in carbohydrates, including structural, stereoisomeric, and configurational isomerism.

-To master the cyclic representation of monosaccharides (Haworth projection), with emphasis on ring formation and anomeric configurations.

I.1. Definition

- Carbohydrates are organic compounds composed mainly of carbon (C), hydrogen (H), and oxygen (O).
- Many carbohydrates follow the empirical formula $C_n(H_2O)_n$, which led to the historical term "hydrates of carbon"; however, this formula is not universally applicable.
- Structurally, carbohydrates are characterized by carbon atoms bearing:

   a. **hydroxyl (-OH)** groups (alcohol functions),
   b. a carbonyl group, either **aldehyde (-CHO)** or **ketone (C=O)** (**Fig. I.1**).

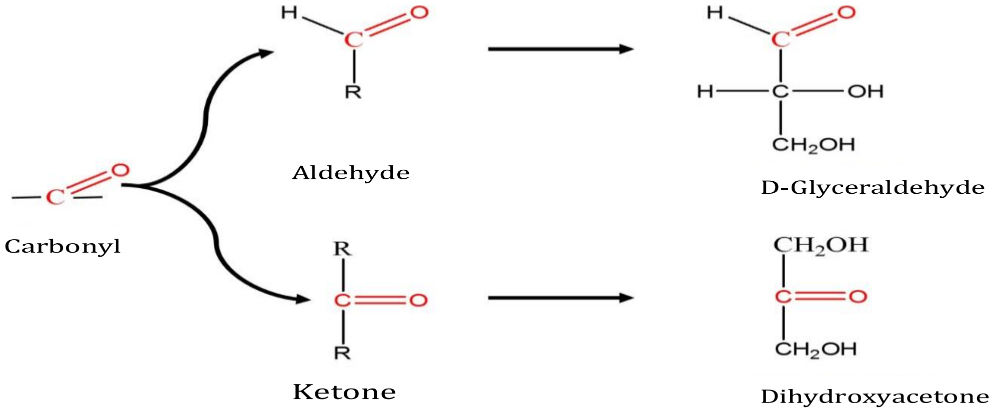


Figure I.1. Carbonyl groups in carbohydrates.

- In addition, some carbohydrates or their derivatives may contain other functional groups, such as:

a. carboxylic (acid) groups,
b. amino groups.

I.2. Carbohydrates classification

Carbohydrates are classified into three major groups:

Monosaccharides, dissacharides , oligosaccharides and polysaccharides **(Fig. I.2)**.

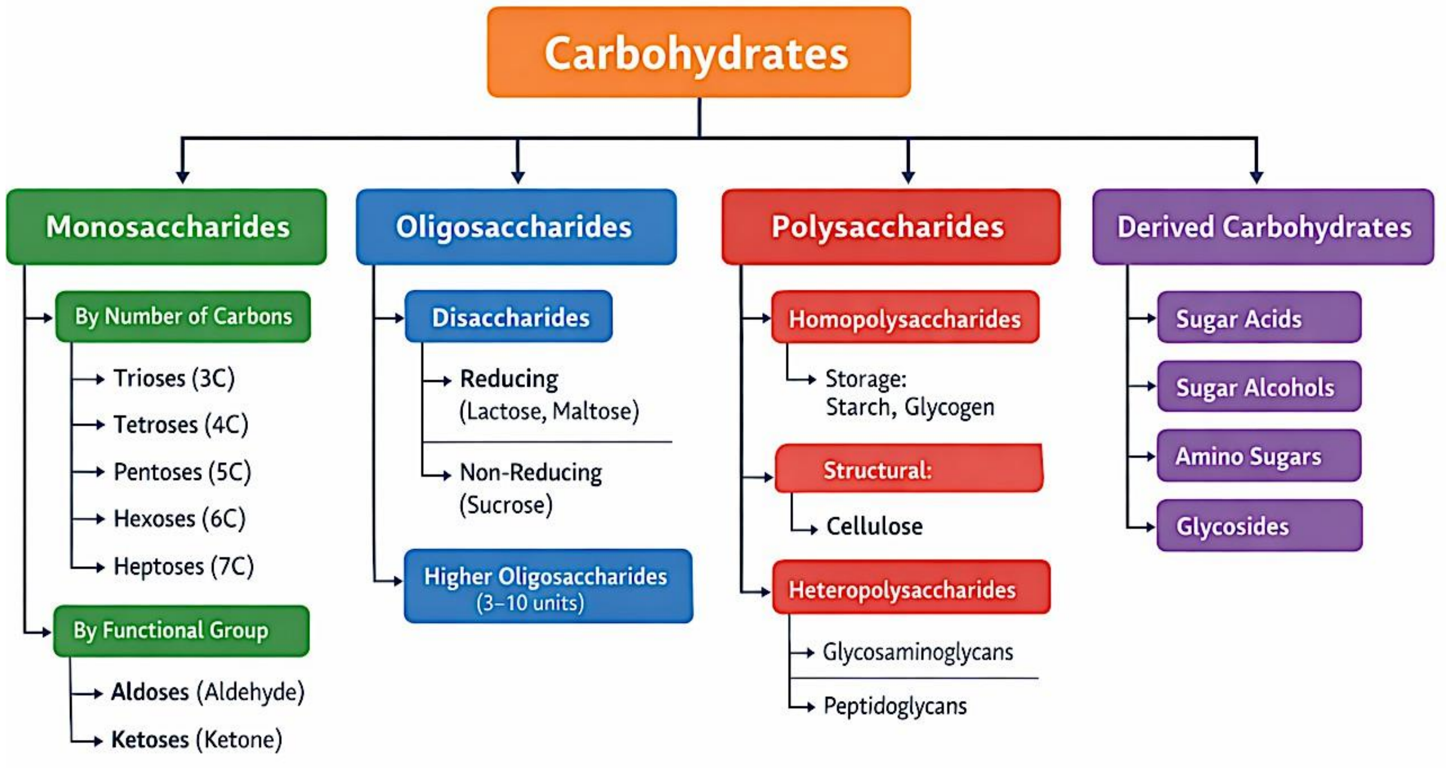


Figure I.2. Carbohydrate classification diagram.

I.3. Monosaccharides

I.3.1. Fischer projection

This is a two-dimensional representation in which all bonds are projected onto the same plane. The chiral carbon (C*) is situated within the plane of the page. The longest carbon chain is oriented vertically, with carbon-carbon bonds directed behind the plane of the page, whereas the non-carbon substituents attached to the chiral carbon are placed horizontally, with their bonds projecting toward the viewer **(see Fig. I.3)**.

This representation is constructed based on D-glyceraldehyde as the reference molecule, through the progressive addition of carbon atoms along the carbon chain.

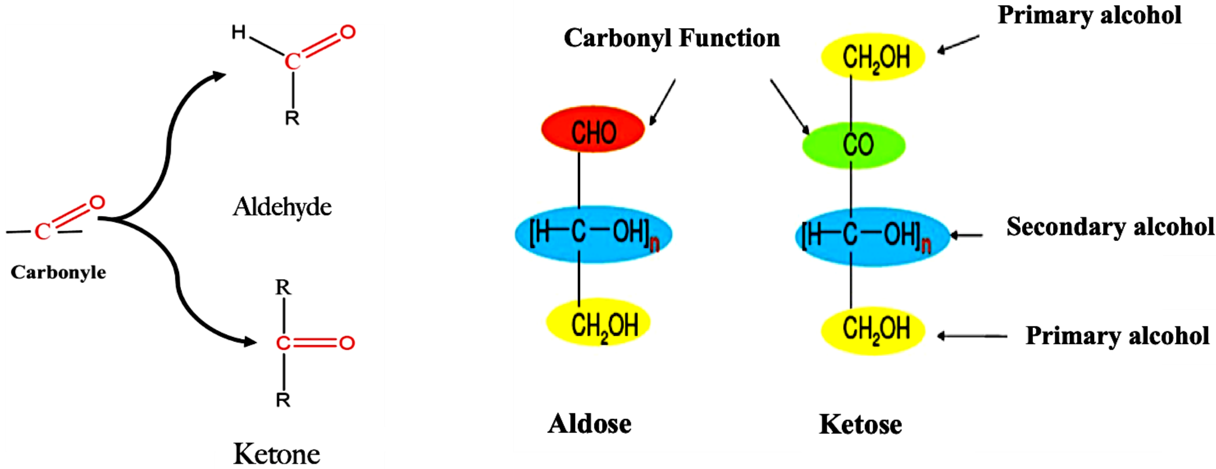


Figure I.3. Fischer projection of carbohydrates.

I.3.2. D and L configuration

The D or L configuration of a monosaccharide is determined by the orientation of the hydroxyl (-OH) group attached to the chiral carbon farthest from the carbonyl group (aldehyde or ketone function), also referred to as the reference chiral carbon.

D Series ________________OH of Cn-1 to the right

L series _______________ OH of Cn-1 to the left **(Fig. I.4)**.

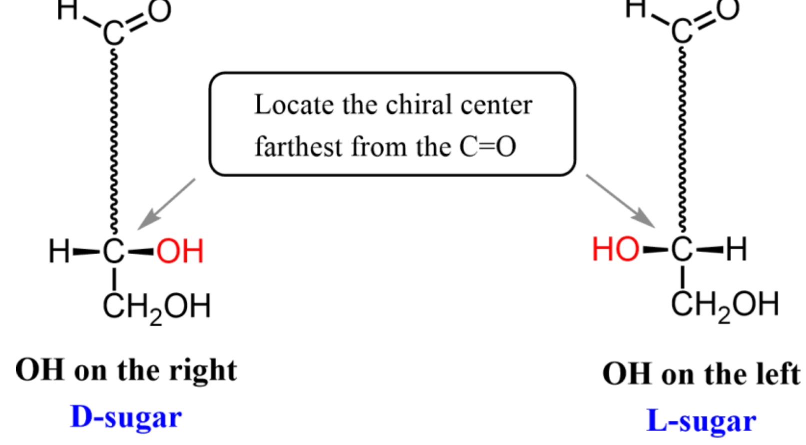


Figure I.4. D and L configurations of carbohydrates.

I.3.3. Kiliani-Fischer synthesis

A)

The number of stereoisomers of linear **aldose** with **n C** atoms is given by: $\mathbf{2^{n-2}}$
Glucose: n=6: $2^{(6-2)} = 2^4 = 16$
8 D-configuration and **8 L-configuration**forms

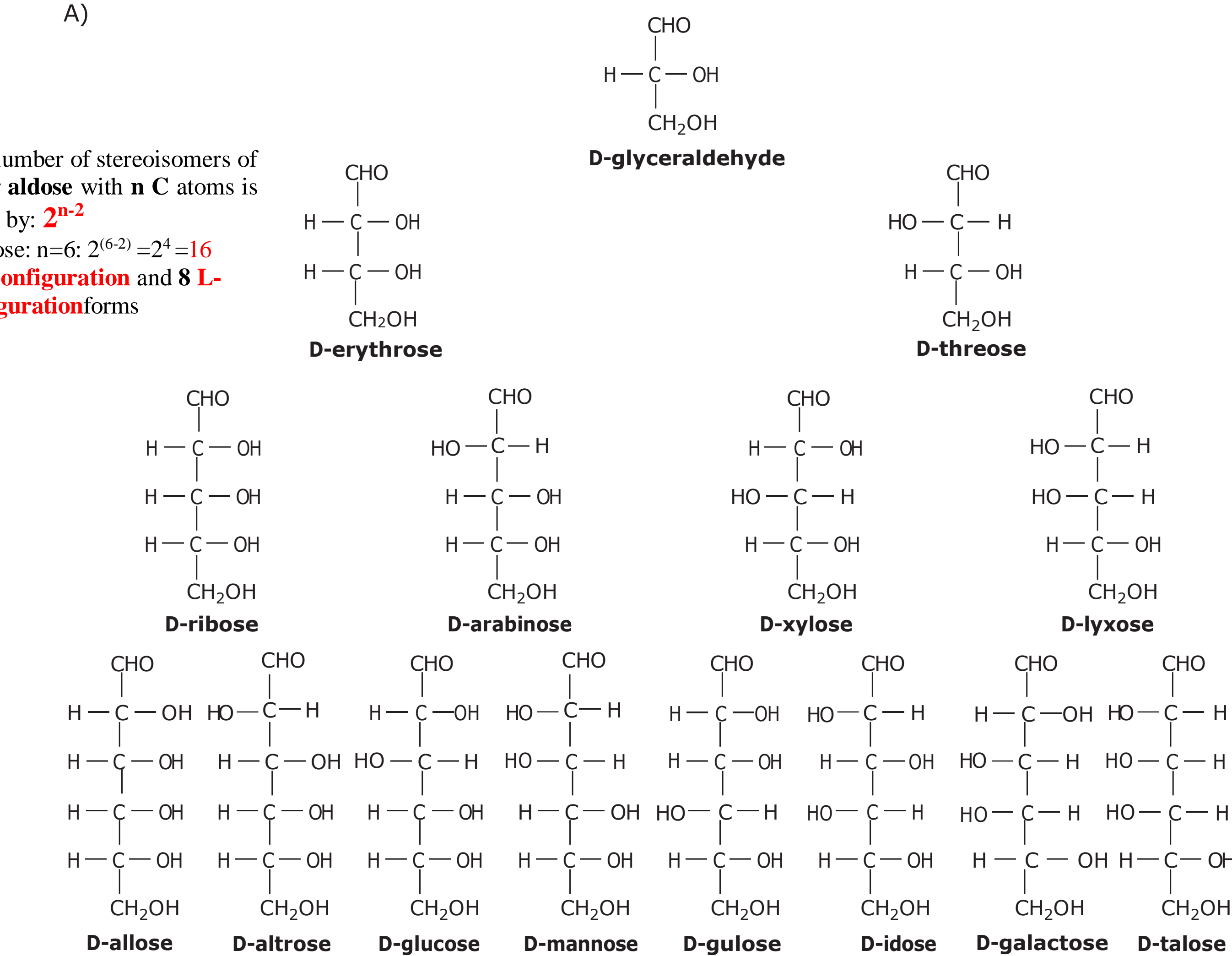


Figure I.5. Kiliani-Fischer synthesis.

1.3.4. Chain lengthening of ketoses

The number of stereoisomers of linear **ketose** with **n C** atoms is given by: **2^{n-3}**
Fructose: n=6: $2^{(6-3)} = 2^3 = 8$
4 D-configuration and **4 L-configuration** forms

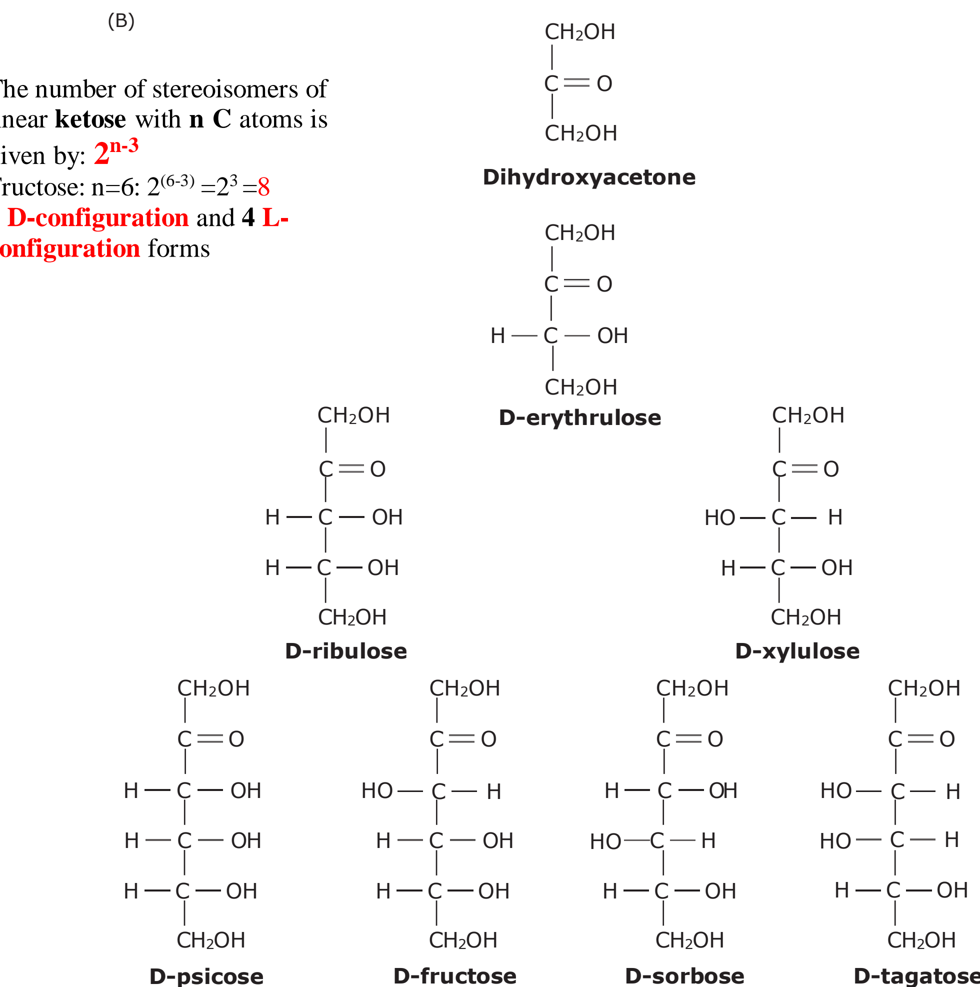


Figure I.6. Lengthening the carbon chain of ketoses.

1.3.5. Isomeric forms

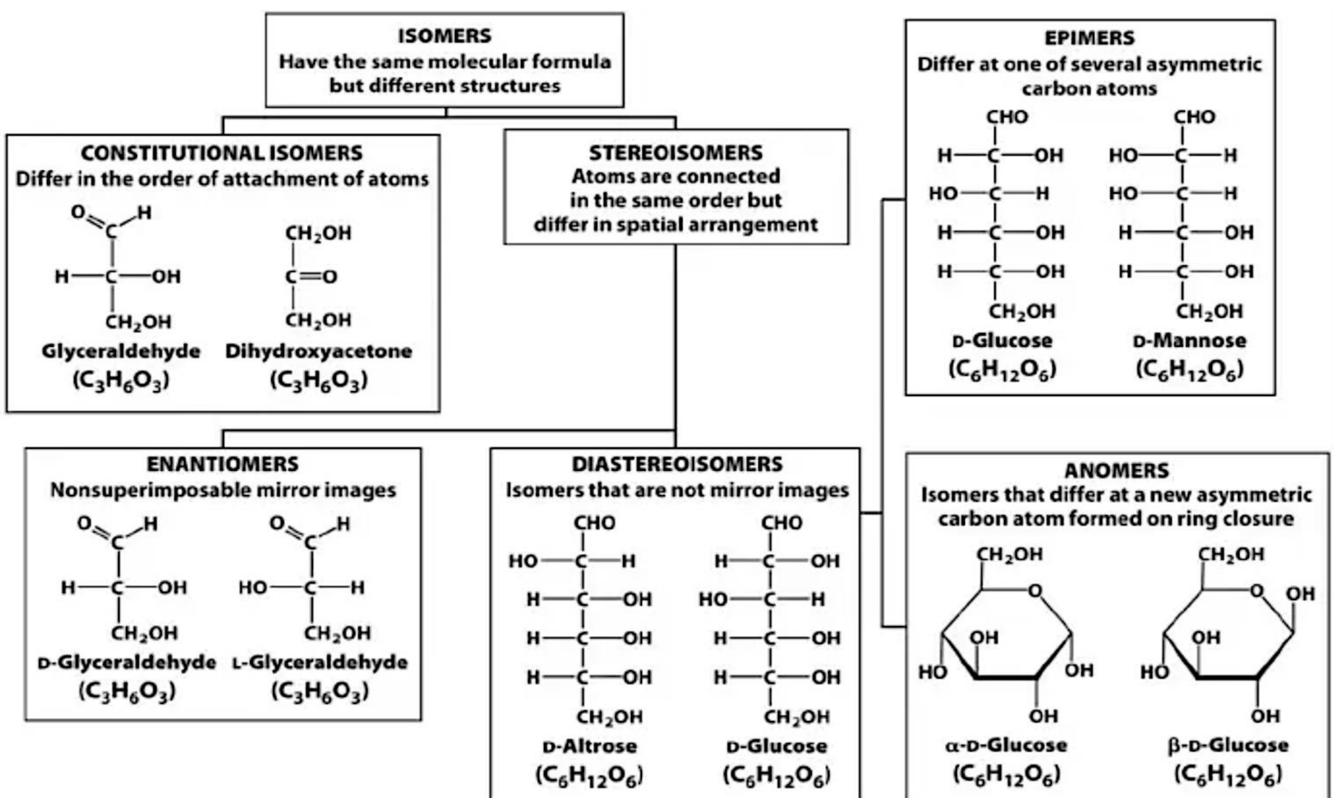


Figure I.7. Diagram illustrating the different isomeric forms.

1.3.6. Optical activity of sugars

Any chiral molecule is optically active, meaning it can rotate plane-polarized light by a specific angle (α); therefore, all monosaccharides except dihydroxyacetone exhibit optical activity due to the presence of at least one asymmetric carbon.

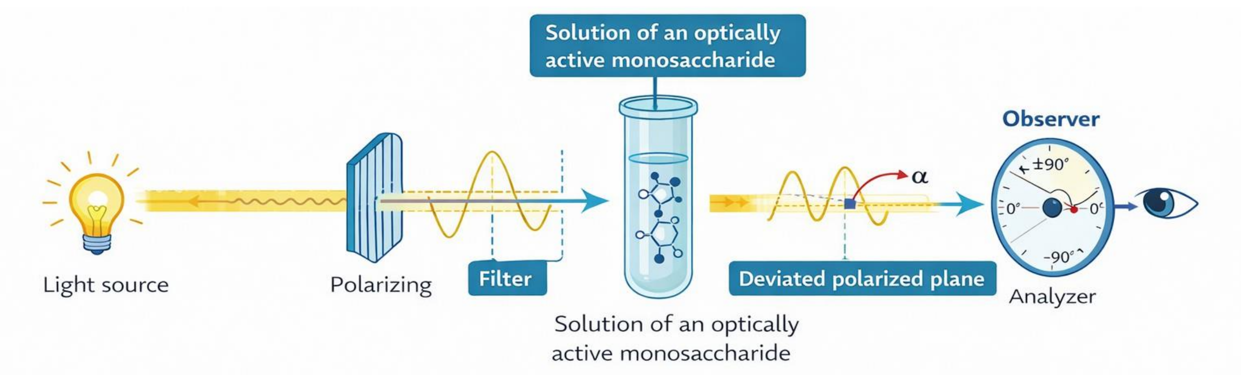


Figure I.8. The polarimeter.

The angle α of deviation of the plane of polarization of the light is given by **Biot's law**:

α of a solution = [α] at 20°C of the solute. l. C

1.3.7. HAWORTH projection

☞ **Cyclization of aldoses**

The reactivity of the aldehyde function leads to an intramolecular hemiacetalization that can take place:

- Between carbons C1 and C5, we obtain a 6-membered heterocycle (O and 5C) called a pyran or pyranose form by analogy with the pyran nucleus **(see Fig. I.9)**.
- Between carbons C1 and C4: a 5-membered heterocycle (O and 4C) is thus obtained, called the furan form or furanose by analogy with the furan nucleus.

☞ **Cyclization of ketoses**

Like aldoses, ketoses can cyclize. In this case the intramolecular hemi-acetalization takes place between the ketone function and a hydroxyl group carried by one of the carbons of the chain. During cyclization, C2 is the anomeric carbon for ketoses.

- Between C2 and C6: a 6-membered heterocycle is thus obtained, called the pyranic form (pyranose).
- Between C2 and C5: a 5-membered heterocycle is thus obtained, called the furan form (furanoses) **(see Fig. I.9)**.

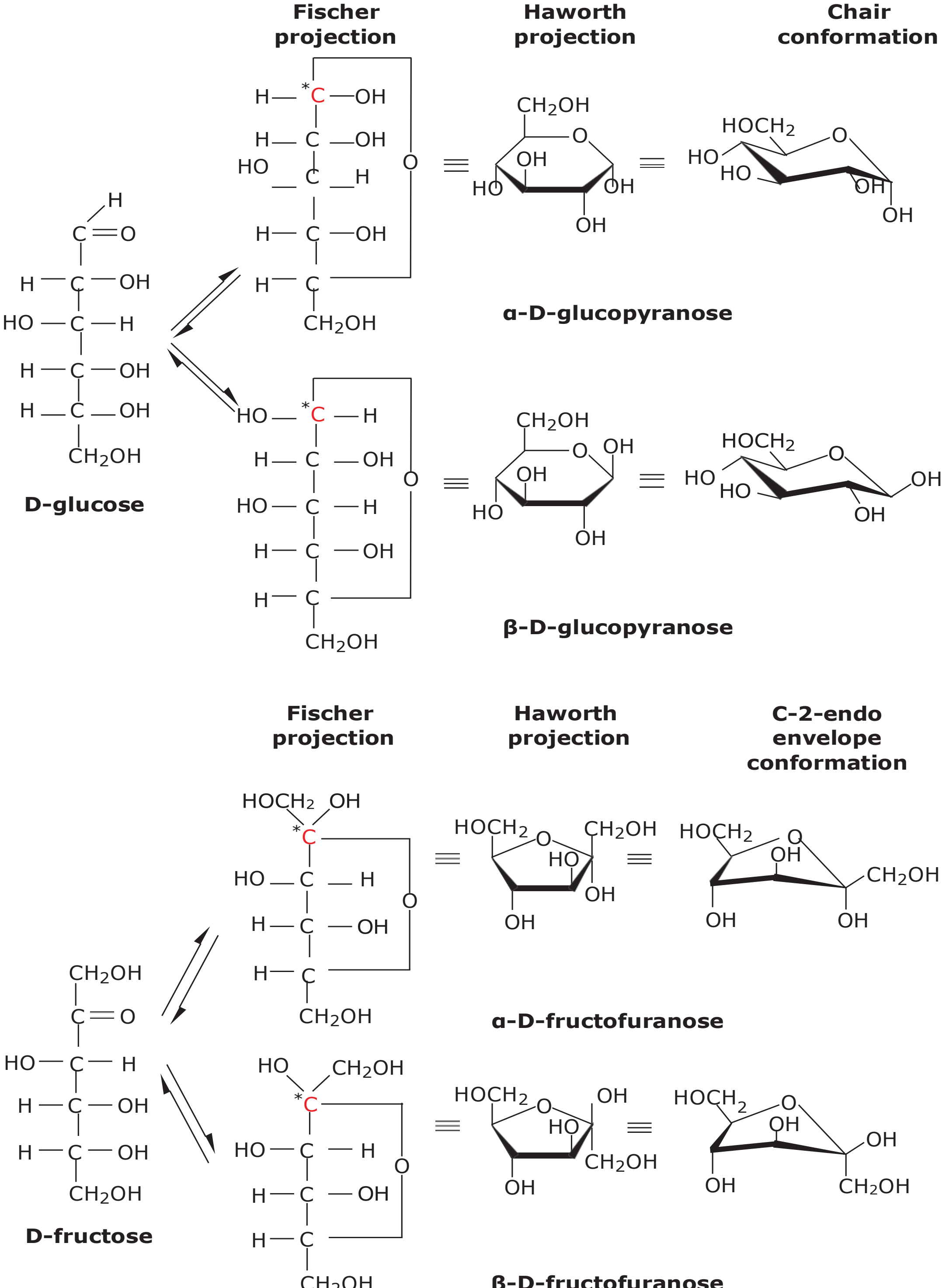


Figure I.9. Cyclic structure of aldoses and ketoses.

1.3.8. Physicochemical properties

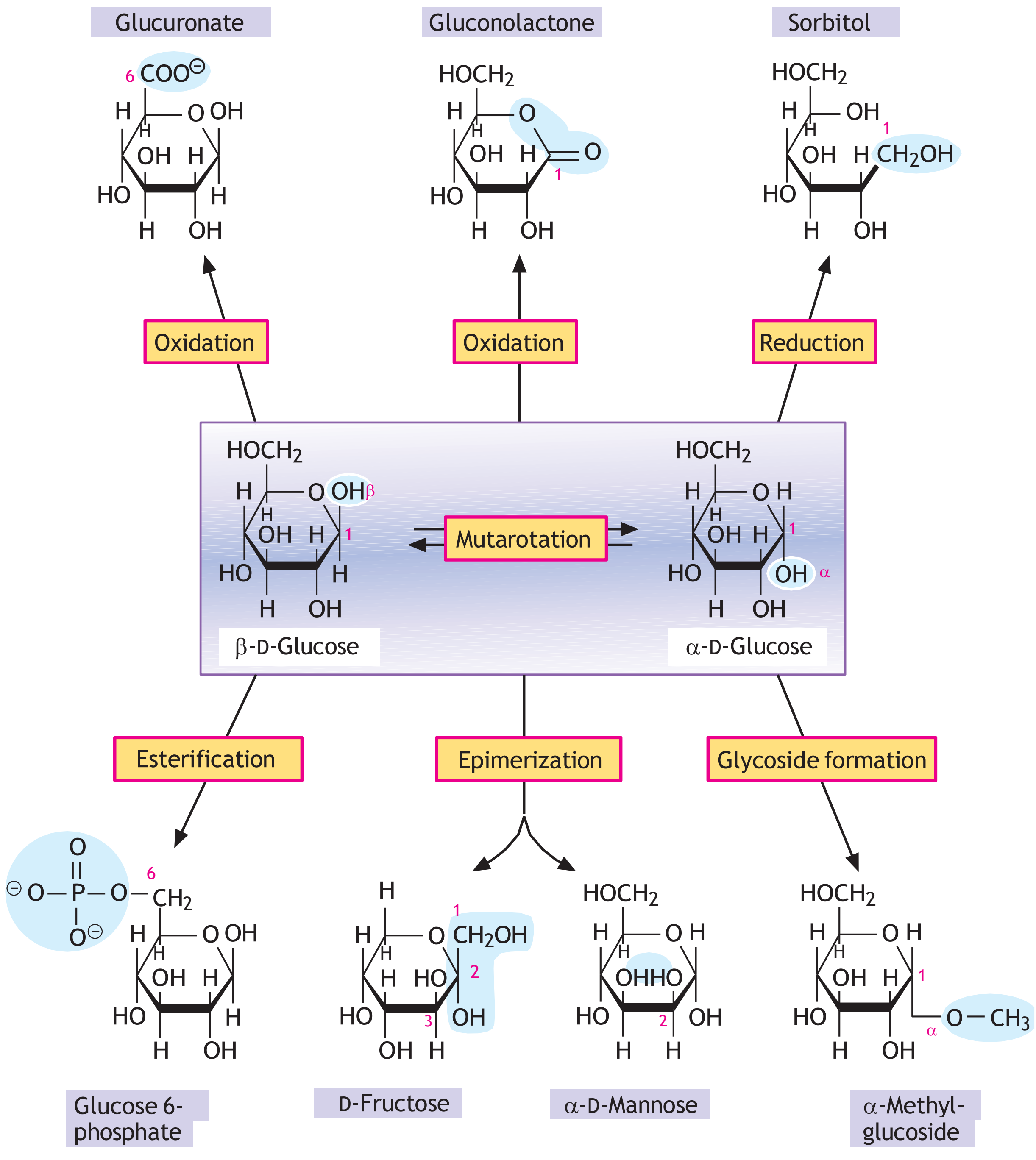


Figure I.10. Some physicochemical properties of sugars.

I.4. Oligosaccharides

Oligosaccharides are formed by creating a glycosidic bond between a sugar engaged by its reducing function and another molecule that can be.

-Glucidic ==> Formation of polysaccharides

1. Oligosaccharides (= 2 to 10 sugars linked)

2. Polysaccharides (= more than 10 sugars linked)

-Non-carbohydrate ==> Formation of glycoconjugates.

I.4.1. The glycosidic bond

Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond.

Three types of bonds can be formed:

-Semi-acetal OH + primary alcohol OH (reducing disaccharide, 1 free semi-acetal)

- Semi-acetal OH + secondary alcohol OH (reducing disaccharide: idem)

- Semi-acetal OH + semi-acetal OH (non-reducing disaccharide, no free hemi-acetal OH)

-Example: D-glucose and D-galactose

I.4.2. Nomenclature

Generically, the name will be:

α/β, D/L X.....osyl (1--->n) α/β, D/L Y.......ose/oside

X: name of the first sugar.

Y: name of the second sugar.

n: carbon number involved in the glycosidic bond.

Osyl: This means that the hemiacetal function of the first sugar is involved in the glycosidic bond.

Ose: This means that the hemiacetal function of the last sugar is free.

Oside: this means that the hemiacetal function of the last monosaccharide is engaged in the glycosidic bond.

I.4.3. Reducing and non-reducing sugars

Any sugar containing a hemiacetal group is a reducing sugar, because it exists in equilibrium with an open-chain form containing a free aldehyde (or ketone). As a result, it can act as a reducing agent, reacting with oxidizing agents such as Cu^{2+} (Fehling's or Benedict's test) and Ag^{+} (Tollens' test).

Examples: glucose, lactose, maltose.

CH_2OH hemiacetal CH_2OH H O H H OH H HO OH OH HO CH_2OH H OH hemiacetal OH H

α-D-glucose D-Fructose

In contrast, sugars lacking a free hemiacetal group are non-reducing sugars. Their structure is "locked" in the cyclic form, and they cannot revert to an open-chain form with a free carbonyl group. Therefore, they do not exhibit reducing properties.

Examples: sucrose, trehalose.

CHOH, H, O, H, acetal, CH_2OH, O, H, OH, H, O, H, HO, OH, acetal, OH, CH_2OH, H, H, OH

Sucrose

I.5. Polysaccharides

Polysaccharides are polymers of monosaccharides linked by glycosidic bonds. They fulfill two main functions in nature:

- Energy reserves (starch, glycogen, etc.)
- Structural role in certain cells (cellulose, chitin...)

-Homopolysaccharides: Composed from the same monosaccharide.

-Heteropolysaccharide: Composed of different types of monosaccharides.

Exercises

Exercise 1

1. Which elements do simple carbohydrates contain, and in what ratio?
2. Based on their molecular formulas, which of the following are not monosaccharides ?

   a) $C_3H_80_3$ **c)** $C_{18}H_{32}O_{16}$ **e)** $C_{16}H_{32}O_2$

   b) $C_{10}H_{18}O_9$ **d)** $C_4H_8O_2$ **f)** $C_6H_{12}O_6$

3. Which of the following monosaccharides is the majority found in the human body?

   a) D-type. **b)** L-type. **c)** L and D-types **d)** None of the above

4. Identify the differences among monosaccharides, disaccharides, and polysaccharides.
5. What is a chiral carbon?

Exercise 2

1. Identify each sugar as an aldose or a ketose and then as a triose, tetrose, pentose, or hexose.

D-glucose	L-ribulose	D-glyceraldehyde	dihydroxyacetone	D-ribose	D-galactose
H–C=O H—C—OH HO—C—H H—C—OH H—C—OH CH_2OH	CH_2OH C=O HO—C—H HO—C—H CH_2OH	H–C=O H—C—OH CH_2OH	CH_2OH C=O CH_2OH	H–C=O H—C—OH H—C—OH H—C—OH CH_2OH	H–C=O H—C—OH HO—C—H HO—C—H H—C—OH CH_2OH

2. What is the meaning of the signs (-) and (+)?
3. Why is dihydroxyacetone optically inactive?
4. What is the specific rotation of an aqueous solution of D-glyceraldehyde at 100 g/L with a rotation of 2.7° at 20°C (the polarimeter tube length is 20 cm)?
5. Given the sugar with the following formula: CHO-$(CHOH)_3$-CH_2OH.
1. Write the compound according to its Fischer projection representation.
2. How many stereoisomers does it present?
6. Identify each sugar as an aldose or a ketose and then as a D-sugar or an L-sugar.

H–C=O HO—C—H HO—C—H H—C—OH CH_2OH	CH_2OH C=O HO—C—H CH_2OH	CH_2OH C=O H—C—OH H—C—OH CH_2OH	H–C=O HO—C—H HO—C—H HO—C—H CH_2OH

7. Indicate whether each of the following sugar pairs is a pair of anomers, epimers, diastereomers, enantiomers, or an aldose-ketose pair:

D-Glyceraldehyde and D-Dihydroxyacetone

D-Glucose and D-Mannose

D-Glucose and D-Fructose

α-D-Glucose and β-D-Glucose

D-Ribose and D-Ribulose

D-Galactose and D-Glucose

D-Mannose and D-Galactose

D-Galactose and L-Galactose

Exercice 3

Given the following sugars:

CH_2OH

A

CH_2OH

CH_2OH

B

1. Are they α or β anomers?
2. Are they in pyranose or furanose form?
3. Are they reducing sugars?
4. Present sugar A in furanose form and sugar B in pyranose form.
5. Represent the symmetrical formula of A with respect to a horizontal plane.
6. Provide the names and formulas of the products obtained from the reduction of sugars A and B using $NaBH_4$. Are they reducing sugars?
7. Give two epimers of A and the products obtained when they are subjected to strong oxidation by HNO_3. Are the products obtained optically active?

Exercise 4

1. For each disaccharide, indicate whether the glycosidic linkage is α or β.

2. Melibiose is a disaccharide that occurs in some plant juices. Its structure is as follows:

a. What monosaccharide units are incorporated into melibiose?

b. What type of linkage (α or β) joins the two monosaccharide units of melibiose?

c. Melibiose has a free anomeric carbon and is thus a reducing sugar. Circle the anomeric carbon and indicate whether the OH group is α or β.

3. Cellobiose is a disaccharide composed of two glucose units joined by a β-1,4-glycosidic linkage.

a. Draw the structure of cellobiose.

b. Is cellobiose a reducing or nonreducing sugar? Justify your answer.

Exercise 5

1. Raffinose is a trisaccharide that occurs freely in many plants. After complete methylation and acid hydrolysis of one millimole, the following products are obtained:

- 1 millimole of 2,3,4,6-tetra-O-methylgalactose
- 1 millimole of 2,3,4-tri-O-methylglucose
- 1 millimole of 1,3,4,6-tetra-O-methylfructose

Yeast invertase catalyzes the hydrolysis of raffinose into melibiose, which is α-D-galactopyranosyl(1→6) α-D-glucopyranose, and β-D-fructofuranose. Write the formula for raffinose.

2. Consider the following pentaholoside:

β-galactosido(1-4)α-glucosido(1-6)α-glucosido(1-4)α-glucosido(1-2)β-fructoside.

- **a.** What is the name of the disaccharide resulting from hydrolysis by α-glucosidase?
- **b.** To determine the pyranose or furanose structure of the different aldoses constituting this pentaholoside, it is subjected to periodic acid (HIO_4). No formaldehyde (HCHO) is formed. What are your conclusions? Knowing that 7 molecules of HIO_4 are necessary to oxidize one molecule of the pentaholoside, write its chemical formula.
- **c.** What compounds are produced by permethylation followed by acid hydrolysis?

Solution

Answer 1

1. Which elements do simple carbohydrates contain, and in what ratio? C, H and O (1/2/1)
2. Which of the following are NOT monosaccharides ?

a) $C_3H_8O_3$ c) $C_{18}H_{32}O_{16}$ e) $C_{16}H_{32}O_2$

b) $C_{10}H_{18}O_9$ d) $C_4H_8O_2$ f) $C_6H_{12}O_6$

3. Which of the following monosaccharides is the majority found in the human body?
   (a) D-type. (b) L-type. (c) LD-types (d) None of the above
4. A monosaccharide is the simplest carbohydrate and cannot be hydrolyzed to produce a smaller carbohydrate; a disaccharide is composed of two monosaccharide units; and a polysaccharide contains many saccharide units.
5. A chiral carbon is a carbon atom that is attached to four different atoms or groups of atoms.

Answer 2

D-glucose	L-ribulose	D-glyceraldehyde	dihydroxyacetone	D-ribose	D-galactose
H–C=O; H–C–OH; HO–C–H; H–C–OH; H–C–OH; CH_2OH	CH_2OH; C=O; HO–C–H; HO–C–H; CH_2OH	H–C=O; H–C–OH; CH_2OH	CH_2OH; C=O; CH_2OH	H–C=O; H–C–OH; H–C–OH; H–C–OH; CH_2OH	H–C=O; H–C–OH; HO–C–H; HO–C–H; H–C–OH; CH_2OH
Aldohexose	Ketopentose	Aldotriose	Ketotriose	Aldopentose	Aldohexose

1. (-) is levorotatory and (+) is dextrorotatory.
2. This is due to the absence of an asymmetric carbon and the presence of a plane of symmetry.
3. Using Biot's law:

$\alpha = [\alpha]_D . l . C.$ $[\alpha]_D^{20°C}$: in °. g^{-1}.ml. dm^{-1}

α: (PR) is the angle of deviation of polarized light. It can be positive (α > 0), meaning the substance is said to be dextrorotatory, or it can be negative (α < 0), meaning the substance is said to be levorotatory.

[α]: (PRS) is the specific rotation of the optically active solute, which is a characteristic constant of the solute measured at a temperature of 20°C using polarized light at a wavelength from the D or Balmer spectral line (λ = 589 nm).

L: is the length of the polarimetric tube or the optical path length (i.e., the length of the solution through which the light passes).

C: is the concentration of the optically active solution.

100 g → **1L (1000 ml)**
X → **1ml** **X= 0.1 g**

20 m = 2 dm

- $[\alpha]_D^{20°C} = \alpha/l. C = 2.7°/ 2 \text{ dm} \times 0.1 \text{ g/ml} = +13.5° \text{ g}^{-1}. \text{ml}^{-1}. \text{dm}^{-1}$

5. Given the sugar with the following formula: $CHO\text{-}(CHOH)_3\text{-}CH_2OH$.

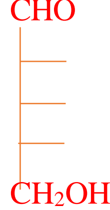
a. The given chemical formula is a semi-structural formula, indicating that the sugar is an aldose because it carries the aldehyde group on carbon 1 (CHO). It consists of five (5) carbons; it is an aldopentose. If the ketone group (C=O) were present on carbon 2, the sugar would then be a ketose. The Fischer projection presents the sugar as follows:

-A vertical line representing the carbon backbone.

-The aldehyde or ketone group must be represented.

-The primary alcohol group (CH_2OH) must be represented.

-The hydroxyl groups (OH) of each secondary alcohol group (CHOH) are represented by horizontal lines. -Hydrogens are not represented.



b. Stereoisomers:

The number of stereoisomers = **2^n,** where n = the number of asymmetric carbons (C*). This pentose has three (3) asymmetric carbons: n = 3. The number of stereoisomers = 2^3 = 8 (four from the D-series and four from the L-series).

Note*: - The number of stereoisomers of an aldose with n carbons is equal to* ***2^{n-2}.***

- The number of stereoisomers of a ketose with n carbons is equal to ***2^{n-3}***.

6. Identify each sugar as an aldose or a ketose and then as a D sugar or an L sugar.

D-aldose	L-ketose	D-ketose	L-aldose

7. Indicate whether each of the following sugar pairs is a pair of anomers, epimers, diastereomers, enantiomers, or an aldose-ketose pair:
    - **D-Glyceraldehyde and Dihydroxyacetone** (aldose-ketose pair)
    - **D-Glucose and D-Mannose** (epimers in C*2)
    - **D-Glucose and D-Fructose** (aldose-ketose pair)
    - **α-D-Glucose and β-D-Glucose** (anomers)
    - **D-Ribose and D-Ribulose** (aldose-ketose pair)
    - **D-Galactose and D-Glucose** (epimers in C*4)
    - **D-Mannose and D-Galactose** (diastereomers in C*2 and C*4)
    - **D-Galactose et L-Galactose** (enantiomers)

Answer 3

1. 2. 3.

-A-

Aldose
D
α
Pyran
Reducing agent

-B-

Ketose
D
β
Furan
Reducing agent

4.

Representation of A in Furanose Form

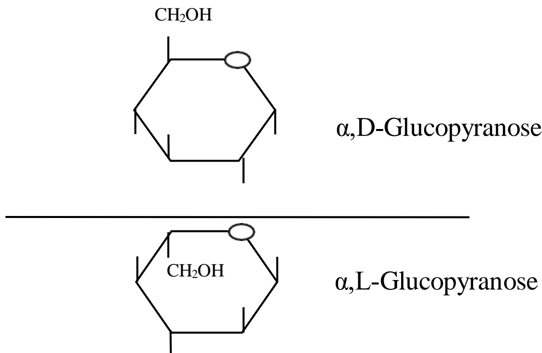
α,D-Glucofuranose

Representation of B in pyranose form

β,D-Fructopyranose

5.

Symmetrical formula of A with respect to a horizontal plane (changing the positions of the different 5 substituents).)



6.

Reduction of sugars A and B by $NaBH_4$.
A : is glucose

CHO + $NaBH_4$ → CH_2OH
CH_2OH CH_2OH

D-Glucose D-Sorbitol

B : is fructose

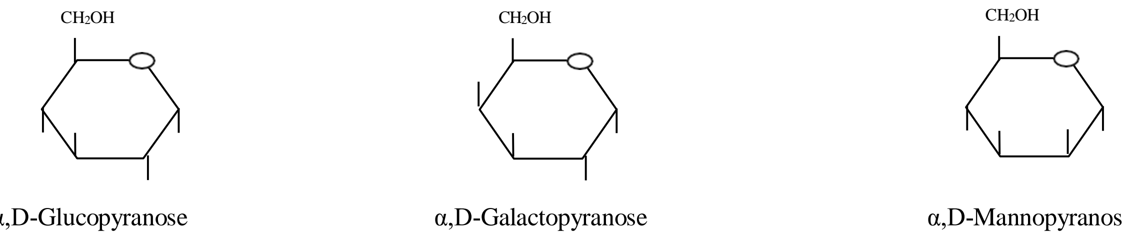
CH_2OH ← $NaBH_4$ — CH_2OH, C=O — $NaBH_4$ → CH_2OH
CH_2OH CH_2OH CH_2OH

D-Mannitol (50%) D-Fructose D-Sorbitol (50%)

The products obtained after the reduction of sugars A and B are not reducing (absence of a free hemiacetal function).

7. Give two epimers of A and the products obtained when they are subjected to strong oxidation by HNO_3. Are the products obtained optically active?

Epimers of compound A.



α,D-Glucopyranose α,D-Galactopyranose α,D-Mannopyranose

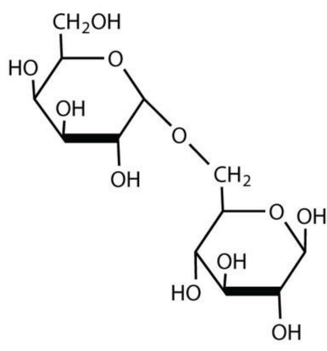
The strong oxidation of D-Galactose and D-Mannose (both sugars have an aldehyde function and therefore a primary alcohol function) results in the formation of aldaric acids.

Answer 4

1. For each disaccharide, indicate whether the glycosidic linkage is α or β.

α 1- α2	β 1-4	β 1-4	α 1- 6

2. Melibiose is a disaccharide that occurs in some plant juices. Its structure is as follows:



a. D-Galactose and. D-Glucose

b. Via α(1→6) linkage

c. Melibiose has a free anomeric carbon and is thus a reducing sugar. Circle the anomeric carbon and indicate whether the OH group is α or β.

β

3. Cellobiose is a disaccharide composed of two glucose units joined by a β 1-4 glycosidic linkage.

a. Draw the structure of cellobiose. galactose and glucose

α-glycosidic linkage (1-4)

b. Is cellobiose a reducing or nonreducing sugar? Justify your answer.

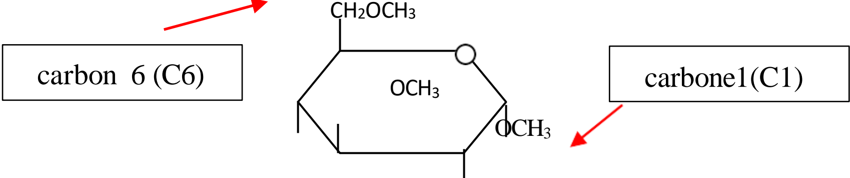
Yes, it is reducing because it has a free hemiacetal group.

Answer 5

1. Methylation is carried out by methyl iodide (ICH_3). The chemical reaction occurs at the level of free hydroxyl groups (OH). Each time there is a free OH, the hydrogen is replaced by a methyl group, giving OCH_3.

Example:

The methylation of free D-glucose (glucose is an aldohexose):



As a result, we have: 1,2,3,4,6 penta-O-methylglucose.

- If the glucose is linked at carbon 1, it cannot be methylated, and the result of its methylation will be: 2,3,4,6 tetra-O-methylglucose.

- Based on this principle and according to the data from the exercise, we find that raffinose is composed of three simple sugars: galactose, glucose, and fructose, with glucose in the middle. Therefore, the formula for raffinose is either Galactose-Glucose-Fructose or Fructose-Glucose-Galactose.

According to the second statement in the exercise, we confirm that raffinose is galactose linked to glucose, which is linked to fructose: Galactose-Glucose-Fructose.

The structure of raffinose

2.a. The enzyme α-glucosidase cuts at the (1,6) bond between two glucose molecules. The name of the disaccharide resulting from this cleavage is: β-D-galactopyranosido(1,4) α-D-glucopyranose.

b. Periodic acid (HIO_4) cleaves the bond between two adjacent carbons that have free OH groups. The result is formic acid (HCOOH) when it cuts at the level of the secondary alcohol functions (CHOH) and formaldehyde (formol, HCHO) when the cleavage occurs at the level of the primary alcohol function (CH_2OH).

When periodic acid is applied to sugars in their cyclic forms, the pyranose form yields formic acid, while the furanose form releases formaldehyde.

The conclusion from the result of the action of periodic acid on this pentaholoside is that the aldoses constituting this sugar are in the pyranose form.

c.The products obtained by permethylation followed by acid hydrolysis

- One molecule of 2,3,4,6 tetra-O-methylgalactose.
- Two molecules of 2,3,6 tri-O-methylglucose.
- One molecule of 2,3,4 tri-O-methylglucose.
- One molecule of 1,3,4,6 tetra-O-methylfructose.

Chapter II.
Structure and Physiochemical Properties of Lipids

Chapter II. Structure and Physicochemical Properties of Lipids

Learning objectives

-To understand the classification and major families of lipids.

-To master the structure and properties of saturated and unsaturated fatty acids.

-To understand the structure and organization of simple and complex lipids.

-To develop the ability to calculate and interpret iodine and saponification indices.

II.1. Definition

Lipids are organic molecules composed primarily of carbon (C), hydrogen (H), and oxygen (O).

They are insoluble in water (hydrophobic) and soluble in nonpolar organic solvents such as benzene, chloroform, and ether.

They are characterized by the presence of at least one fatty acid or hydrophobic hydrocarbon chain within their structure.

II.2. Classification

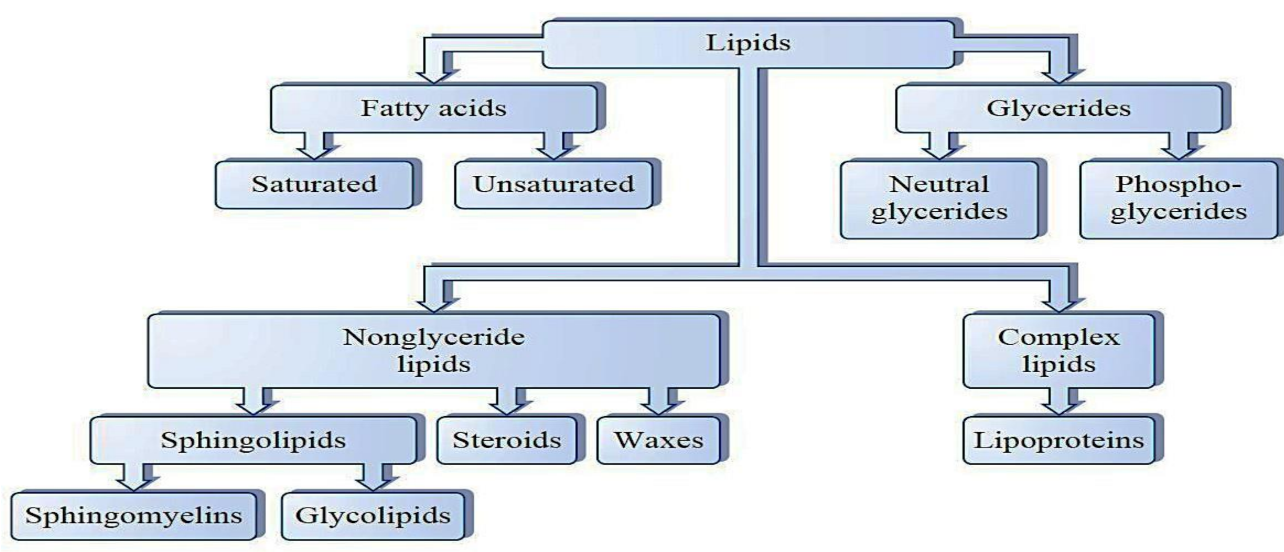


Figure II.1. Lipid classification diagram.

II.3. Fatty acids

II.3.1. Definition

Fatty acids are carboxylic acids (R-COOH) with a long hydrophobic hydrocarbon chain, usually containing an even number of carbon atoms.

They are typically:

- Monocarboxylic
- Linear and unbranched
- Either saturated (no double bonds) or unsaturated (one or more double bonds)

Although they range from 4 to 38 carbons, fatty acids with 16 and 18 carbons are the most common.

Table II.1. Characteristics of saturated and unsaturated fatty acids.

Numbering of the carbons	H_3C (18, ω) – CH_2 (17) – CH_2 (16) – CH_2 (15) – CH_2 (14) – CH_2 (13) – CH_2 (12) – CH_2 (11) – CH_2 (10) – CH_2 (9) – CH_2 (8) – CH_2 (7) – CH_2 (6) – CH_2 (5, δ) – CH_2 (4, γ) – CH_2 (3, β) – CH_2 (2, α) – $COOH$ (1)
Saturated fatty acids	
Nomenclature	Number of carbon atoms + the suffix "anoic"
Symbol	Cn:0 (0 indicates that the chain is saturated)
General formula	$C_nH_{2n}O_2$
Example	Palmitic acid (hexadecanoic) $C_{16}H_{32}O_2$: C_{16}:0
Unsaturated fatty acids	
Nomenclature	Number of carbon atoms + number of double bonds + suffix enoic
Symbol	**Cn:m $\Delta^{(p, p', \ldots)}$** , Cn: number of carbons m Δ: number of double bonds (p, p', …): positions of the double bonds in normal numbering
General formula	$\mathbf{C_nH_{2n-2x}O_2}$ (x: the number of double bonds).
Example	Linolenic acid C18: 3 $\Delta^{9,12,15)}$: octadeca-9,12,15-trienoic acid.

II.3.2. Physicochemical properties

a. Solubility

Lipids are generally insoluble in water due to their nonpolar nature but are soluble in organic solvents.

Some lipids are amphipathic, containing both hydrophilic and hydrophobic regions, allowing them to form micelles, lipid bilayers, liposomes, and emulsions, which are essential for biological functions.

b. Melting point

The melting point of fatty acids increases with the length of the carbon chain and decreases with the number of double bonds. Thus, saturated fatty acids tend to be solid, while unsaturated fatty acids are generally liquid at room temperature.

Table II. 2. Chemical properties of lipids.

Chemical properties	Reaction	Saponification Value and Iodine value
Saponification	X = Na, K, etc. $H{-}O{-}C(=O){-}R_1 + XOH \rightleftharpoons X{-}O{-}C(=O){-}R_1 + H_2O$ Free Fatty Acid, Base, Soap, Water	***SV = (MW KOH * 1 g) / MW of Fat***
Halogenation	$CH_3{-}(CH_2)_4{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_7{-}COOH$ Linoleic acid $\downarrow 2I_2$ $CH_3{-}(CH_2)_4{-}CH(I){-}CH(I){-}CH_2{-}CH(I){-}CH(I){-}(CH_2)_7{-}COOH$ Stearate-tetra-iodinate	**IV = (MW I_2 * 100)/MW of fat**

Table II.3. Structure, systematic name, trivial name, and symbol of fatty acids.

Numerical symbol	Structure	Systematic name[a]	Trivial name[a]
4:0	CH_3-$[CH_2]_2$-COOH	Butanoic	Butyric
6:0	CH_3-$[CH_2]_4$-COOH	Hexanoic	Caproic
8:0	CH_3-$[CH_2]_6$-COOH	Octanoic	Caprylic
10:0	CH_3-$[CH_2]_8$-COOH	Decanoic	Capric
12:0	CH_3-$[CH_2]_{10}$-COOH	Dodecanoic	Lauric
14:0	CH_3-$[CH_2]_{12}$-COOH	Tetradecanoic	Myristic
16:0	CH_3-$[CH_2]_{14}$-COOH	Hexadecanoic	Palmitic
16:1	CH_3-$[CH_2]_5CH{=}CH[CH_2]_7$-COOH	9-Hexadecenoic	Palmitoleic
18:0	CH_3-$[CH_2]_{16}$-COOH	Octadecanoic	Stearic
18:1(9)	CH_3-$[CH_2]_7CH{=}CH[CH_2]_7$-COOH	*cis*-9-Octadecenoic[b]	Oleic
18:1(11)[c]	CH_3-$[CH_2]_5CH{=}CH[CH_2]_9$-COOH	11-Octadecenoic	Vaccenic
18:2(9,12)[c]	CH_3-$[CH_2]_3(CH_2\ CH{=}CH)_2[CH_2]_7$-COOH	*cis*,*cis*-9,12-Octadecadienoic[b]	Linoleic
18:3(9,12,15)[c]	CH_3-$(CH_2CH{=}CH)_3[CH_2]_7$-COOH	9,12,15-Octadecatrienoic	(9,12,15)-Linolenic
18:3(6,9,12)[c]	CH_3-$[CH_2]_3(CH_2CH{=}CH)_3[CH_2]_4$-COOH	6,9,12-Octadecatrienoic-	(6,9,12)-Linolenic
18:3(9,11,13)[c]	CH_3-$[CH_2]_3(CH{=}CH)_3[CH_2]_7$-COOH	9,11,13-Octadecatrienoic	Eleostearic
20:0	CH_3-$[CH_2]_{18}$-COOH	Icosanoic	Arachidic
20:2(8,11)[c]	CH_3-$[CH_2]_6(CH_2CH{=}CH)_2[CH_2]_6$-COOH	8,11-(*E*)Icosadienoic	
20:3(5,8,11)[c]	CH_3-$[CH_2]_6(CH_2CH{=}CH)_3[CH_2]_3$-COOH	5,8,11-(*E*)Icosatrienoic	
20:4(5,8,11,14)[c]	CH_3-$[CH_2]_3(CH_2CH{=}CH)_4[CH_2]_3$-COOH	5,8,11,14-(*E*)Icosatetraenoic	Arachidonic
22:0	CH_3-$[CH_2]_{20}$-COOH	Docosanoic	Behenic
22:6(4,7,10,13,16,19)[c]	CH_3-$(CH_2CH{=}CH)_6[CH_2]_2$-COOH	*all*-*cis*-4,7,10,13,16,19-Docosahexaenoic acid	Cervonic acid (DHA)
24:0	CH_3-$[CH_2]_{22}$-COOH	Tetracosanoic	Lignoceric

II.4. Simple lipids

They are esters of fatty acids classified according to the alcohol:

- **Triglycerides or triacylglycerols** are esters of glycerol.
- **Waxes** are esters of long-chain alcohols (fatty alcohols).
- **Sterol esters** are esters of sterols (polycyclic alcohols).

II.4.1. Glycerides (acylglycerols)

Esters of glycerol and fatty acids. Glycerol has 3 esterification positions.

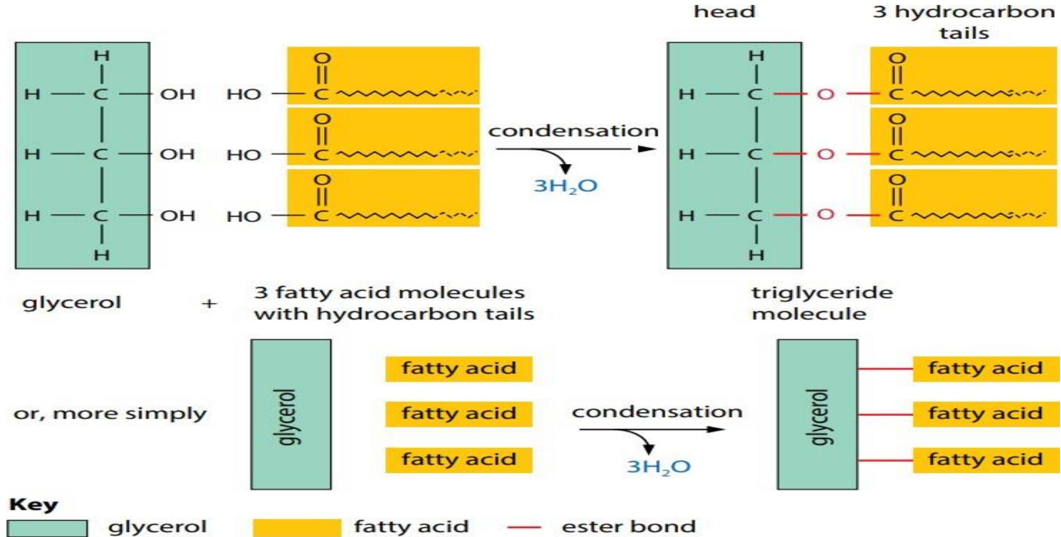


Figure II.2. Synthesis of glycerides.

➔ **Physicochemical properties**

Table II.4. Enzymatic hydrolysis and saponification of lipids.

Physicochemical properties	Reaction
Enzymatic hydrolysis	$R_2-CO-O-{}^*CH(CH_2-O-CO-R_1)(CH_2-O-CO-R_3) \xrightarrow{lipase} 2\,R-CO_2H + R-CO-O-CH(CH_2OH)(CH_2OH)$ Triglyceride — fatty acids. — monoglyceride
Saponification SV = (nMW KOH * 1 g) / MW of Fat	$H-C(-O-CO-R')(CH_2O-CO-R)(CH_2O-CO-R'') \xrightarrow{KOH} H-C(-OH)(CH_2OH)(CH_2OR) + K^{\oplus}\,{}^{\ominus}O-CO-R + K^{\oplus}\,{}^{\ominus}O-CO-R' + K^{\oplus}\,{}^{\ominus}O-CO-R''$

II.4.2. Waxes

The lipid alcohols are also not hydrolyzable. They include long-chain alcohol (**Fig. II.3**).

$$CH_3(CH_2)_{14}-C(=O)-OH + H-O-(CH_2)_{29}CH_3 \longrightarrow CH_3(CH_2)_{14}-C(=O)-O-(CH_2)_{29}CH_3 + H_2O$$

fatty acid (Palmitic acid)

long-chain alcohol (Melissyl alcohol)

palmitic acid portion

long-chain alcohol portion

a wax (Melissyl palmitate)

Figure II.3. Formation of waxes.

II.4.3. Sterol esters

These result from the esterification of fatty acids with sterols (the most representative sterol being cholesterol).

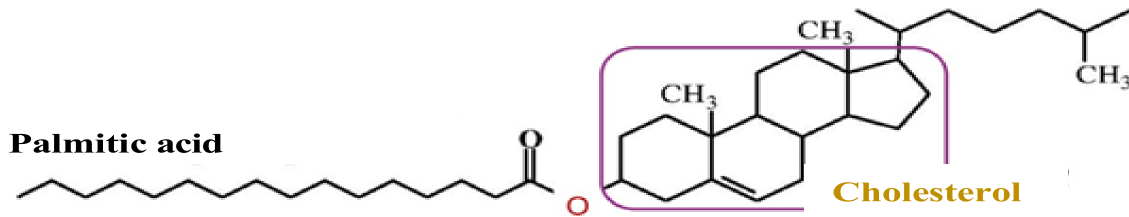


Figure II.4. Cholesteryl palmitate.

II.5. Complex lipids

II.5.1. Glycerophospholipids

Glycerophospholipids are lipids composed of fatty acids and an alcohol in which the molecule also contains additional functional groups such as phosphate (P), nitrogen (N), or sulfur (S).

In these compounds, one of the primary hydroxyl groups of glycerol is esterified with phosphoric acid (H_3PO_4) rather than with a fatty acid.

Typically, a saturated fatty acid is esterified at position sn-1, while an unsaturated fatty acid is esterified at position sn-2 of the glycerol backbone.

The simplest representative of this class is phosphatidic acid **(Fig. II.5).**

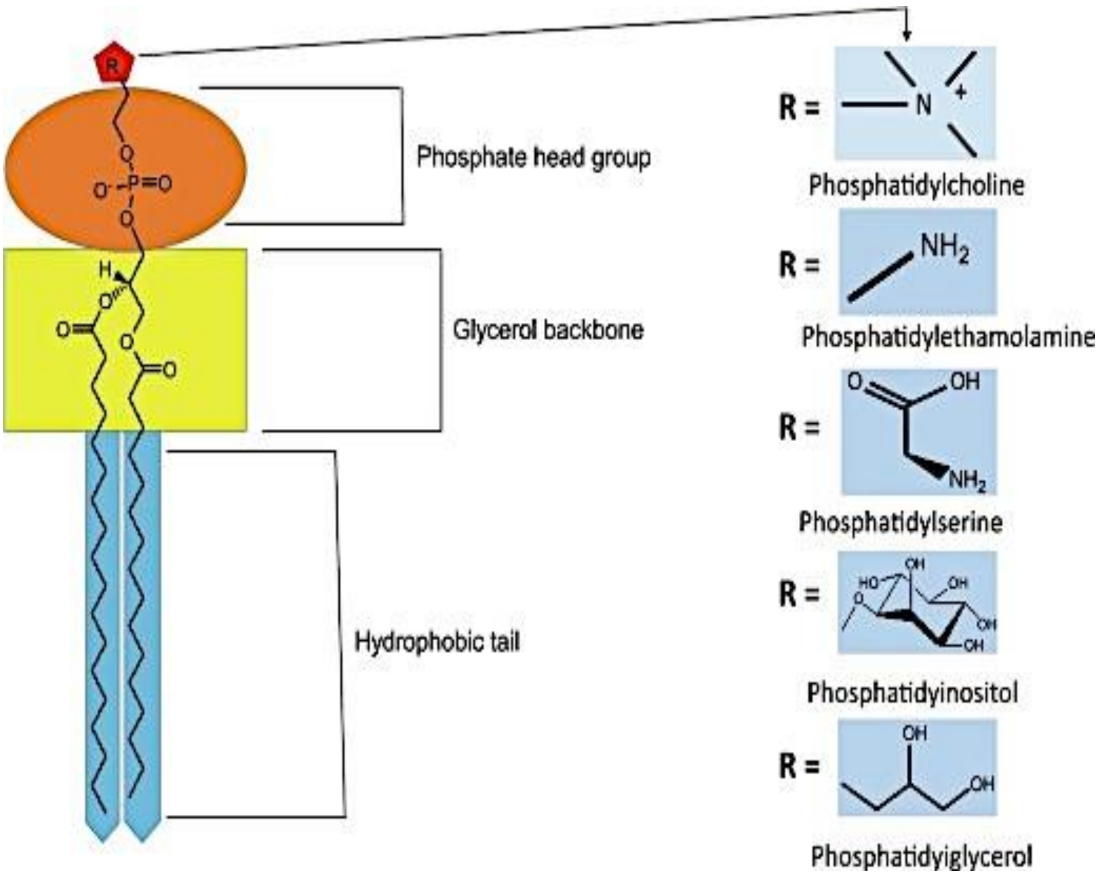


Figure II.5. Different types of glycerophospholipids.

➔ **Enzymatic hydrolysis**

Glycerophospholipids can be hydrolyzed by four specific enzymes: phospholipases A1, A2, C, and D **(see Fig. II.6**).

- **Phospholipase A1 (PLA1):** catalyzes the hydrolysis of the ester bond at the sn-1 position, releasing the fatty acid linked to the primary alcohol of glycerol.
- **Phospholipase A2 (PLA2):** catalyzes the hydrolysis of the ester bond at the sn-2 position, releasing the fatty acid linked to the secondary alcohol of glycerol.
- **Phospholipase C (PLC):** cleaves the bond between glycerol and the phosphate group, producing diacylglycerol (DAG) and a phosphorylated head group.
- **Phospholipase D (PLD):** cleaves the bond between the phosphate group and the polar head group (alcohol other than glycerol), generating phosphatidic acid and a free alcohol.

Phospholipase A_1 (free fatty acid and lysophospholipid)

Phospholipase A_2 (free fatty acid and lysophospholipid)

Phospholipase D (phosphatidic acid)

Phospholipase C (diacylglycerol and inositol triphosphate)

Figure II.6. Enzymatic hydrolysis of glycerophospholipids.

II.5.2. Glyceroglycolipids

In glyceroglycolipids, the C1 and C2 positions of glycerol are esterified with fatty acids, while the hydroxyl group at C3 is not esterified but instead is linked to a carbohydrate (monosaccharide or oligosaccharide) via a glycosidic bond.

II.5.3. Sphingolipids

Sphingolipids are lipids built on a sphingosine, which is a long-chain (18 carbons), diolamine formed from the condensation of palmitic acid (16C) and the amino acid serine (3C), containing a trans double bond at position C4.

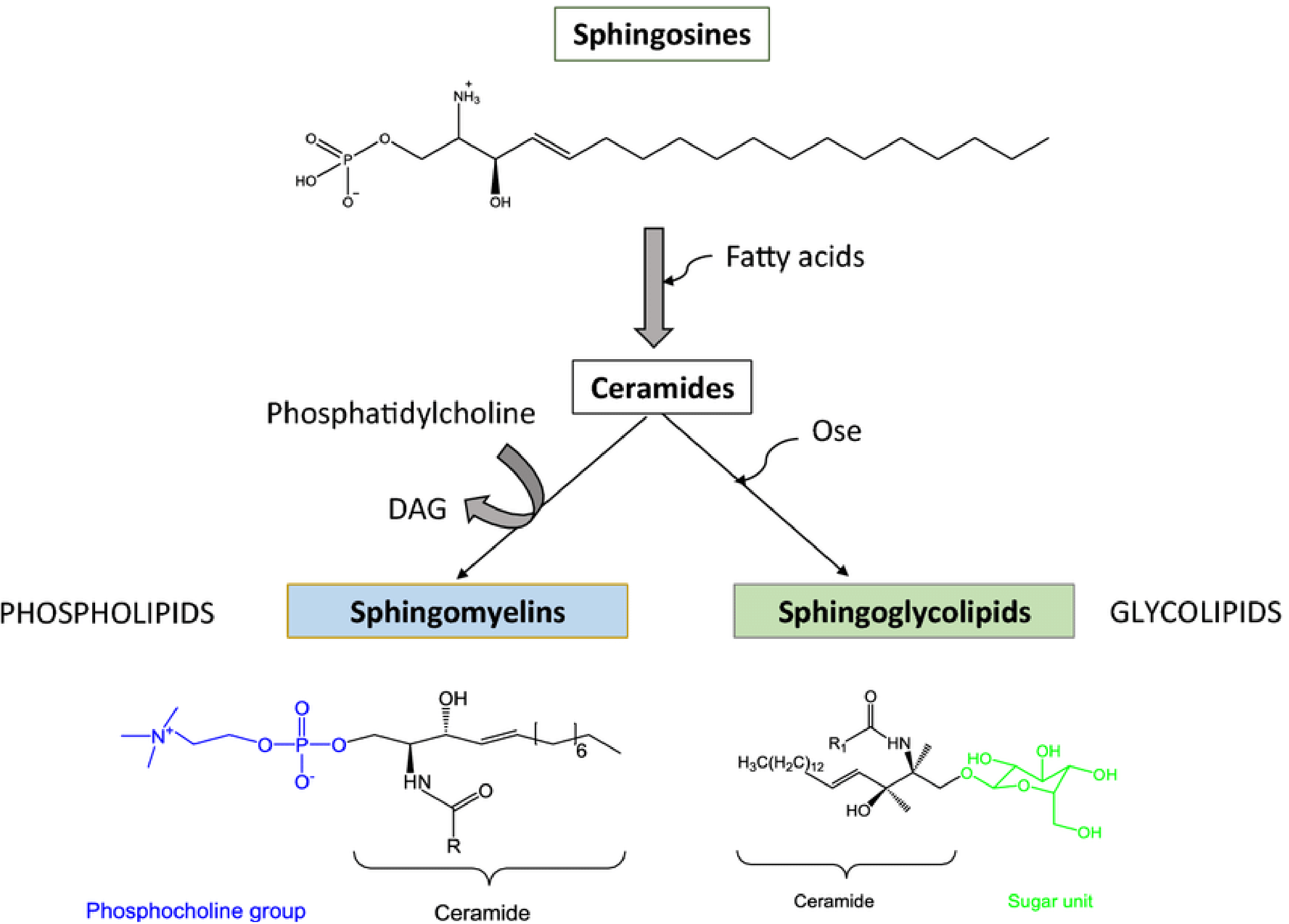


Figure II.7. Structure of sphingolipids.

Exercises

Exercise 1

1. Write the semi-developed chemical formula of the following fatty acids:

C12:0; C18:1 Δ^9; C18:3 $\Delta^{9, 12, 15}$

2. Give the common name (origin) of the 3 fatty acids.
3. What is the difference between the 3 fatty acids?
4. What differentiates FA2 from FA3?
5. Represent these 3 chemical formulas using the ω nomenclature.

Exercise 2

Given the following fatty acids: (FA1: $C_{25}H_{51}COOH$; FA2: $C_{15}H_{29}COOH$; FA 3: $C_{17}H_{31}COOH$)

1. Which fatty acid has the highest melting point? Justify.
2. Write the reaction of FA1 with KOH and calculate its saponification value.
3. Calculate the iodine value of FA2.

Exercise 3

A fatty acid with 20 carbons (C=20) gives the following products under the effect of $KMnO_4$: HOOC-(CH_2)3-COOH + 3(HOOC-CH_2-COOH) + $_3$HC-(CH_2)4-COOH

1. Deduce the effect of $KMnO_4$ on the fatty acid.
2. Identify this lipid and evaluate its iodine value.

Exercise 4

The analysis of a monoglyceride gives an SV = 186.67; the fatty acid composing it fixes 1 molecule of I_2.

1. Determine the molecular weight of the monoglyceride. Determine the formula and name of the fatty acid composing it. Provide the chemical formula and the complete name of the monoglyceride.
2. Which digestive enzyme is capable of hydrolyzing TG?

Exercise 5

Part A

1. To which types of complex lipids do the two compounds below belong?

2. Present the action of phospholipases (A1, A2, C, and D) on molecule B and which constituents are obtained after specific enzymatic actions?

$CH_3-(CH_2)_{12}-CH=CH-CH-OH$

$CH_3-(CH_2)_{16}-CO-NH-CH$

$CH_2-O-P(=O)(O^-)-O-CH_2-CH_2-N^+(CH_3)_3$

A

16 COO-CH2

18 10 9 COO-CH

$CH_2-O-P(=O)(O^-)-O-CH_2-CH_2-N^+(CH_3)_3$

B

Part B: Given the following compound.

1. What is the nature of the bond between the oligosaccharide and the rest of the molecule?

2. How is this compound oriented in the plasma membrane?

3. List the products obtained from this compound by the action of a β-glucosidase.

CH_2OH CH_2OH CH_2OH OH

$O-CH_2-CH-CH-CH=CH-(CH_2)_{12}-CH_3$

NH

$CO-(CH_2)_{22}-CH_3$

$NH-CO-CH_3$

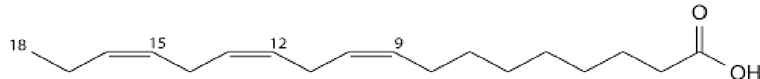
Solutions

Answer 1

1. Semi-developed chemical formulas.

$CH_3(CH_2)_{10}-\overset{\overset{\displaystyle O}{\|}}{C}-OH$,

$CH_3(CH_2)_7CH{=}CH(CH_2)_7\overset{*}{C}O_2H$,



2. Common names (lauric acid, oleic acid, linolenic acid respectively).
3. This is the degree of unsaturation.
4. FA2 is monounsaturated, while FA3 is polyunsaturated; FA2 is produced by the body, while FA3 is an essential fatty acid.
5. Second nomenclature (FA2 = C18:1 ω 9, FA3 = C18 :3 ω 3, 6, 9)

Answer 2

1. It is FA1 (Justification: a saturated fatty acid with a long 26C hydrocarbon chain; the other two fatty acids are unsaturated FAs, where double bonds lower the melting point).
2. Molecular weight of FA1 is 396 g, so SV = 141.41 mg.
3. Molecular weight of FA2 = 254 g, so IV= 100 g.

Answer 3

1. KMnO4 is a strong oxidant that causes double bond cleavage.
2. FA: it is arachidonic acid C20:4 Δ 5,8,11,14, MW = 304 g, IV = 334.21 g.

Answer 4

1. Molecular weight of monoglyceride = 299.99 ≈ 300 g; fatty acid: MW FA = MW monoglyceride - MW glycerol + MW H_2O, MW FA = 226, $C_nH_{2n-2}O_2$, so the FA is C14:1 Δ9, which is myristoleic acid; and the monoglyceride is myristoleylglycerol.
2. It is pancreatic lipase.

Answer 5

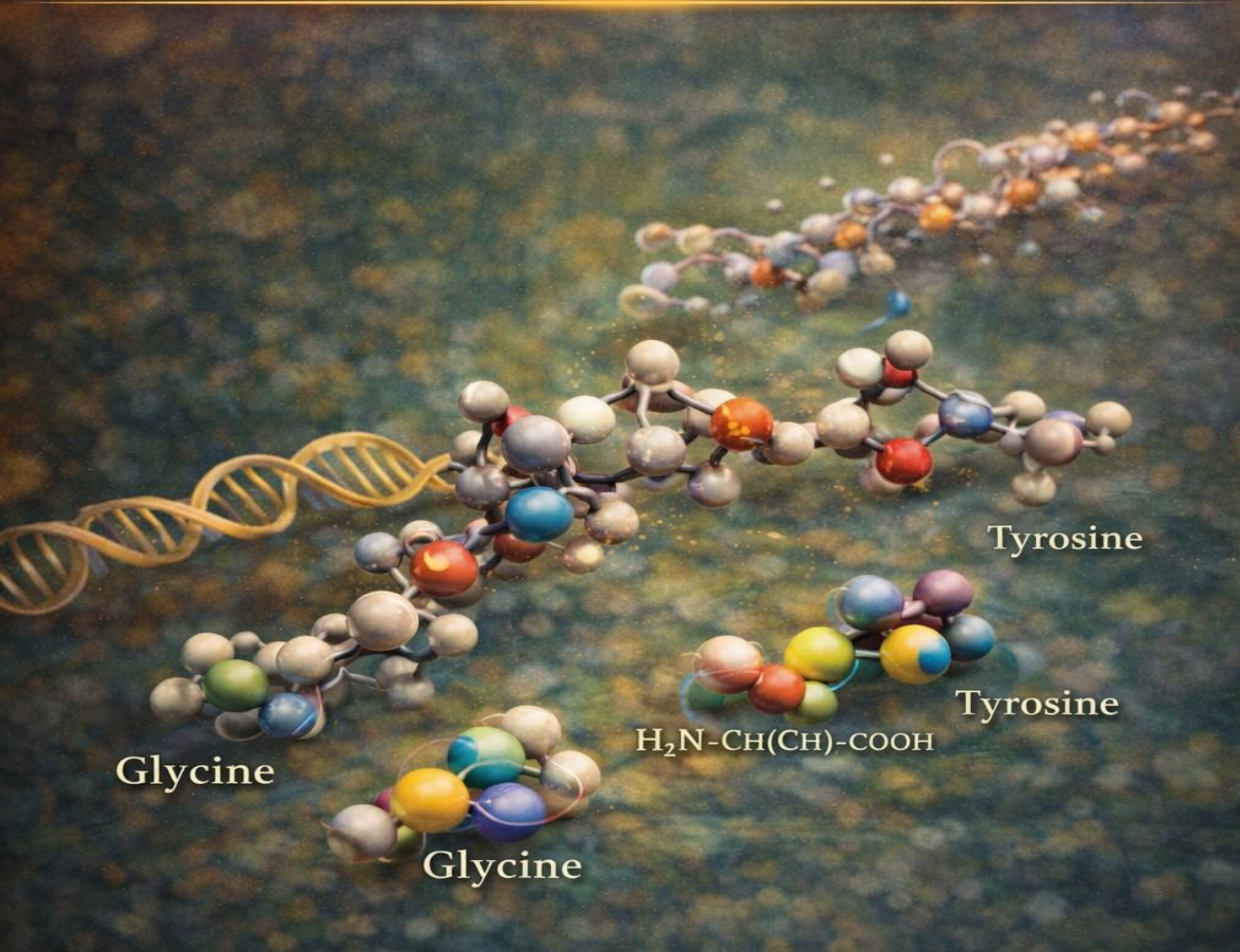
Part A

1. Complex lipids.
2. PLA1 eliminates saturated FA1 in position 1 or alpha, forming lysophosphatidylcholine; PLA2 eliminates unsaturated FA2 in position 2 or beta, forming lysophosphatidylcholine; PLC releases a diglyceride (1-palmitoyl, 2-oleoyl glycerol) and phosphocholine; PLD leads to the release of phosphatidic acid and choline.

Part B

1. β-glycosidic bond.
2. The carbohydrate part is a hydrophilic part that orients outward from the membranes, while the rest of the molecule anchors in the phospholipid bilayer of the membranes.

Chapter III.
Structure and Physiochemical Properties of Amino Acids and Peptides



Chapter III. Structure and Physicochemical Properties of Amino Acids and Peptides

Learning objectives

- To understand the classification of amino acids based on their chemical properties.
- To develop the ability to calculate the isoelectric point (pI) of amino acids.
- To master the different ionization states of amino acids as a function of pH.
- To understand the principles and techniques used for the separation of amino acids.
- To understand the methods used for peptide sequencing and the determination of amino acid sequences.

III.1. Definition

Amino acids are organic molecules containing an amino group (-NH_2) and a carboxyl group (-COOH), which are the basic building blocks of proteins.

Peptides are short chains of amino acids linked by **peptide bonds**.

Proteins are large, complex macromolecules composed of one or more polypeptide chains, folded into specific structures to perform biological functions.

III.2. Amino acids

All common amino acids are α-amino acids, with both an amino (-NH_2) and a carboxyl (-COOH) group attached to the same α-carbon. They differ by their side chain (R group), which determines their properties **(see Fig. III.1**).

In addition to the 20 standard amino acids, other modified or non-protein amino acids exist. Standard amino acids are represented by three-letter and one-letter codes.

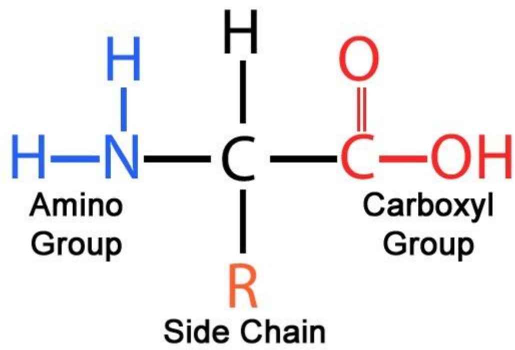


Figure III.1. Structure of amino acids.

III.2.1. Classification of amino acids

Nonpolar/hydrophobic amino acids		
	Abbreviation	Symbol
Glycine	Gly	G
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Proline	Pro	P

Nonpolar/hydrophobic AROMATIC amino acids		
	Abbreviation	Symbol
Phenylalanine	Phe	F
Tryptophan	Trp	W

Polar/hydrophilic AROMATIC amino acids		
	Abbreviation	Symbol
Tyrosine	Tyr	Y

Polar/hydrophilic, uncharged amino acids		
	Abbreviation	Symbol
Serine	Ser	S
Threonine	Thr	T
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q

Polar/hydrophilic, CHARGED amino acids			
	Abbreviation	Symbol	
Aspartic acid	Asp	D	(-)
Glutamic acid	Glu	E	(-)
Lysine	Lys	K	(+)
Arginine	Arg	R	(+)
Histidine	His	H	(+)

Figure III.2. Classification of amino acids based on polarity.

III.2.2. Physicochemical properties

- Amino acids are water-soluble molecules
- Solutions of amino acids are generally colorless.
- Most amino acids absorb ultraviolet light at wavelengths below 230 nm.
- Aromatic amino acids (tyrosine, tryptophan, and phenylalanine) absorb strongly at around 280 nm
- Amino acids exist in solution predominantly as zwitterions, carrying both positive and negative charges depending on the pH.

a. Isoelectric point of amino acids

The isoelectric point (pI) of an amino acid is the pH at which it carries no net electric charge.

For amino acids with non-ionizable side chains, the pI is calculated as the average of the two pKa values:

- **pKa_1** (carboxyl group)
- **pKa_2** (amino group)

Thus: **$pI = (pKa_1 + pKa_2) / 2$**

For amino acids with ionizable side chains, three pKa values are present.

- For acidic amino acids, the pI is calculated by averaging the two lowest pKa values.
- For basic amino acids, the pI is calculated by averaging the two highest pKa values.

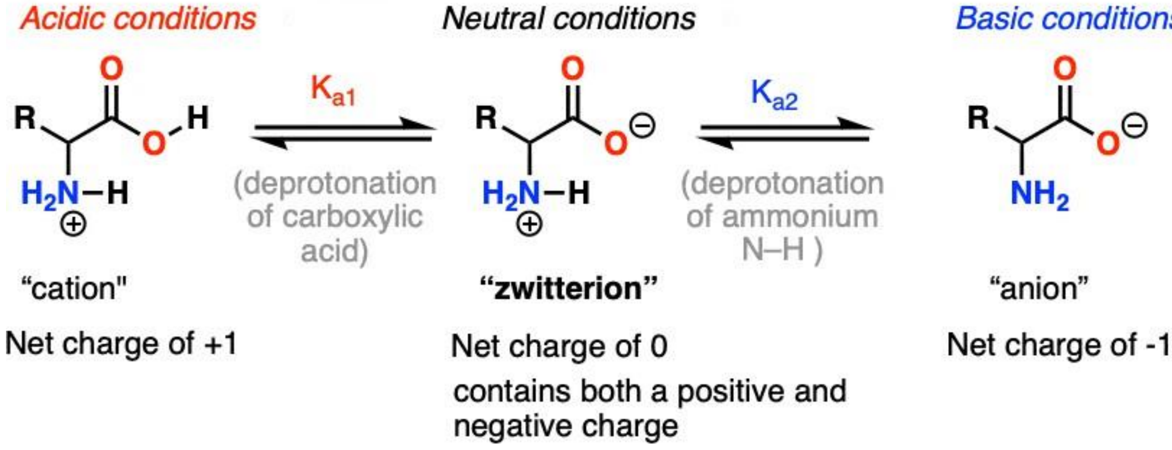


Figure III.3. Different ionic forms of amino acids.

III.2.3. Separation and detection amino acids

a. Thin layer chromatography

A separation technique based on differences in polarity, where amino acids migrate on a plate and are visualized (e.g., with ninhydrin) as colored spots **(see Fig. III.4)**.

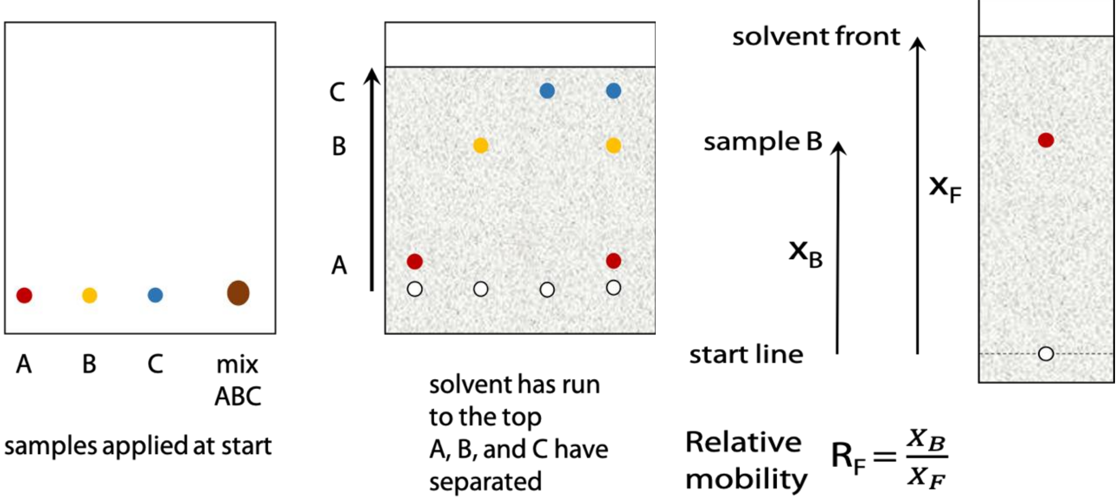


Figure III.4. Determining the relative mobility of sample B.

b. Ion-exchange chromatography

Ion-exchange chromatography is a technique that separates amino acids based on their net charge, using a charged resin. There are two types: cation-exchange chromatography, which binds positively charged molecules, and anion-exchange chromatography, which binds negatively charged molecules **(Fig. III.5)**.

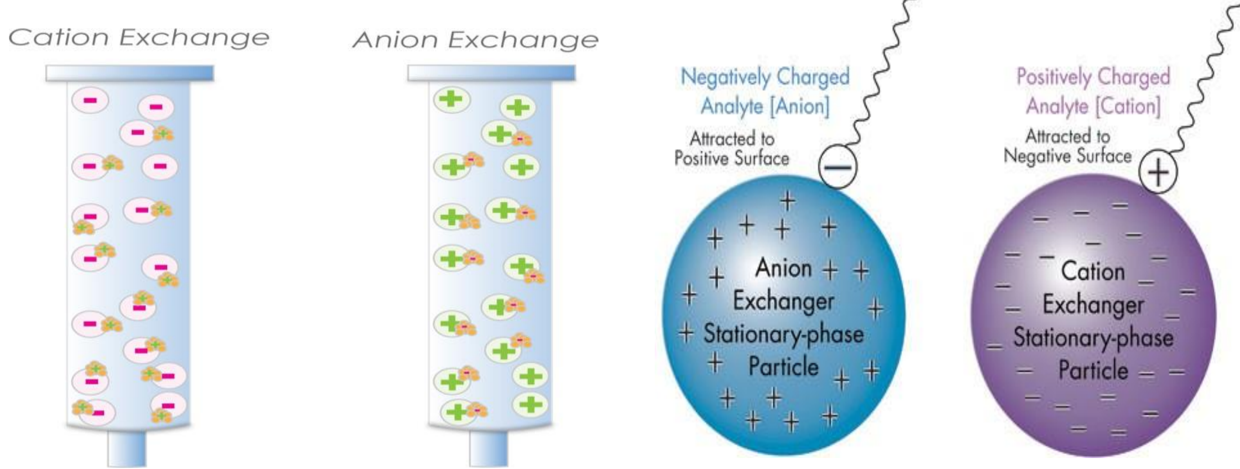


Figure III.5. Cation and anion exchange chromatography.

c. Electrophoresis

Electrophoresis of amino acids is a separation technique based on their net charge, where amino acids migrate in an electric field at different rates depending on the pH of the medium relative to their pI **(see Fig. III.6)**.

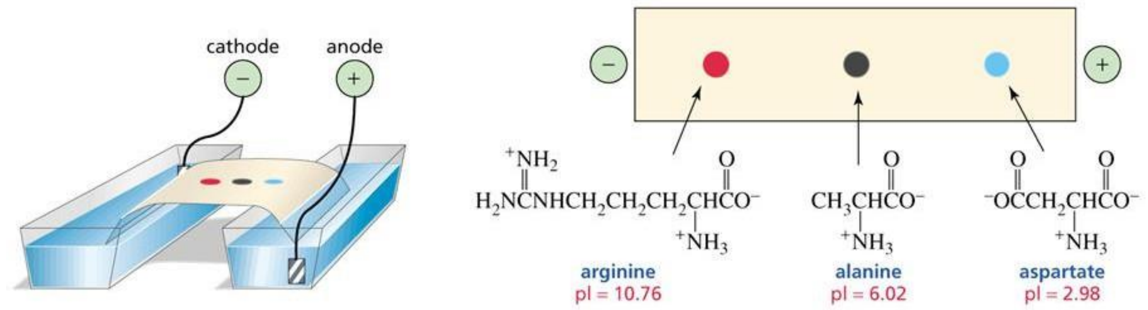


Figure III.6. Electrophoresis.

III.3. Peptides

Peptides are chains of amino acids linked by peptide bonds, forming a linear sequence that defines the primary structure and is written from the N-terminus to the C-terminus. The peptide bond is a covalent amide bond formed between the carboxyl group of one amino acid and the amino group of another, through a condensation reaction with the release of water (H_2O) **(Fig. III.7)**.

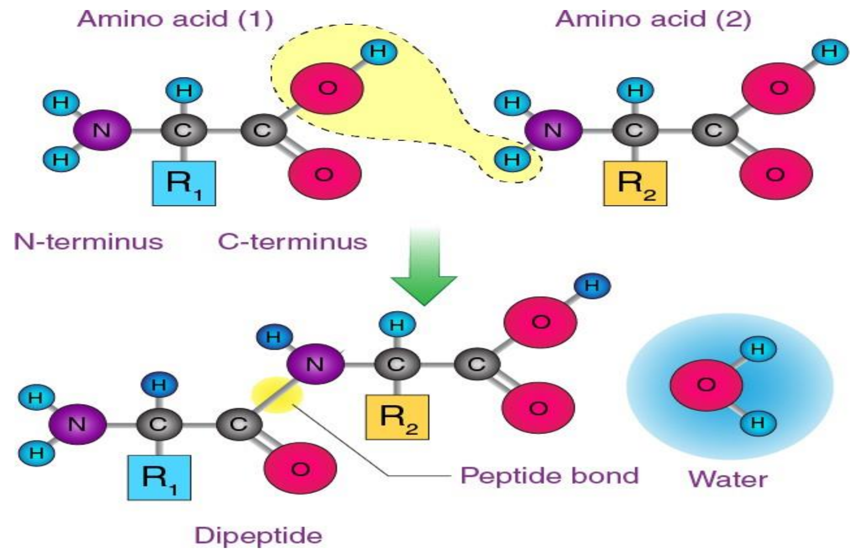


Figure III.7. Formation of peptide bonds and peptides.

III.3.1. Peptides sequencing

Table III.1. Steps of peptide sequencing.

Step / Method	Principle	Purpose / Outcome
1. Reduction of disulfide bonds	Break S-S bonds (e.g., β-mercaptoethanol)	Separates polypeptide chains
2. Hydrolysis (partial)	Chemical or enzymatic cleavage	Produces smaller peptide fragments
3. Specific cleavage	Enzymes (trypsin, chymotrypsin) or chemicals (CNBr)	Generates overlapping fragments
4. N-terminal identification	Edman degradation, Sanger method	Identifies first amino acid
5. C-terminal identification	Carboxypeptidases	Identifies last amino acid
6. Fragment analysis	Chromatography / sequencing	Determines fragment sequences
7. Sequence reconstruction	Alignment of overlapping fragments	Obtains complete peptide sequence

Table III.2. Key enzymes and reagents.

Type	Example	Specificity
Endopeptidase	Trypsin	Cuts after Lys, Arg
	Chymotrypsin	Cuts after aromatic AA
	Pepsin	Cuts before aromatic AA
Exopeptidase	Aminopeptidase	Removes N-terminal AA
	Carboxypeptidase	Removes C-terminal AA
Chemical	CNBr	Cuts after Met
Edman degradation	Phenylisothiocyanate (PITC)	Sequential removal and identification of N-terminal amino acids (stepwise sequencing)
Sanger method	DNFB (2,4-dinitrofluorobenzene)	Labels N-terminal amino acid → complete hydrolysis → identification of DNP-AA
Dansylation	Dansyl chloride (DNS-Cl)	Forms fluorescent derivative → hydrolysis → identifies first amino acid

Exercises

Exercise 1

Using the ionization pK values given below, write the ionization equation, and calculate the pHi values of the amino acids:

Amino Acid	pKa (α-COOH)	pKa (α-NH_2)	pKa (R group)
Leucine	2.3	9.7	—
Glutamic Acid	2.1	10.0	4.3
Lysine	2.2	9.2	10.5
Tyrosine	2.2	9.1	10.0

Exercise 2

A mixture of amino acids containing aspartic acid, cysteine, alanine, and arginine is subjected to two electrophoreses in pH buffers of 3 and 6 respectively.

1. Give the principle of the electrophoresis technique

2. Give the result obtained in the form of an electropherogram and justify your answer.

Exercise 3

A mixture of amino acids indicated in the first exercise was placed in the ion exchange chromatography. The chromatography support is composed of sulfonic resin (sulfonate groups: SO_3^-). Elution is carried out using a solution with an increasing pH gradient.

- Give the order of elution of the different amino acids.

Exercise 4

Consider the following peptide: $_2$HN-Asp-Lys-Tyr-Arg-Ala-COOH (pHi Asp = 2.9/pHi Lys = 9.7/pHi Tyr = 5.6/pHi Arg = 10.8/pHi Ala = 6.1); after acid hydrolysis, we want to separate the mixture of amino acids by ion exchange chromatography.

1. What type of resin and what pH range of buffer (medium) should be chosen to elute only basic amino acids and retain other amino acids.

Exercise 5

Let's say a mixture of three peptides:

A) His-Gly-Pro-Lys

B) Glu-Leu-Cys-Asp

C) Ala-Gly-Ile-Ser

They are subjected to zone electrophoresis at pH = 6.

- Indicate the position of the three peptides using a diagram. Justify your answer.

Exercise 6

Let a heptapeptide (P) be formed from different amino acids and not contain Trp.

- The action of chymotrypsin on P gives a tripeptide A and a tetrapeptide B.

- The action of aminopeptidase on A releases a hydroxylated amino acid possessing two asymmetric carbons.

-The action of carboxypeptidase on A releases an apolar aromatic amino acid.

-Tripeptide A contains a 4-carbon acidic amino acid in the center.

-The action of DNFB on tetrapeptide B followed by hydrolysis releases the compound DNP-Val followed by basic DNP-AA with a guanidine group.

-Reduction of B by $LiBH_4$ followed by acid hydrolysis releases 3 amino acids and the following compound: $_2HN-CH-CH_2OH$

$$
\begin{array}{l}
{}_2HN-\underset{|}{CH}-CH_2OH \\
\quad\ \ (CH_2)_2-CH_2OH
\end{array}
$$

-Tetrapeptide B contains an amino acid without optical activity.

1. Give the sequence of P, briefly explaining each of the steps.

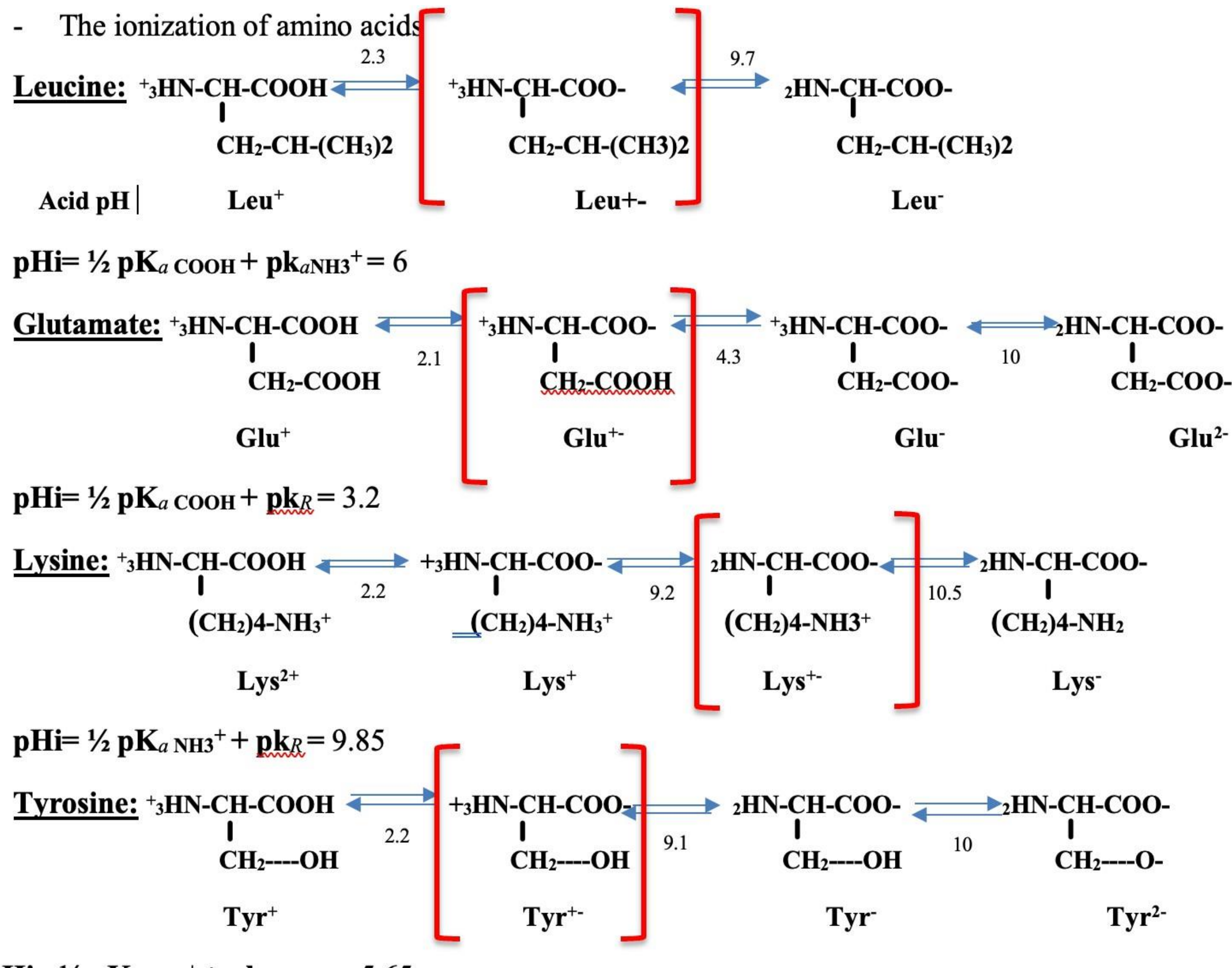
2. Give the developed formula of this peptide.

Exercise 7

We want to determine the molecular weights of a protein, knowing that the protein contains 0.2% His and that the MW of His is 155, calculate the minimum MW of the protein. Ultracentrifugation of the protein gave a MW of 390 000. Indicate the number of His molecules included in the composition of this peptide.

Solutions

Answer 1



Answer 2

pHi Cys (blue) = 5.1 / pHi Asp (yellow) = 2.9 / pHi Ala (red) = 6.1 / pHi Arg (green) = 10.8

1. Principle of electrophoresis: Aims to separate charged molecules through a gel (a polymer) under the effect of an electric field. Cationic molecules (+) move towards the cathode (−)
2. Electropherogram at pH=3

Cathode (-) Anode (+)

Electrogram at pH=6

Overall charge + neutral (+-) overall charge -

pH < pHi pH = pHi pH > pHi

Answer 3

-Separation of the following amino acids (Leu, Glu, Lys, Tyr) using ion exchange chromatography using a sulfonic resin (counter ion with a positive charge), so it is cation exchange chromatography.

-The order of elution: first is aspartic acid, then tyrosine, then Leu, and last is Lys.

Answer 4

-Anion exchange resin and a basic pH (pH = 12): in this pH all the amino acids are negatively charged; then the pH decreases (decreasing pH) and the first one to come out of the column is the amino acid Arg, followed by Lys.

Answer 5

Calculate the pHi of each peptide:

A) (9.2) $^{+}{}_3$HN-His-Gly-Pro-Lys-COOH (2.2) ⇄ (2.2) $^{+}{}_3$HN-His-Gly-Pro-Lys-COO- ⇄ (6)

His side chain: NH^+ (6); Lys side chain: NH_3^+ (10.5)

Pep A^{3+} ; Pep A^{2+}

$^{+}{}_3$HN-His-Gly-Pro-Lys-COO- [N (6), NH_3^+ (10.5)] ⇄ (9,2) $_2$HN-His-Gly-Pro-Lys-COO- [N (6), NH_3^+ (10.5)] ⇄ (10.5) $_2$HN-His-Gly-Pro-Lys-COO- [N (6), NH_2 (10.5)]

Pep A $^{+}$; [Pep A $^{+-}$] ; Pep A $^{-}$

pHi (pep A) = 9.2+ 10.5/ 2= 9.85

In the same way pep B and C

Pep B: $^{+}$**3HN-Glu-Leu-Cys-Asp-COOH (pKa_{NH2} Glu = 10 ;pK_R Glu = 4.3 ; pK_R Cys= 8.2 ; pK_R Asp = 3.7 ; pKa_{COOH} Asp= 2)**

- Pep B^+ ⇄ (2) **[Pep B^{+-}]** ⇄ (3.7) **Pep B^-** ⇄ (4.3) **Pep B^{2-}** ⇄ (8.2) **Pep B^{3-}** ⇄ (10) **Pep B^{4-}**

pHi (pep B) = 2+ 3.7/ 2= 2.85

Pep C: $^{+}{}_{3}$**HN-Ala-Gly-Ile-Ser-COOH (pKa_{NH2} Ala = 9.9; pKa_{COOH} Ser= 2.2)**

Pep C^+ ⇄ (2.2) **[Pep C^{+-}]** ⇄ (9.9) **Pep C^-**

pHi (pep C) = 2.2+ 9.9/ 2= 6.05

Cathode — Anode

pH= 6

-pHi pep A (🟡) = 9.85 > pH ½ = 6 so pep A is positively charged and migrates towards the cathode.

-pHi pep B (🔴) = 2.85 < pH ½ = 6 so pep B is negatively charged and migrates towards anode.

- pHi pep C (🟢) = 6.05 = pH ½ = 6 therefore pep C has a neutral overall charge, and it does not migrate.

Answer 6

-Heptapeptide —Chymotrypsin→ tripep A + tetrapep B

-Pep A —Aminopeptidase A.→ hydroxylated AA with 02 asymmetric carbons ($_2$HN-Thr)

- Pep A —Carboxypeptidase AA→ aromatic apolar (Either Phe or Trp but in the exercise he mentions that our peptide does not contain Trp so it is Phe). (-Phe-COOH)

- Tripeptide A contains a 4-carbon acidic amino acid (Asp) in the center.

So pepA: 2HN-Thr-Asp-Phe-COOH

- Tetrapep B. —DNFB→ DNP-Val + DNP-AA basic with guanidine group (His)

So, the first in the N-terminal is the AA Val followed by His

- -Pep B —$LiBH_4$→ 3 AA+ $_2$HN-CH-CH_2OH (alcohol obtained after reduction of AA Glu)
with side chain $(CH_2)2$-CH_2OH on the CH

The reduction of free carboxylic functions so the last one is AA Glu.

-Pep B contains an amino acid without optical activity; it is glycine or glycol.

So, Pep B: $_2$HN-Val-His-Pro-Glu-COOH

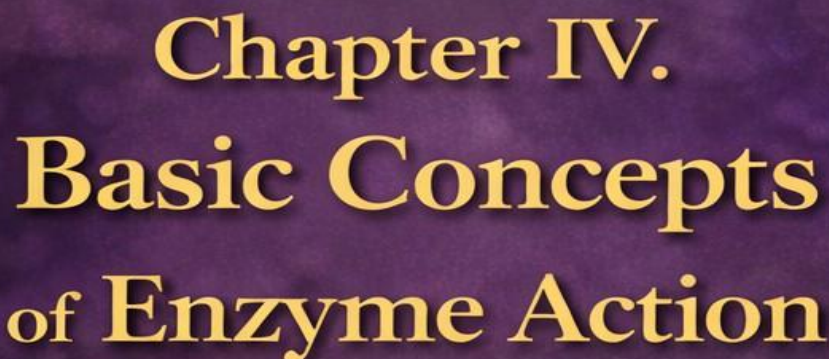
Heptapeptide: $_2$HN-Thr-Asp-Phe-Val-His-Pro-Glu-COOH.

Answer 7

Protein contains 0.2% His and MW His = 155

MW protein = 100X155/0.2 = 7750

MW protein = 390,000 so the number of His molecules included in the composition of this peptide is 5 His molecules (39000/7750=5)



Chapter IV. Basic Concepts of Enzyme Action

Chapter IV. Basic Concepts of Enzyme Action

Learning objectives

-To describe the mechanism of enzyme action, including enzym-substrate complex formation.

-To understand the concepts of active site, specificity, and catalytic efficiency.

-To analyze the factors affecting enzyme activity (temperature, pH, substrate concentration).

-To understand enzyme kinetics, including the Michaelis-Menten model and key parameters (Km and Vmax).

-To differentiate between types of enzyme inhibition (competitive, non-competitive, uncompetitive).

IV.1. Definition

Enzymes are biological catalysts, mostly proteins, that accelerate chemical reactions without being consumed in the process.

IV.2. Classification

Enzymes are classified based on the type of reaction they catalyze.

The first number of the EC code defines the main class (**Fig. IV.1**).

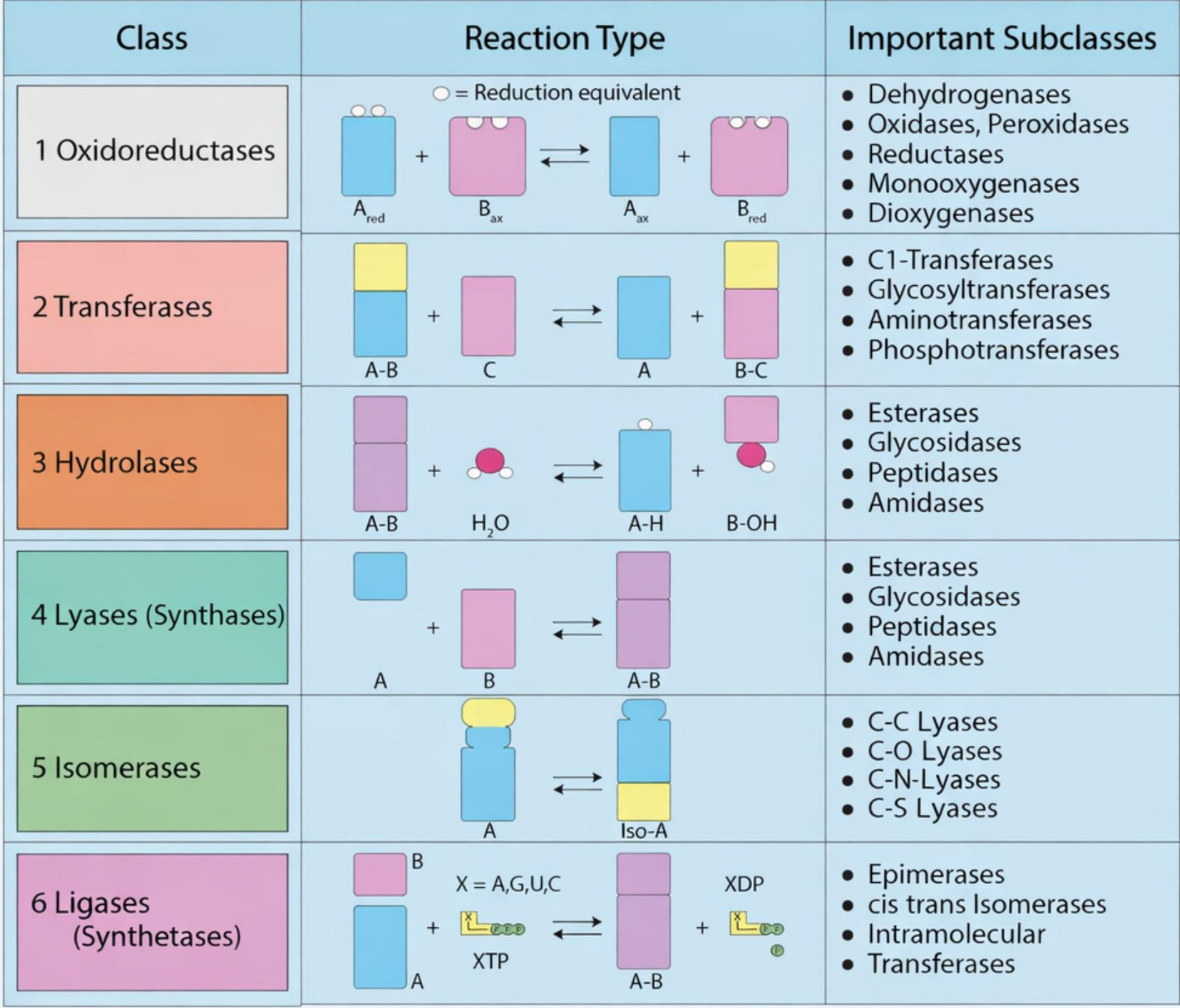


Figure IV.1. Classes of enzymes.

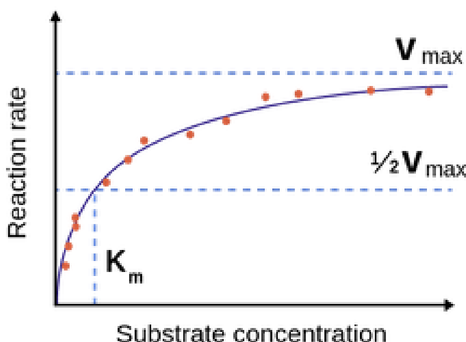
IV.3. Enzyme kinetics

Enzyme kinetics studies the rate of enzyme-catalyzed reactions and how it is affected by factors such as substrate concentration, enzyme concentration, pH, and temperature. It describes the formation of the enzyme–substrate complex and is commonly analyzed using the Michaelis-Menten model, which defines key parameters such as Vmax (maximum velocity) and Km (substrate affinity) **(Fig. IV.2)**.

The Lineweaver-Burk transformation is the double reciprocal form of the Michaelis–Menten equation. It is obtained by taking the reciprocal of both sides:

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

It is used to determine Km and Vmax more easily and to analyze enzyme inhibition (**Fig. IV.2**).



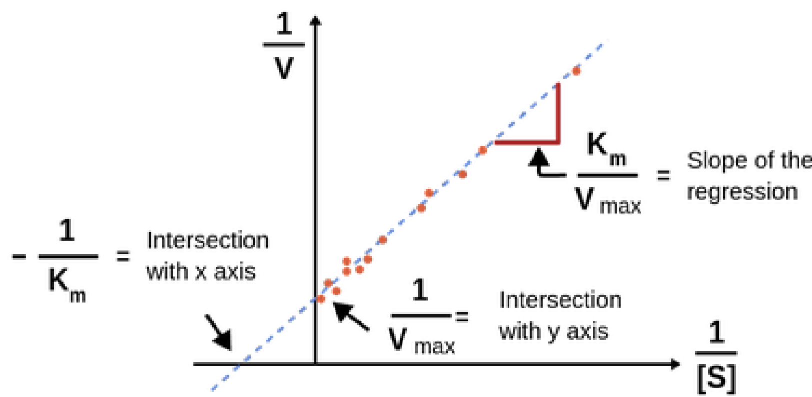


Figure IV.2. Michaelis-Menten curve and Lineweaver-Burk transformation.

IV.4. Reversible inhibition

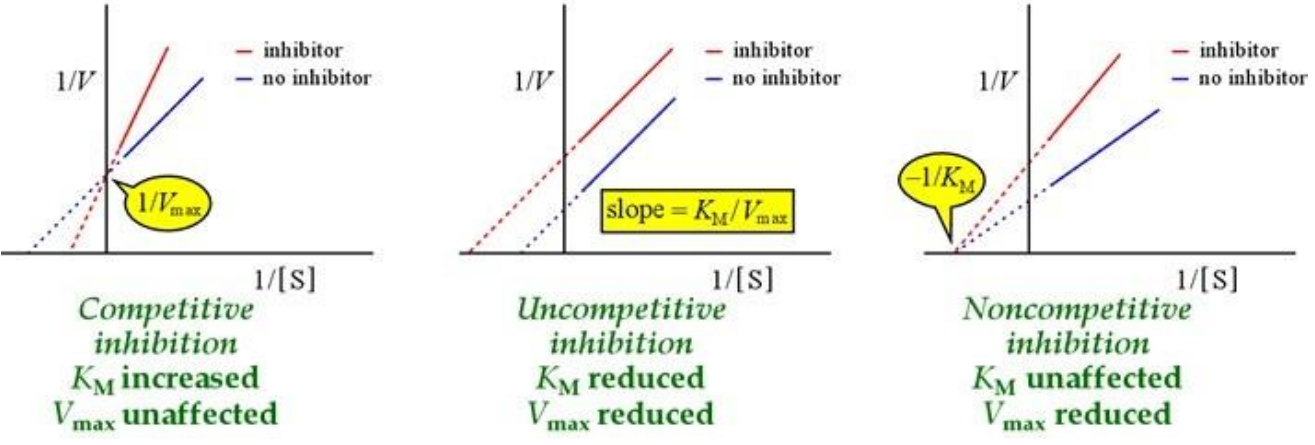


Figure IV.3. Competitive, uncompetitive, and noncompetitive inhibition.

Exercises

Exercise 1

To which class of enzymes do the enzymes catalyzing the following reactions belong:

1. Glyceraldehyde-3-phosphate + NAD^+ +P_i → NADH + H^+ + 1,3-bisphosphoglycerate
2. Glucose 6-P ⟷ Fructose 6-P
3. ATP + D-Hexose → ADP + D-Hexose phosphate.
4. Glucose 6-phosphate + H_2O → Glucose + Pi
5. CH_3-$(CH_2)_n$-COOH + CoASH + ATP → CH_3-$(CH_2)_n$-CO~SCoA + AMP + 2Pi
6. Glucose-6-P ⟷ Glucose-1-P
7. CH_3-$(CH_2)_n$-CH=CH-$(CH_2)_n$'-CO~SCoA + H_2O ⟷ CH_3-$(CH_2)_n$-CHOH-CH_2-$(CH_2)_n$'-CO~SCoA
8. Glycogen + phosphate → glycogen $_{(n-1)}$ + α-D-glucose phosphate

Exercise 2

Glucokinase catalyzes the reaction: D-glucose + ATP ⇌ D-glucose 6-phosphate + ADP. The Michaelis constant of *Bacillus stearothermophilus* glucokinase is given for the following substrates:

Substrates	ATP	TTP	GTP	UTP	CTP
K_m (M)	6×10^{-5}	6×10^{-4}	1.2×10^{-3}	4.5×10^{-3}	3.6×10^{-3}

1. Identify the class of the enzyme.
2. Rank the substrates in order of increasing apparent affinity for glucokinase.

Exercise 3

1. A cell extract C contains 28 mg of enzymatic protein per mL.

   Ten microliters of this extract catalyze the conversion of 0.70 µmol of substrate in 5 minutes, under standard conditions of pH and temperature, and at a substrate-saturating concentration during the experiment.

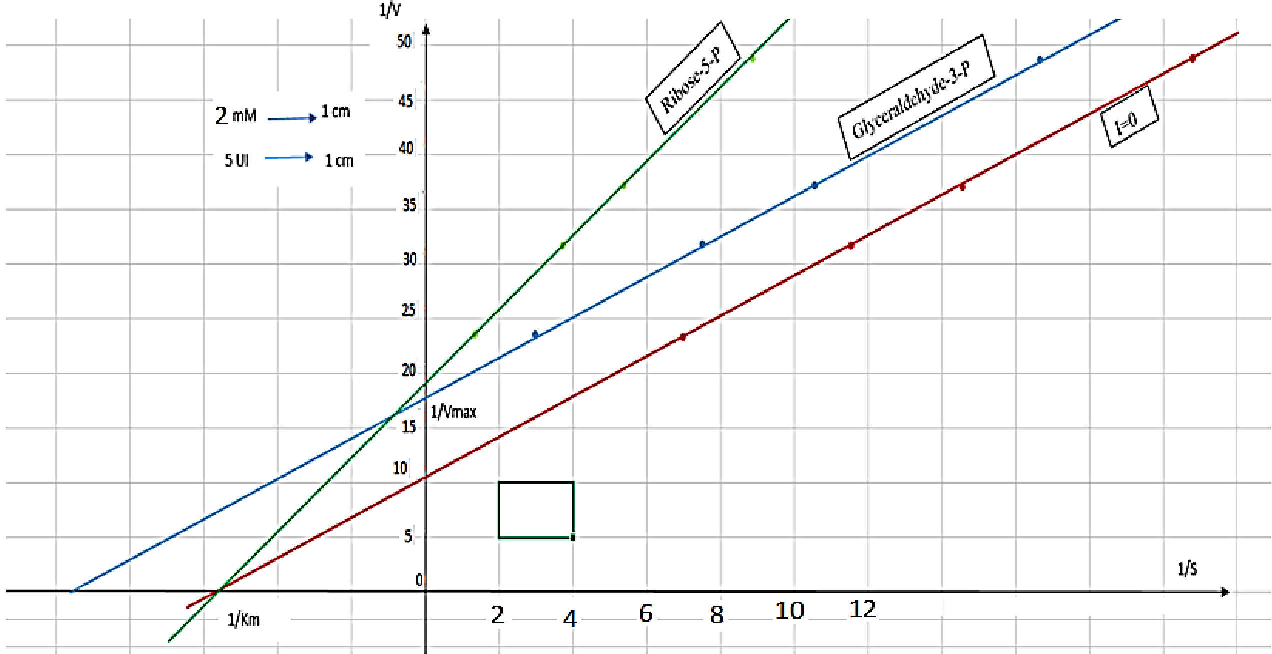
   a) Calculate the enzymatic activity of extract C in µmol of substrate converted per minute and per mg of protein.
   b) Why is the experiment carried out under defined conditions of pH, temperature, and at a substrate-saturating concentration for the duration of the assay?

2. What does the molecular enzymatic activity (MEA) represent? What information would be required to calculate it?

Exercise 4

We monitor the catalysis of glucose-6-phosphate into phosphogluconic acid by the enzyme glucose-6-phosphate dehydrogenase, first in the absence and then in the presence of two different substrates (glyceraldehyde-3-phosphate at a concentration of $[I_1] = 4.10^{-5}$ mM and ribose-5-phosphate at a concentration of $[I_2] = 4.10^{-3}$ mM

The results obtained allowed us to plot the Lineweaver-Burk plot shown below.



1. Graphically determine Km and Vmax in each case.
2. Determine the type of inhibition for each inhibitor.
3. Determine the Ki for each of the two inhibitors.

Solutions

Answer 1

1- Oxidoreductase. 2- Isomerase. 3- Transferase. 4- Hydrolase
5- Ligase (synthetases). 6- Isomerase. 7- Lyase. 8- Transferase

Answer 2

1. **Class of enzymes:** Glucokinase is a transferase.
2. **Ranking according to affinity:** Km = 1/affinity.

Substrates	ATP	TTP	GTP	UTP	CTP
K_m (M)	6×10^{-5}	6×10^{-4}	1.2×10^{-3}	4.5×10^{-3}	3.6×10^{-3}
Affinity	16.66×10^3	1.66×10^3	0.83×10^3	0.22×10^3	0.27×10^3

The order: UTP, CTP, GTP, TTP, ATP.

Answer 3

1. Extract C: 28 mg of enzyme → 1 mL = 1000 µL

   X → 10 µL

   Therefore, the amount of enzyme is 28×10^{-2} mg

Enzymatic activity (EA):

We have 0.70 µmol / 5 min → therefore, EA = 0.70 / 5 = 0.14 µmol / 1 min.

0.14 µmol/min → 28×10^{-2} of enzyme

SA → for 1 mg of enzyme

Thus, SA = 0.5 µmol of substrate transformed per min per mg of enzyme (or IU).

2. The enzyme is placed under standard conditions (pH, temperature, and saturating substrate concentration) that allow the enzyme to reach its optimal activity.

3. MEA (Molecular Enzymatic Activity)

Number of moles of substrate transformed per minute per mole of enzyme. We would need to know the enzyme's molecular weight.

Answer 4

1. Determine graphically K_M and V_{max}

$1/Km = -5.8$ ⟶ $Km = 17.24 * 10^{-2}$ mM

$1/Vmax = 10.5$ ⟶ $Vmax = 9.52 * 10^{-2}$ UI

$1/Km_{(Glyceraldehyde\text{-}3\text{-}P)} = -9.5$ ⟶ $Km_1 = 10.52 * 10^{-2}$ mM

$1/Vmax_{(Glyceraldehyde\text{-}3\text{-}P)} = 17$ ⟶ $Vmax_1 = 5.88 * 10^{-2}$ UI

$1/Km_{(Ribose\text{-}5\text{-}P)} = -5.8$ ⟶ $Km_2 = 17.24 * 10^{-2}$ mM

$1/Vmax_{(Ribose\text{-}5\text{-}P)} = 19.2$ ⟶ $Vmax_2 = 5.20 * 10^{-2}$ UI

2. Determine the type of inhibition for each inhibitor.

$Km > Km_1$ ⟶ K_{m1} decrease ⟶ $K_{m1} = K_m / \alpha$

$Vmax > Vmax_1$ ⟶ **V_{max} decrease** ⟶ $V_{max1} = V_{max} / \alpha$

So, **Glyceraldehyde-3-P** is an **Uncompetitive inhibitor.**

$Km = Km_2$ ⟶ same affinity

$Vmax > Vmax_2$ ⟶ **V_{max} decrease** ⟶ $V_{max2} = V_{max} / \alpha$

So, **Ribose-5-P** is a **Noncompetitive inhibitor**

3. Determine the Ki for each of the two inhibitors.

Known that: $\alpha = 1 + ([I])/K_I$ ⟶ $\alpha - 1 = [I]/K_I$ ⟶ $Ki = [I]/(\alpha - 1)$

a) Glyceraldehyde-3-P is an uncompetitive inhibitor ($[I] = 4*10^{-5}$ mM)

$K_{m1} = K_m / \alpha$ ⟶ $\alpha = Km/Km_1 = 17.24 * 10^{-2} / 10.52.16 * 10^{-2} = 1.63$

$Ki = [I]/(\alpha - 1)$ ⟶ **Ki** $= 4.10^{-5} / (1.63-1) = 6.34 * 10^{-5}$ **mM**

b) Ribose-5-P is a noncompetitive inhibitor ($[I] = 4*10^{-3}$ mM)

$Vmax_2 = Vmax / \alpha$ ⟶ $\alpha = Vmax/Vmax_2 = 9.52 * 10^{-2} / 5.20 * 10^{-2} = 1.83$

$Ki = [I]/(\alpha - 1)$ ⟶ **Ki** $= 4.10^{-3} / (1.83-1) = 4.81 * 10^{-3}$ **mM**

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