

Introduction into Biochemistry

Summer 2023

Course information



Recommended textbooks

 Marks' Basic Medical Biochemistry: A Clinical Approach 5th Edition, by Michael Lieberman (Author), Alisa Peet MD (Author), 2018

Instructors

- Prof. Mamoun Ahram
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Outline



- Biochemistry:
 - Introduction
 - Acids and bases, pH, buffer
 - Macromolecules (carbohydrates, lipids, amino acids, proteins)
 - Protein structure-function relationship (part I: fibrous proteins: collagen, elastin, and keratins; part II: globular proteins (myoglobin, hemoglobin, and immunoglobulins))

Midterm

- Enzymes (introduction, kinetics, mechanisms of regulation, cofactors)
- Protein analysis
- Molecular biology (online/recorded):
 - Restriction endonucleases recombinant DNA technology; polymerase chain reaction (PCR);
 DNA sequencing, protein-protein interactions; CRISPR-CAS9; DNA microarrays; RNA-seq)

References



Main References:

- The lectures
- Marks' Basic Medical Biochemistry by M. Lieberman A. Marks and Alisa Peet, Lippincott, Williams and Wilkins, 2017. 5th edition.
- The Cell: A Molecular Approach, Geoffrey M. Cooper and Robert E. Hausmann, 8th edition, Sinauer Associates, 2019.

Extra:

- NCBI Bookshelf:
 - (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books)
- The Medical Biochemistry Page:
 - (https://themedicalbiochemistrypage.org/)

Office hours for Prof. Ahram

- Location: School of Medicine, building 1, first floor (Sunday and Wednesday 3:30-4:30)
- Note: If I am not in my office, try the lab on the first floor. The map to the lab is on the office's door. Simply ask for me.



Biochemistry & chemical composition of living organisms

Biochemistry = understanding life

- Know the chemical structures of biological molecules
- Understand the biological function of these molecules
- Understand the interaction and organization of different molecules within individual cells and whole biological systems
- Understand bioenergetics (the study of energy flow in cells)

Biochemistry in medicine:

- explains all disciplines
- diagnose and monitor diseases
- design drugs (new antibiotics, chemotherapy agents)
- understand the molecular bases of diseases

Biochemistry is for the elite

Molecular biology & biochemistry



Physiology and pharmacology

Pathology and microbiology

Anatomy and histology

Chemical elements in living creatures

- The human body is composed mainly of ~30 elements.
- Four primary elements: carbon, hydrogen, oxygen, and nitrogen (96.5% of an organism's weight)
- Then, calcium and phosphorus (that's 98.5%).
- Others exist in trace amounts but are essential, elements (mostly metals).

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Li	Be											B	C	N	.0.	F	Ne
Na	Mg											AI x MI	Si	P	* S #}	LI II	
ĸ	Ca	SC SC	II Ti Janu Tanka		Cr	Mn	Fe	Co	Ni	, Cu I	Zn	Ga	Ge	As	Se	Br	M Kr sa Krolat
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Trace elements believed to be essential for bacteria, plants or animals



Possibly essential trace elements for some species

TABLE 2.1	Element	s of the Human E	Body			
Name		Symbol	Percentage of Body Weight			
	Major Ele	nents (Total 98.5%)				
Oxygen		0	65.0			
Carbon		С	18.0			
Hydrogen		Н	10.0			
Nitrogen		N	3.0			
Calcium		Ca	1.5			
Phosphorus		Р	1.0			
	Lesser El	lements (Total 0.8%)				
Sulfur		S	0.25			
Potassium		К	0.20			
Sodium		Na	0.15			
Chlorine		Cl	0.15			
Magnesium		Mg	0.05			
Iron		Fe	0.006			
	Trace Ele	ements (Total 0.7%)				
Chromium	Cr	Molybdenum	Мо			
Cobalt	Co	Selenium	Se			
Copper	Cu	Silicon	Si			
Fluorine	F	Tin	Sn			
lodine	1	Vanadium	V			
Manganese	Mn	Zinc	Zn			

Important terms

- Electronegativity
- Covalent bonds
 - Polar vs. non-polar covalent bonds
 - Single vs. multiple
- Non-covalent interactions
 - electrostatic interactions
 - hydrogen bonds (donor and acceptor)
 - van der Waals interactions
 - Hydrophobic interactions
 - Hydrophobic versus hydrophilic molecules
 - Backbone of a molecule
- Nucleophile versus electrophile



Important properties of bonds

- Bond strength (amount of energy that must be supplied to break a bond)
- Bond length: the distance between two nuclei
- Bond orientation: bond angles determining the overall geometry of atoms
- The three-dimensional structures of molecules are specified by the bond angles and bond lengths for each covalent linkage.

Bond	HF	HCI	HBr	HI
Length	0.917 Å	1.275 Å	1.415 Å	1.609 Å
Strength	136 kcal/mol	103 kcal/mol	87 kcal/mol	71 kcal/mol
Strength	571 kJ/mol	432 kJ/mol	366 kJ/mol	298 kJ/mol



Polarity of covalent bonds

- Covalent bonds in which the electrons are shared unequally in this way are known as polar covalent bonds. The bonds are known as "dipoles".
 - Oxygen and nitrogen atoms are electronegative
 - Oxygen and hydrogen
 - Nitrogen and hydrogen
 - Not carbon and hydrogen

Water is an excellent example of polar molecules, but not CO₂.



What are the non-covalent interactions?

They are reversible and relatively weak.

Electrostatic interactions (charge-charge interactions):

- They are formed between two charged particles.
- These forces are quite strong in the absence of water.



contact distance

Distance

Energy

- charge around an atom changes with time.
 - The strength of the attraction is affected by distance.

Hydrophobic interactions

- Self-association of nonpolar compounds in an aqueous environment
- Minimize unfavorable interactions between nonpolar groups and water



Properties of noncovalent interactions

- Reversible
- Relatively weak
- Molecules interact and bind specifically.
- Noncovalent forces significantly contribute to the structure, stability, and functional competence of macromolecules in living cells.
- Can be either attractive or repulsive
- Involve interactions both within the biomolecule and between it and other molecules of the surrounding environment



Carbon

The road to diversity and stability

Properties of carbon (1)

- It can form four bonds, which can be single, double, or triple bonds.
- Each bond is very stable.
 - strength of bonds: triple > double > Single)
- They link C atoms together in chains and rings.
 - These serve as a backbones.

HO



Properties of carbon (2)

- Carbon bonds have angles giving molecules three-dimensional structures.
- In a carbon backbone, some carbon atoms rotate around a single covalent bond producing molecules of different shapes.
- The electronegativity of carbon is between other atoms.
 - It can form polar and non-polar molecules.
- Pure carbon is not water soluble, but when carbon forms covalent bonds with electronegative elements like O or N, the molecule becomes soluble.



Functional groups



(Groups attached to a carbon skeleton)

Class of Compound	General Structure ^a	Functional Group Structure	Functional Group Name	Example
Alkanc	RCH ₂ -CH ₃		Carbon–carbon and carbon-hydrogen single bonds	H ₃ C-CH ₃
Alkene	RCH=CH ₂)c=c(Carbon-carbon double bond	$H_2C = CH_2$
Alcohol	ROH	-OH	Hydroxyl group	CH ₃ OH
Thiol	RSH	—SH	Thiol or sulfhydryl group	CH₃SH
Ether	R-O-R	$-\mathbf{o}$.	Ether group	CH ₃ -O-CH ₃
Amine ^b]	RNH ₂ R ₂ NH R ₃ N	-N	Amino group	H ₃ C-NH ₂
Imine ^b	R=NH	C=N-H	Ιπίοο group	H ₃ C C=NH H ₃ C
Aldehyde	о R-С-Н	O U C-H	Carbonyl group	CH ₃ C
Ketone	$\mathbf{R} = \mathbf{C} - \mathbf{R}$		Carbonyl group	CH ₃ CCH ₃
Carboxylic acid ^b	R-COOH	⊸С−он	Carboxyl group	сн3с

0 о Ester R-C-OR -OR Ester group CH₃C-OCH₃ Η O Amide R-C-NH2 -CAmide group CH₃C+NH₂ н 0 0 Phosphoric acid^b HO-P-OH НО-Р-ОН Phosphoric acid group HO-P-OH ÓH ÓH ÔH 0 0 0 Phosphoric acid R-O-P-OH -O-P-OH Phosphoester group CH3OP-OH ester or phosphoryl group OH ÓН ÓН n 0 0 Phosphoric acid R - O - P - O - P - OH-O - P - O - P - OHPhosphoric anhydride $CH_3O - \ddot{P} - O - \ddot{P} - OH$ anhydride^b group ÒН ÓН ÓН ÓН ÓН ÓН 0 O 0 0 0 Carboxylic acid-phosphoric acid R-C-O-P-OH $-\ddot{C}-O-\ddot{P}-OH$ Acyl-phosphoryl CH₃C -O - P - OHmixed anhydride^b anhydride ÓΗ ÓH ÔH

"R refers to any carbon-containing group.

^b These molecules are acids or bases and are able to donate or accept protons under physiological conditions. They may be positively or negatively charged.



Water

Properties of water (1)

CO,

- Water is a polar molecule as a whole because of:
 - the different electronegativities between Hydrogen and oxygen
 - It is angular.
- Water is highly cohesive.
- Water molecules produce a network.^H
- Water is an excellent solvent because:
 - it is small.
 - it weakens electrostatic forces and hydrogen bonds among polar molecules.





Dipole-dipole interaction





Dipole-charge interaction









Properties of water (3)



- It is reactive because it is a nucleophile.
 - A nucleophile is an electron-rich molecule that is attracted to positivelycharged or electron-deficient species (electrophiles).



Properties of water (4)

Water molecules are ionized to become a positively-charged hydronium ion (or proton), and a hydroxide ion:

$H_2O + H_2O \leftarrow \longrightarrow H_3O^{\oplus} + OH^{\Theta}$ Note: $H_3O^+ = H^+$

Brønsted-Lowry acids and bases

Acid: a substance that produces H⁺ when dissolved in water
 H⁺ Reacts with water producing hydronium ion (H₃O⁺).

Base: a substance that produces OH⁻ when dissolved in water.

Types of acids and bases

- The Brønsted-Lowry acid: any substance (proton donor) able to give a hydrogen ion or a proton (H⁺) to another molecule.
 - Monoprotic acid: HCl, HNO₃, CH₃COOH
 - Diprotic acid: H₂SO₄
 - Triprotic acid: H₃PO₃
- Brønsted-Lowry base: any substance that accepts a proton (H⁺) from an acid.
 - NaOH, NH₃, KOH

Water = amphoteric



Substances that can act as an acid in one reaction and as a base in another are called amphoteric substances.

- Example: water
- With ammonia (NH₃), water acts as an acid because it donates a H⁺ to NH₃.
 NH₃ + H₂O ↔ NH₄⁺ + OH⁻
- With hydrochloric acid, water acts as a base.

 $HCI+H_2O \rightarrow H_3O^+ + CI^-$

Ampho = 'both' or 'dual'

Acid/base strength



- Acids differ in their ability to release protons.
 - Strong acids dissociate 100%.
- Bases differ in their ability to accept protons.
 - Strong bases have a strong affinity for protons.
- For multi-protic acids (H_2SO_4, H_3PO_4) , each proton is donated at different strengths.

		ACID	BASE		
100		HCI	Cl	ee.	1
ionized in	5	H ₂ SO ₄	HSO4	ligilt	ŝ
H ₂ O	ŝ	HNO ₃	NO3	Neg	ase
4. 6 .60. D		H+ (aq)	H ₂ O		lie
		HSO4	SO42		inc
		H ₃ PO ₄	H2PO4		£
1		HF	F		euč
		HC2H3O2	C2H3O2		str
	ak	H ₂ CO ₃	HCO3	eak	Se
Ses	Ň	H ₂ S	HS	Ň	Ba
eas		H2PO4	HPO42		
JC 10		NH4 ⁺	NH ₃		
		HCO ₂	CO35.		÷
at	2	HPO42	PO43-		
trei		H ₂ O	OH.		2
qs	lible	HS	S ²	Bu	100 percent
Aci	aglig	OH	02	Stro	protonated
1	ž	H ₂	H	0,) in H ₂ O

Rule

Conjugate acid-base pair



 $\begin{array}{c} CH_{3}COOH + H_{2}O \rightleftharpoons H_{3}O^{+} + CH_{3}COO^{-} \\ Weak acid & Weak base & Strong acid & Strong base \\ \hline \\ Conjugate acid-base pair \end{array}$

- The stronger the acid, the weaker the conjugate base.
- Strong vs. weak acids
 - Strong acids and bases are one-way reactions

 $HCI \rightarrow H^+ + CI^-$

 $NaOH \rightarrow Na^+ + OH^-$

• Weak acids and bases do not ionize completely $HC_2H_3O_2 \leftrightarrow H^+ + C_2H_3O_2^ NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$

Equilibrium constant



Acid/base solutions are at constant equilibrium.

• We can write equilibrium constant (K_{eq}) for such reactions HA <--> H⁺+ A⁻

$$K_a = \frac{[\mathrm{H}_3\mathrm{O}^+] \cdot [\mathrm{A}^-]}{[\mathrm{H}\mathrm{A}]}$$

The value of the K_a indicates the direction of the reaction.

When K_a is greater than 1 the product side is favored (more acidic).

Note: $H_3O^+ = H^+$

When K_a is less than 1 the reactants are favored (less acidic).

What is pKa?



pK_a = -log K_a

The lower the pKa is, the more acidic it is

TABLE 2.4 Dissociation constants and pK_a values of weak acids in aqueous solutions at 25°C

Acid	<i>K</i> _a (M)	p <i>K</i> _a
HCOOH (Formic acid)	1.77×10^{-4}	3.8
CH ₃ COOH (Acetic acid)	1.76×10^{-5}	4.8
CH ₃ CHOHCOOH (Lactic acid)	1.37×10^{-4}	3.9
H ₃ PO ₄ (Phosphoric acid)	7.52×10^{-3}	2.2
$H_2PO_4^{\bigcirc}$ (Dihydrogen phosphate ion)	6.23×10^{-8}	7.2
$HPO_4^{\textcircled{O}}$ (Monohydrogen phosphate ion)	2.20×10^{-13}	12.7
H ₂ CO ₃ (Carbonic acid)	4.30×10^{-7}	6.4
HCO ₃ [⊖] (Bicarbonate ion)	5.61×10^{-11}	10.2
NH_4^{\oplus} (Ammonium ion)	5.62×10^{-10}	9.2
CH ₃ NH ₃ ⊕ (Methylammonium ion)	2.70×10^{-11}	10.7

TABLE 9.4 KA AND PKA VALUES FOR SELECTED ACIDS



Name	Formula	Ka	pKa
Hydrochloric acid	HCl	$1.0 imes 10^7$	-7.00
Phosphoric acid	H_3PO_4	$7.5 imes 10^{-3}$	2.12
Hydrofluoric acid	HF	$6.6 imes 10^{-4}$	3.18
Lactic acid	CH ₃ CH(OH)CO ₂ H	$1.4 imes 10^{-4}$	3.85
Acetic acid	CH ₃ CO ₂ H	$1.8 imes 10^{-5}$	4.74
Carbonic acid	H ₂ CO ₃	4.4×10^{-7}	6.36
Dihydrogenphosphate ion	$H_2PO_4^-$	$6.2 imes 10^{-8}$	7.21
Ammonium ion	$\mathrm{NH_4}^+$	5.6×10^{-10}	9.25
Hydrocyanic acid	HCN	$4.9 imes 10^{-10}$	9.31
Hydrogencarbonate ion	HCO3-	5.6×10^{-11}	10.25
Methylammonium ion	CH ₃ NH ₃ ⁺	$2.4 imes 10^{-11}$	10.62
Hydrogenphosphate ion	HPO_4^{2-}	4.2×10^{-13}	12.38

Expression



- Solutions can be expressed in terms of its concentration or molarity.
- Acids and bases can also be expressed in terms of their normality (N) or equivalence (Eq).



Moles of a solution are the amount in grams in relation to its molecular weight (MW or a.m.u.).

moles = grams / MW

A molar solution is where the number of grams equal to its molecular weight (moles) in 1 liter of solution.

M = moles / volume (L)

 Since (mol = grams / MW), you can calculate the grams of a chemical you need to dissolve in a known volume (L) of water to obtain a certain concentration (M) using the following formula:

grams = M x volume (L) x MW





How many grams do you need to make 5M NaCl solution in 100 ml (MW 58.4)?

grams = 58.4 x 5 M x 0.1 liter = 29.29 g

Equivalents



- When it comes to acids, bases and ions, it is useful to think of them as equivalents.
- An equivalent is the amount of moles of hydrogen ions that an acid can donate .
 - or a base can accept.
- A 1 g-Eq of any ion is defined as the molar mass of the ion divided by the ionic charge.

Examples



For acids:

- 1 mole HCl = 1 mole [H⁺] = 1 equivalent
- 1 mole $H_2SO_4 = 2$ moles $[H^+] = 2$ equivalents
 - 1 eq of $H_2SO_4 = \frac{1}{2}$ mol (because 1 mole gives two H^+ ions)
- For ions:
- One equivalent of Na⁺ = 23.1 g
- One equivalent of Cl⁻ = 35.5 g
- One equivalent of Mg²⁺ = (24.3)/2 = 12.15 g

Remember: One equivalent of any acid neutralizes one equivalent of any base.



Equivalents = n x M x volume (L)

One equivalent of any acid neutralizes one equivalent of a base.

Based on the equation above, since x eq of an acid is neutralized by the same x eq of a base, then (n x M x vol) of an acid is neutralized by (n x M x vol) of a base.

This is done in a process known as Titration. Titration is the slow addition of one solution of a known concentration to a known volume of another solution of unknown concentration until the reaction reaches neutralization.

Problem 1



10.92 Titration of a 12.0 mL solution of HCl requires 22.4 mL of 0.12 M NaOH. What is the molarity of the HCl solution?

Note that each one produces 1 mole of H⁺ or OH⁻, so 1M of HCl is equal to 1M of NaOH, so 1M HCl produces 1M of H⁺, which is neutralized by 1M NaOH, which produces 1M OH⁻

Eq of acid = Eq of base N x M1 x Vol1 = n x M2 x Vol2 1 x 0.12 x 22.4 = 1 x M2 x 12 M2 = (0.12 x 22.4) / 12 M2 = 0.224 M

Problem 2



10.93 What volume of 0.085 M HNO₃ is required to titrate 15.0 mL of 0.12 M Ba(OH)₂ solution?

- Note that 1 mole of HNO₃ produces 1 mole of H⁺, but 1 mole of Ba(OH)₂ produces 2 moles of OH⁻. In other words, the n is different.
- Also, remember that Equivalents = n x M x volume (L), where n is the number of charges or the number of H + (or OH-) the acid or base can produce or accept.

Titration means that we an acid to a base slowly. At one point during titration, the acid and the base neutralize or cancel each other. In other words, "to titrate" means "to neutralize". At the point of neutralization, the concentration of H+ is equal to the concentration of OH-. The best way to calculate how much acid is needed to neutralize a base (or the opposite) is to calculate the equivalents.

Eq of acid = Eq of base N x M1 x Vol1 = n x M2 x Vol2 1 x 0.085 x Vol = 2 x 0.12 x 15 Vol = (2 x 0.12 x 15) / 1 x 0.085 Vol = 42.35 mL

lonization of water



- Solution Water dissociates into hydronium (H_3O^+) and hydroxyl (OH^-) ions.
- Sor simplicity, we refer to H_3O^+ as H^+ and write the reaction equilibrium as:

$H_2O \Longrightarrow H^+ + OH^-$

The equilibrium constant Keq of the dissociation of water is:

$$C_{eq} = \frac{[H^{\oplus}][OH^{\Theta}]}{H_2O}$$

The equilibrium constant for water ionization under standard conditions is 1.8 x 10⁻¹⁶ M.





Since there are 55.6 moles of water in 1 liter, the product of the hydrogen and hydroxide ion concentrations results in a value of 1 x 10⁻¹⁴ for:

$K_{eq} (55.5 \text{ M}) = [H^{\oplus}] [OH^{\Theta}]$

This constant, Kw, is called the ion product for water

$$K_{w} = [H^{\oplus}][OH^{\ominus}] = 1.0 \times 10^{-14} M^{2}$$

$[H^+]$ and $[OH^-]$



- For pure water, there are equal concentrations of [H⁺] and [OH⁻], each with a value of 1 x 10⁻⁷ M.
- Since Kw is a fixed value, the concentrations of [H⁺] and [OH⁻] are inversely changing.
- If the concentration of H⁺ is high, then the concentration of OH- must be low, and vice versa. For example, if [H⁺] = 10⁻² M, then [OH⁻] = 10⁻¹² M

