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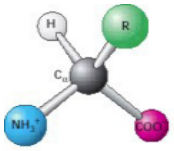
Biochemistry

Peptides and Proteins structure 1

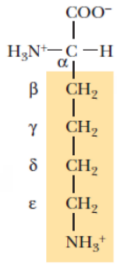
❖ Amino acids

- Proteins are polymers of α-amino acids
- An amino acid consists of:
  - A central carbon atom (**α-carbon**) linked to four groups
  - An amino group (-NH2),
  - A carboxylic acid group (-COOH),
  - A hydrogen atom, and
  - A specific R group (the **side chain**)



α-carbon is chiral, which can be rotated forming L & D enantiomers
- L: The **amino group** on the left
- D: The **amino group** on the right
- Only L amino acids naturally make up **proteins**
- D-isomers aren't used naturally to form proteins but they present in **bacterial cell walls**

- Only 20 amino acids are used to make up our proteins
- Amino acids can be classified according to their size, shape, charge, Hydrogen bonding capacity, Hydrophobic character, chemical reactivity of functional groups

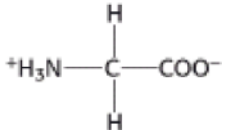


- The atoms of the side chain are designated by the Greek alphabets (**β, γ, δ and ε**) carbons
  - The last carbon is **ω-carbon**

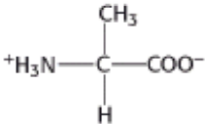
❖ Types of amino acids according their R groups

1- Non-polar amino acids

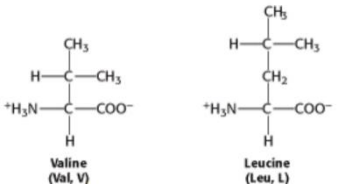
- **Glycine (Gly, G)**
  - Derivative of **acetic acid** or **aminoethane**
  - It is the simplest amino acid with only a H atom in the R-group
  - The only achiral amino acid



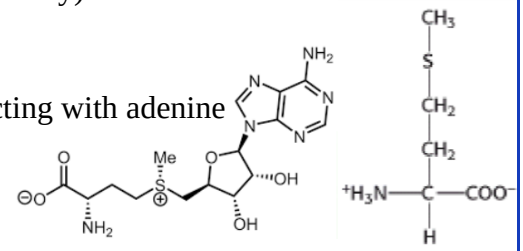
- **Alanine (Ala, A)**
  - It R-group is methyl (CH_3)



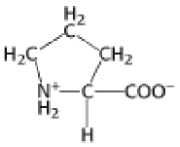
- **Valine (Val, V)**
- **Leucine (Leu, L)**
- **Isoleucine (Ile, I)**
  - Valine, Leucine and isoleucine are branched amino acids
  - They are essential amino acids (can't be synthesized inside our body)



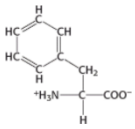
- **Methionine (Met, M)**
  - It is the precursor of SAM (S-Adenosyl-L-Methionine) by reacting with adenine
    - ✓ SAM is a methyl donor in the reactions
  - It contains a **thioether group**



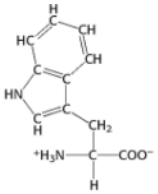
- **Proline (Pro, P)**
  - The only amino acid containing a secondary amine
    - ✓ It is considered an imino acid



- **Phenylalanine (Phe, F)**
  - It contains a Phenyl (benzene) ring



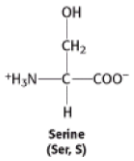
- **Tryptophan (Trp, W)**
  - It contains indole ring which is 2 fused rings with an amino group



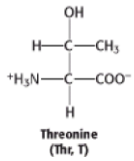
Aliphatic non-polar amino acids:
- Alanine, Valine, Leucine, Isoleucine, Methionine and Proline

Aromatic non-polar amino acids:
- Phenyl and tryptophan

2- Polar, Hydrophilic, Neutral Amino Acids

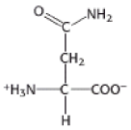


Serine (Ser, S)

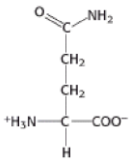


Threonine (Thr, T)

- **Serine (Ser, S)**
- **Threonine (Thr, T)**
  - Their R-groups contain OH which make them polar and reactive

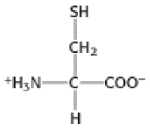


Asparagine (Asn, N)

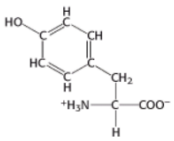


Glutamine (Gln, Q)

- **Asparagine (Asn, N)**
- **Glutamine (Gln, Q)**
  - They have Amide group ($CONH_2$)



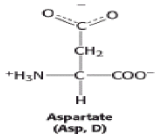
- **Cysteine (Cys, C)**
  - Contains Sulfhydryl or thiol (-SH) making it reactive



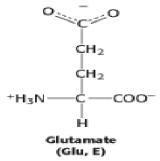
- **Tyrosine (Tyr, Y)**
  - Has a benzene ring attached to OH
  - Derived from Phenylalanine

All polar amino acids are aliphatic except **Histidine** and **tyrosine**

3- Polar, Hydrophilic, Negative Amino Acids (Acidic)



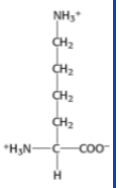
Aspartate (Asp, D)



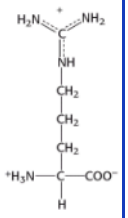
Glutamate (Glu, E)

- **Aspartic acid (Asp, D)**
- **Glutamic acid (Glu, E)**
  - They have Carboxyl group (COO^-)

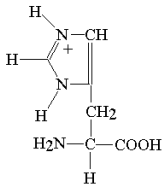
4- Polar, Hydrophilic, Positive Amino Acids (basic)



- **Lysine (Lys, K)**
  - Has 4 carbons in the side chain which terminate with a primary amino group (NH_3^+)



- **Arginine (Arg, R)**
  - Terminates with a guanidinium group



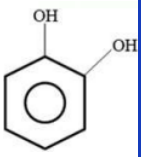
- **Histidine (His, H)**
  - Has an imidazole ring (an aromatic ring with 2 N)

- **Notes:**
  - Methionine encoded by the **starting codon**
  - The largest amino acid is **Tryptophan**

❖ Biological significance of amino acids

- α-nitrogen atom of amino acids is a primary source for many nitrogenous compounds (such as hormones, neurotransmitter and biologically active peptide)
- Amino acids can be used to form different types of molecules

♦ Tyrosine



- It is converted into
  - Catecholamine neurotransmitters such as dopamine, norepinephrine and epinephrine which are used in flight or fight conditions
  - Melanin which is a pigment of skin color and it includes eumelanin and pheomelanim
  - Thyroxine hormone which has 4 Iodide atoms, produced by thyroid gland, and it regulates metabolism

- **Cheese** contains high amounts of **tyramine**, which mimics (similar to) epinephrine
  - For many people a cheese omelet in the morning is a favorite way to start the day
  - Tyrosine is converted into Tyramine by tyrosine decarboxylase (TDC-1)

♦ Tryptophan

- Tryptophan serves as the precursor for the synthesis of Neurotransmitters (**Serotonin** & **Melatonin**)
  - Melatonin: produced by pineal gland it is the sleep hormone (regulated day-night cycle)
  - Serotonin: a Sedative-neurotransmitter and it is also called 5-hydroxytryptamine

♦ Histidine

- It forms a neurotransmitter by histidine decarboxylase forming **histamine**
- Histamine functions as an allergic mediator causing asthma (constriction of smooth muscles), regulate physiological function in the gut, acts as a neurotransmitter, contributes into inflammatory response

♦ Glutamate

- It is a precursor of:
  - GABA (γ-aminobutyric acid)
    - ✓ It is an **inhibitory** neurotransmitter that reduces neuronal excitability in the CNS, so it has relaxing, anti-anxiety and anti-convulsive effects
    - ✓ Synthesized in the brain and can't cross the BBB (blood brain barrier)
  - Gla (γ-Carboxyglutamate)
    - ✓ Synthesized by the carboxylation of glutamate in some clotting factors with the aid of vitamin K
    - ✓ This carboxylation process is important for clotting process because it introduces extra negative charges, performing more attraction with calcium ions and so more clotting
  - Mono-sodium glutamate (MSG)
    - ✓ It is a flavor enhancer used in Asian food
    - ✓ It can cause physiological reactions in some people (chills, headaches and dizziness)
    - ✓ It can cause Chinese restaurant syndrome

♦ Arginine

- L-arginine is precursor of nitric oxide (NO) gas which is synthesized in a reaction derived by **NADPH**
  - Nitric oxide has a role is Vasodilation, Inhibition of platelet adhesion, inhibition of leukocyte adhesion, anti-proliferative action, scavenging superoxide anion (anti-inflammatory)

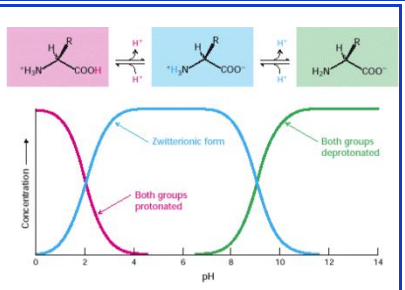
♦ Lysine & proline

- They are hydroxylated and then become parts of **collagen** (a structural protein)
- Post-translational modifications (hydroxylation) of lysine & proline gives collagen more strength

❖ Ionization of amino acids

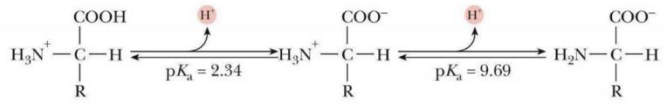
- Amino acids have:
  - Carboxyl group (pKa = about 2)
  - Amino group (pKa = about 9)
    - ✓ These groups can be protonated or deprotonated depending on the pH

- At the physiological pH (about 7.4):
  - Carboxyl: pH > pKa and so it is deprotonated (COO^-)
  - Amino: pH < pKa and so it is protonated (NH_3^+)



Isoelectric zwitterion

- ✓ So, At physiological pH amino acids (<u>without</u> ionizable R group) have a net charge = +1 + -1 = 0 (Neutral)
  - **Zwitterion (isoelectric):** A molecule with <u>opposite charges</u> & the net charge is **Zero**



- If pH = carboxyl pKa: 50% protonated (COOH) and 50% deprotonated (COO^-)
- If pH = amine pKa: 50% protonated (NH_3^+) & 50% deprotonated (NH_2)

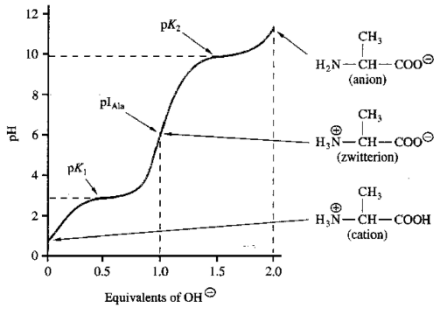
- **Note:**
- R groups can be either ionizable or non-ionizable
  1) **Non-ionizable:** These R groups can't ionize these amino acids (non-polar and some polar) have **<u>only 2 pKa</u>** values (of carboxyl and amine groups of the backbone)
  2) **Ionizable:** can ionize affecting the pH and the total charge of the amino acid, they have their own pKa values, such as the groups of these amino acids:
     - ✓ Aspartate, glutamate, Lysine, Arginine & Histidine, Tyrosine, Serine & Threonine, Cysteine

Amino Acid	Side Chain pK_a [3]	pI
Arginine	12.5	10.8
Aspartic Acid	4.0	3.0
Cysteine	8.0	5.0
Glutamic Acid	4.1	3.2
Histidine	6.0	7.5
Lysine	11.0	10

- **Isoelectric (Zwitterion) point (pI):** It is pH where the net charge of a molecule is zero
- It is calculated (for amino acids without ionizable R groups) by taking the average of the carboxyl pKa & Amine pKa

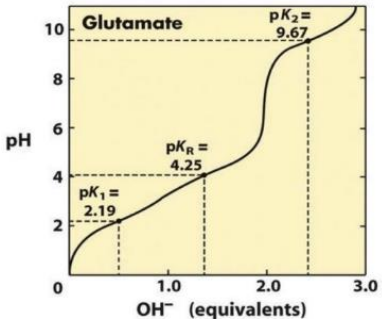
$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

- **<u>Example:</u>**
- Alanine is a non-polar amino acid, so it has only 2 pKa values that determine the pI:
  - pI = (2.34 + 9.69)/2 = 6
  - At pH = pKa_1 (pka of carboxyl)
    - ✓ 50% of alanine molecules are Neutral (Zwitterion) & 50% are positively charged (cation)
  - At pH = pKa_2 (pKa of Amine group)
    - ✓ 50% of alanine molecules are Neutral (Zwitterion) & 50% are Negatively charged (anion)



- **<u>Examples:</u>**

  1) Glutamate

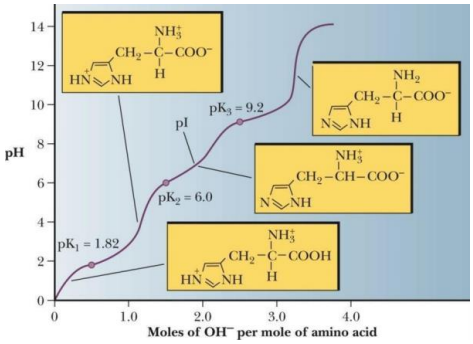


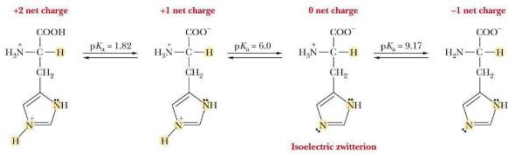
- Pka of R group (which contains carboxyl) = 4.2
  - At low pH
    - ✓ All groups are protonated (COOH, NH_3^+, COOH)
    - ✓ So it is positively charged (cation)
  - At pH between pKa of carboxyl & R group
    - ✓ pH > pKa_1 → deprotonated → COO^-
    - ✓ pH < pKa_R & pKa_2 → protonated → NH_3^+ & COOH
    - ✓ So net charge = 0 (Zwitterion)

    pI = (2.19 + 4.1)/2 = 3.22

2) Histidine

- $pKa_R = 6$
  - At low pH
    - ✓ All groups are protonated (COOH, NH_3^+, NH^+)
    - ✓ So it is positively charged (cation)
  - At pH between pKa of carboxyl & R group
    - ✓ pH > pKa_1 → deprotonated → COO^-
    - ✓ pH < pKa_R & pKa_2 → protonated → NH_3^+ & NH^+
    - ✓ So, the net charge +1 → positively charged (cation)
  - At pH between pKa of R group & amine group
    - ✓ pH > pKa_1 & pKa_R → deprotonated → COO^- & N
    - ✓ pH < pKa_2 → protonated → NH_3^+
    - ✓ So net charge = 0 (Zwitterion)
  - pI = (9.2 + 6)/2 = 7.6

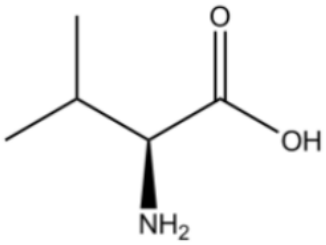




- **<u>Questions:</u>**
  - What is the pH of conjugate base/acid of glutamate at pH 4.5 (pKa = 4.25):
    - ✓ **1.78**
  - What is the total charge of lysine at pH = 7 (pka_R = 11):
    - ✓ **+1**

Past papers

1. What is the amino acid in the image below:



A. Glu
B. Asn
C. Pro
D. Val
E. Leu

2. What is the net charge of " Ile-His-Ser-Glu-Arg-Ala-His " peptide at pH=6?

A. +2
B. +1
C. 0
D. -1
E. -2

3. What is the charge of cys at physiological pH (pKa_R= 8)?

A. +2
B. +1
C. 0
D. -1
E. -2

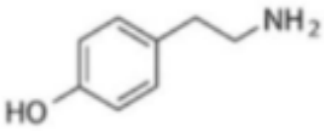
4. Which of the following is a secondary amine?

A. Gly
B. Gln
C. Glu
D. Cys
E. Pro

5. Which of the following is polar uncharged?

A. Arg
B. Leu
C. Phe
D. Tyr
E. Met

6. The following structure represents a molecule that:



A. Cannot be considered an amino acid
B. Can produce Epinephrine
C. Is produced by decarboxylation of Histidine
D. Can produce Serotonin
E. Is produced by hydroxylation of phenylalanine

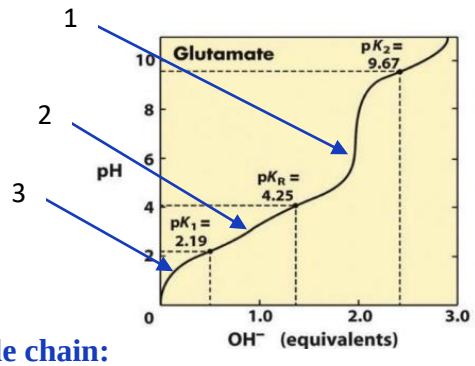
7. (Glu) is the 3 –letter code of:

A. glutamine
B. glutamic acid
C. cysteine
D. none of the above

8. One of the following is essential amino acid that the body cannot synthesize:

A. leucine
B. valine
C. serine
D. A+B
E. all of the above

9. The point that represents the zwitterion is



A. 1
B. 2
C. 3
D. 1 and 2

10. The amino acid that consists of a charged amino group in its side chain:

A. His
B. Lys
C. Asp
D. Gln
E. Pro

11. What's different about Pro from any other amino acids?

A. It's structure of a ring
B. Contains 3 carbons in its chain
C. It has a secondary amine
D. Possess 3 double bonds
E. All the above

12. Which group has the correct classification

A. {Ala, Val, Gli, Leu} nonpolar
B. {Ser, Asn, Arg} polar
C. {Gln, Trp, Met} uncharged
D. B+C

13. The amino acid arginine contains a guanidino R-group and has pKa values of 2.2, 9.0, and 12.5. A sample of arginine is titrated from pH=1.0 to pH=14.0 with NaOH. At pH=2.2

A. all of the amino acid molecules will be in the fully protonated form
B. half of the amino acid molecules will be in the fully protonated form
C. all of the amino acid molecules will be in the zwitterion form
D. half of the amino acid molecules will be in the zwitterion form

14. Which property is shared by both arginine and aspartate as each is titrated with NaOH from pH=1.0 to pH=14.0?

A. Both will require the same number of NaOH equivalents to complete the titration
B. Both will have the same number of equivalence points at the same pH values
C. Both will have the same net charge at pH=1.0
D. Both will have the same net charge at pH=14.0

15. The amino acids have a carboxyl group with a pK around _____, and an amino group with a pK near _____

A. 1.1 and 12.1
B. 6.5 and 8.0
C. 3.3 and 10.5
D. 9.0 and 2.5
E. 2.0 and 9.5

16. When the amino acid alanine (the R group is: CH3) is added to a solution with a pH of 7.3 , alanine becomes:

A. A cation
B. non-polar
C. a zwitterion
D. an isotope

17. The isoelectric point of an amino acid is defined as:

A. The pH where the molecule carrier no net electric charge
B. The pH where the carboxyl group is uncharged
C. The pH where the amino group is uncharged

D. The pH of maximum electrolytic mobility
E. $-log10(pKi + pKJ)$

18. Which of the following amino acids has a net charge of +2 at low pH?

A. Aspartic acid
B. alanine and glutamic acid
C. arginine and lysine
D. leucine

19. Which has a net charge of -2 at high pH?

A. Aspartic acid and glutamic acid
B. alanine
C. arginine and lysine
D. leucine

20. For a solution of tyrosine molecules at pH = 10.2 (pKa_R= 10.4)

A. all the α-carboxyl groups will be uncharged
B. all the α-amino groups will be uncharged
C. all the phenolic R-groups will be uncharged
D. all the ionizable groups will be uncharged

21. The amino acid tyrosine contains a phenolic R-group and has pKa values of 2.2, 9.0, and 10.2. A sample of tyrosine is titrated from pH = 1.0 to pH = 14.0 with NaOH. At which pH will all the amino acid molecules be in their fully protonated form?

A. 1.0
B. 2.2
C. 5.6
D. 9.0

22. At which pH will half the amino acid (without ionizable R groups) molecules have a +1 charge?

A. 10.2
B. 9.0
C. 2.2
D. 1.0