جامعة الملك سعود
King Saud University

College of Science

Department of Biochemistry

Protein Biochemistry (BCH 303)

Chapter 1

Amino acids

AMINO ACIDS

Learning goals

- To know the structure and naming of all 20 protein amino acids
- To know the chemical properties of each aminoacid.
- To understand the ionization behavior of each amino acids at different pH's.
- To know the general pKa's of amino acids: their carboxyls, aminos, the R-group weak acids.

AMINO ACIDS

They are organic molecules that are the building block of proteins.

There are 20 of L-α-amino acids commonly found in proteins (they have a carboxyl group and an amino group bonded to the same carbon atom [the α-carbon]).

The 20 α-amino acids of proteins are often referred as the natural or standard amino acids.

Proteins are polymers of amino acids joined by a specific type of covalent bond called **peptide or amide bond.**

AMINO ACIDS (CONT.)

The large diversity of the thousands of proteins arises from the intrinsic properties of the 20 natural amino acids.

These properties are:

- the capacity to polymerize,
- novel acid–base properties,
- varied structure and chemical functionality in the amino acid side chains, and
- chirality.

The term "residue" is used in protein to express the aminoacid that bind other by peptide bond through the loss of the elements of water (dehydration) when one amino acid is joined to another.

NATURAL AMINO ACIDS

There are too many amino acids. But the amino acids that form proteins are called natural amino acids. The number of natural amino acids is Twenty.

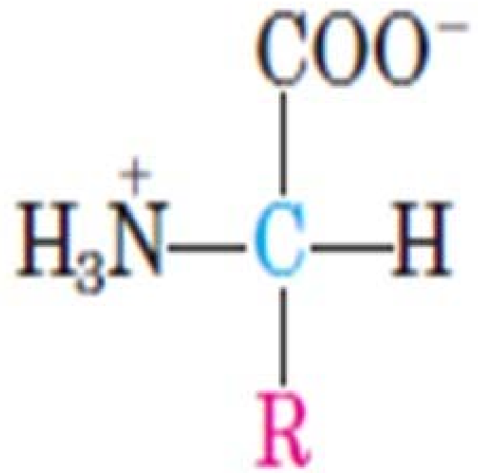
The first discovered amino acid was asparagine, in 1806.

The last of the was threonine in 1938.

In addition to these 20 amino acids there are many less common ones. Some are residues modified after a protein has been synthesized; others are amino acids present in living organisms but not as constituents of proteins.

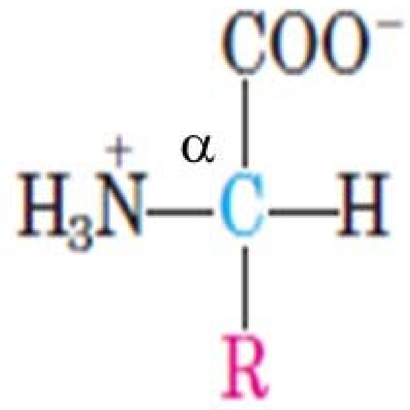
Natural Amino Acids Share Common Structural Features

- It contains tetrahedral alpha (α) carbon (C_α), which is covalently linked to four groups:
  - α - amino group ($-NH_3^+$)
  - α - carboxyl group ($-COO^-$)
  - hydrogen atom and
  - a variable side chain called **R** group.



- So, it contains both the acidic part (-COOH) and the basic part ($-NH_2$).

The side chain in the natural amino acids "R group"



It binds to α-carbon

Varies from amino acid to other. It has:

- different structure
- different size,
- different electric charge,

So, it influences the solubility of the amino acids in water.

It influences the protein structure through its effect on the 3D structure, protein binding and biological activity.

The R group or side chain **(red)** attached to the *α- carbon* ***(blue)*** *is different in each amino acid*

- All amino acids except proline (which contains imino group) have free unsubstituted α-amino group.

- The common amino acids of proteins have been assigned three-letter abbreviations and one letter symbols.

  example:

  Alanine: Ala or A

  Serine : Ser or S

  Glycine : Gly or G

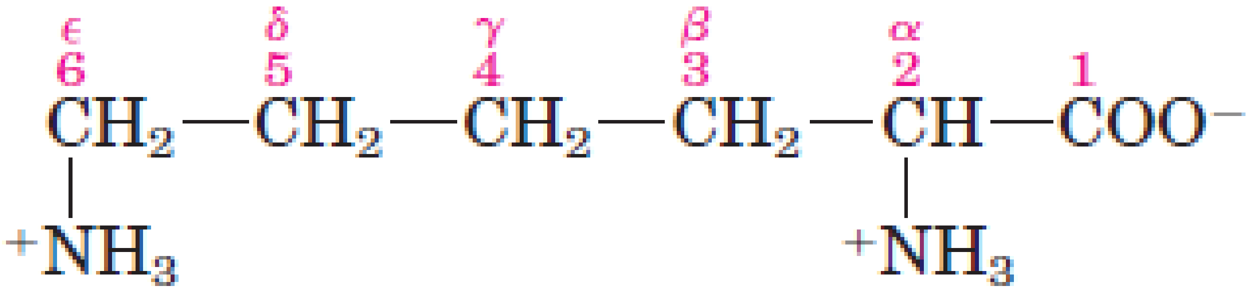
A complete set of abbreviations is listed in the following table

Name of Amino Acid	Three letter code	One letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

- **Numbering of carbon atoms in the aminoacids:**
- Two methods are used to identify the carbons in an amino acid.
  1- The additional carbons in an R group are commonly designated α, β , γ, δ and so forth, proceeding out from the α *carbon*.

  2- For most other organic molecules, carbon atoms are simply numbered from one end, giving highest priority (C-1) to the carbon with the substituent containing the atom of highest atomic number.

  - The carboxyl carbon of an amino acid would be C-1 and the α *carbon would be C-2*.



Lysine (K)

Classification of amino acids

Amino acids can be classified according to three criteria:

↓

A) on the basis of their R-group (depend on the polarity of their R-group).

B) Nutritional classification

C) Metabolic classification

A) Classification on the basis of their R-group:

[depend on the polarity of their R-groups [tendency to interact with water at biological pH (near pH 7.0)]

- The polarity of the R-groups varies widely from non-polar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble).

1) Amino acids with non-polar (hydrophobic) R-groups.
2) Amino acids with aromatic R-groups .
3) Amino acids with polar, uncharged R-group
4) Amino acids with positively charged (basic) R-group.
5) Amino acids with negatively charged (acidic) R-group.

A) Classification on the basis of their R-group:

1- Non-polar amino acids (with hydrophobic R-group)

- The R-group in this class of amino acids are non-polar and hydrophobic.

- Glycine has the simplest structure.
- Glycine
  Alanine
  Proline
  Valine — have non-polar, aliphatic R-group
  Leucine
  Isoleucine
  Methionine
- Methionine is **sulfur**-containing amino acid
- Proline has an aliphatic side chain with a distinctive **cyclic structure.**

The 20 common amino acids of proteins.

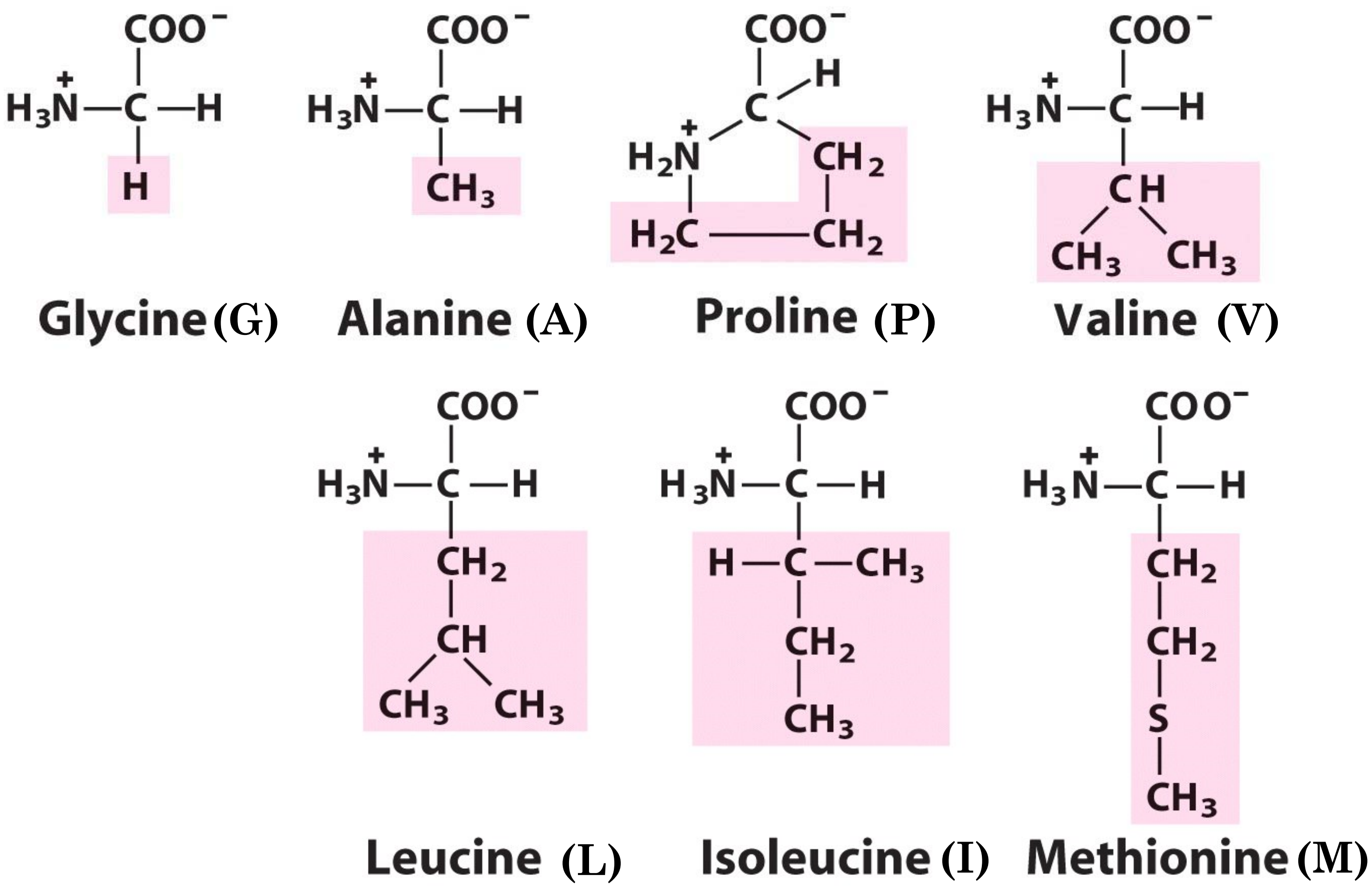
The structural formulas show the state of ionization that would predominate at pH 7.0.

The unshaded portions are those common to all the amino acids;

the portions shaded in red are the R groups.

The R group of proline form a cyclic structure and it N-form imino group instead of the amino group in the rest of amino acids

Nonpolar, aliphatic R groups



Which of the following is set apart from other amino acids for containing a secondary alpha amino group?

a. lysine b. valine c. glutamine d. proline

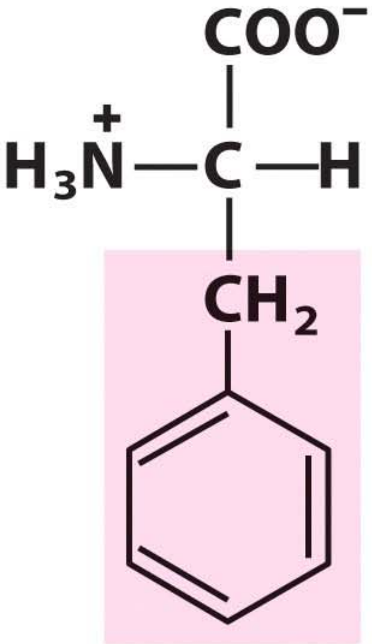
A) Classification on the basis of their R-group (Cont.):

2- Amino acids with aromatic R-groups :

- They are:
  - phenylalanine
  - tryptophan
  - tyrosine

- The hydroxyl group of tyrosine can form hydrogen bonds.

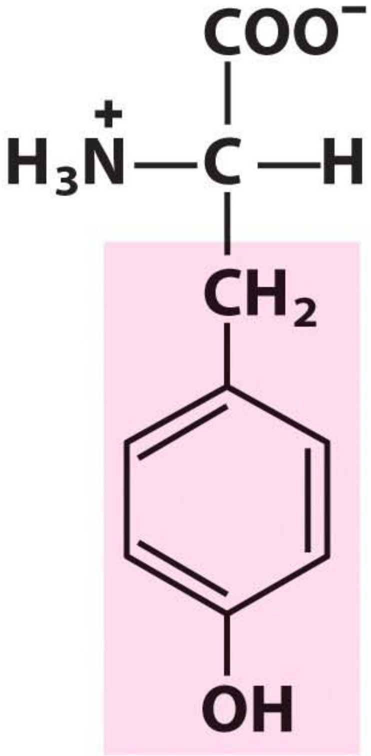
Aromatic R groups



Phenylalanine

(F)

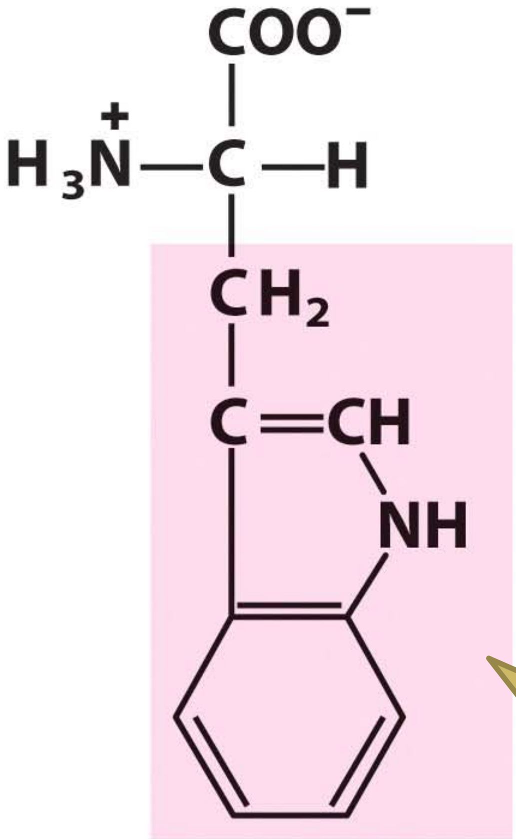
Nonpolar
Hydrophobic



Tyrosine

(Y)

Polar
Hydrophobic but more hydrophilic than Phe



Name this group?
Indole

Tryptophan

(W)

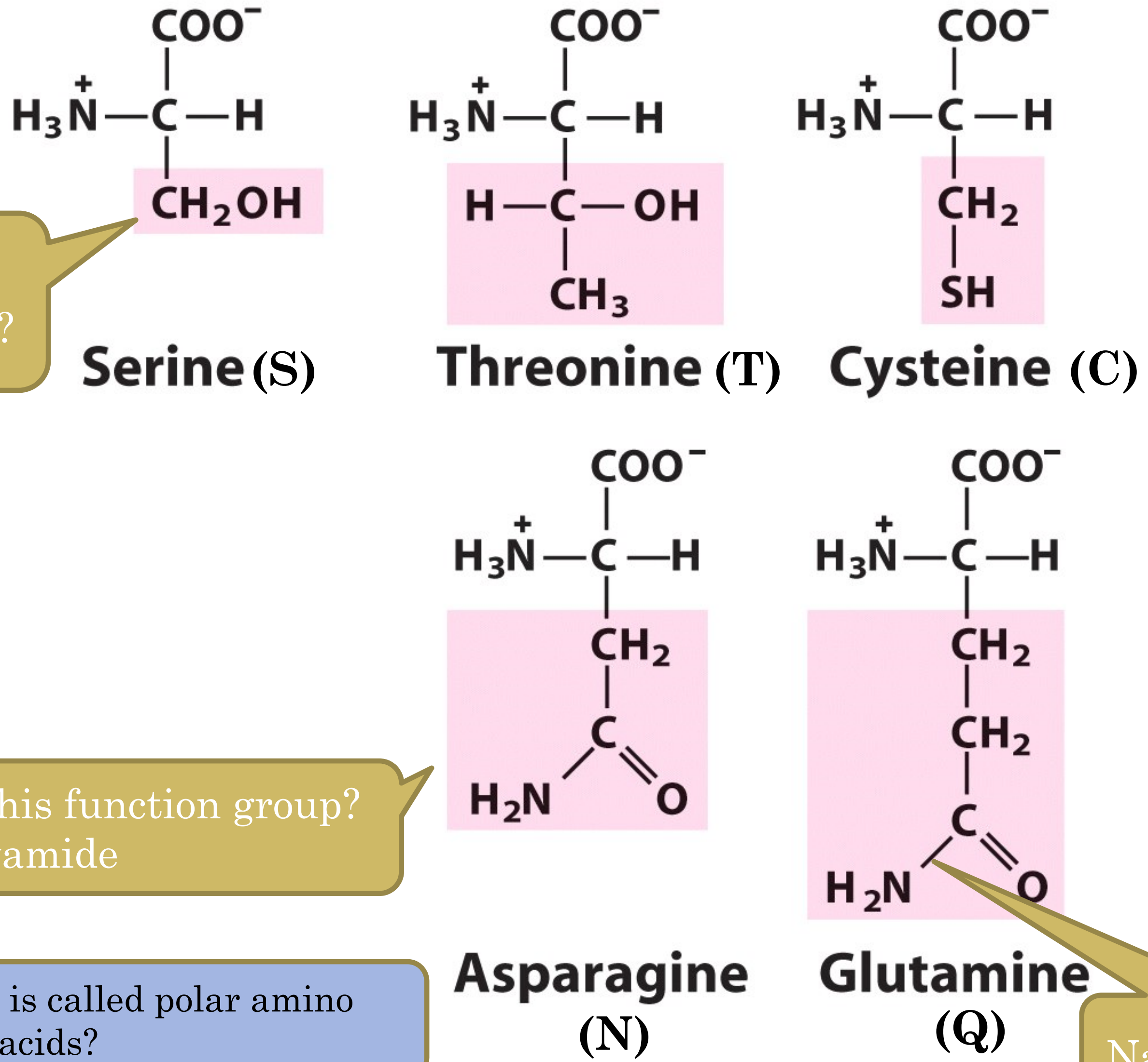
Nonpolar
Hydrophobic

A) Classification on the basis of their R-group (Cont.):

3) Amino acids with polar, uncharged R-group:

- The R-groups of these amino acids are more soluble in water, or more hydrophilic, than those of the non-polar amino acids, because they contain functional groups that form hydrogen bonds with water.
- This class of amino acids includes:
  - serine
  - threonine
  - cysteine
  - asparagine
  - glutamine

Polar, uncharged R groups



Name this function group?

Name this function group?
carboxyamide

Name this bond

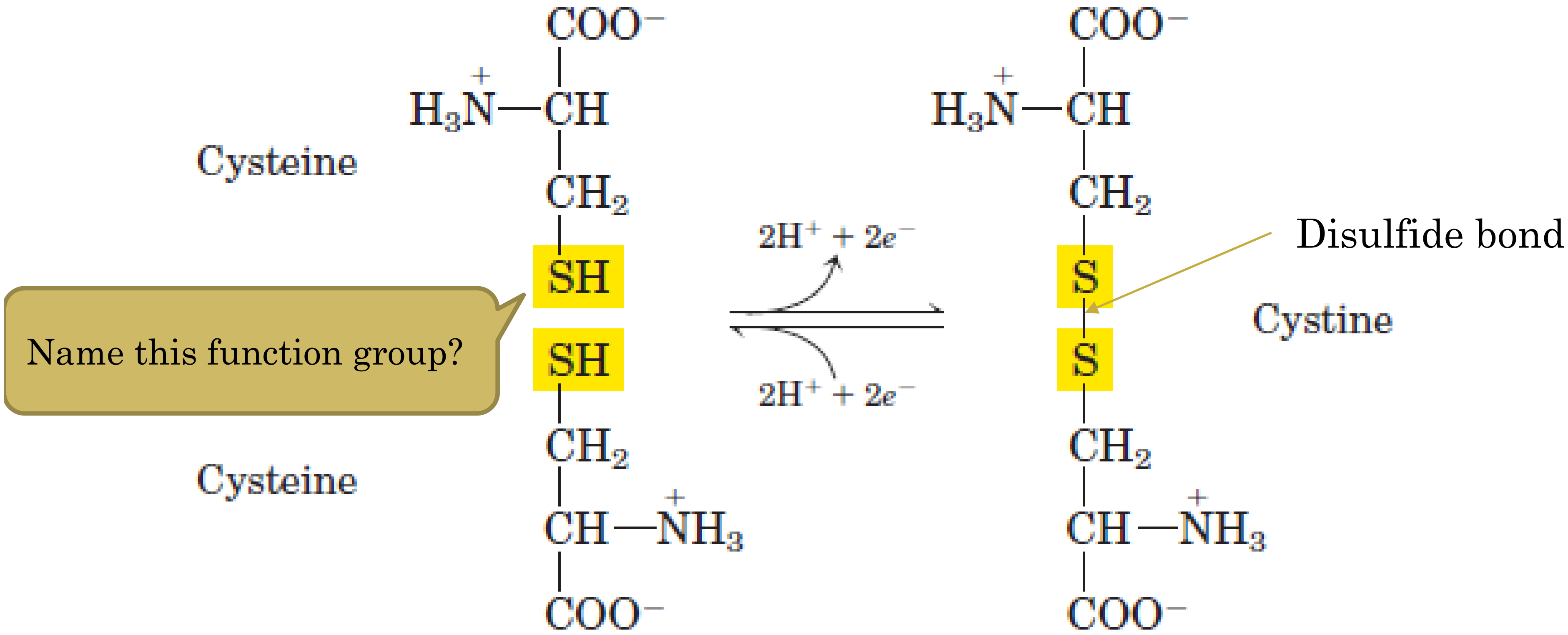
Why this group is called polar amino acids?

- The polarity of serine and threonine is contributed by their hydroxyl groups.
- The polarity of cysteine is contributed by its sulfhydryl group (thiol group).
- The polarity of aspargine and glutamine is contributed by their amide groups.

- Aspargine is amide of aspartic acid (aspartate).
- Glutamine is amide of glutamic acid (glutamate).

- Cysteine is readily oxidized to form covalently linked dimeric amino acid called **cystine,** in which two cysteine molecules or residues are joined by **a disulfide bond.**

Cysteine can disulfide bond



Two molecules of cysteines can bind to form cystine (Dicystein). Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine. Disulfide bonds between Cys residues stabilize the structures of many proteins.

A) Classification on the basis of their R-group (Cont.):

4) Amino acids with positively charged (basic) R-group:

- The most hydrophilic R-groups are those that are either positively or negatively charged.
- The amino acids in which the R-groups have significant positive charge at pH 7.0 are:

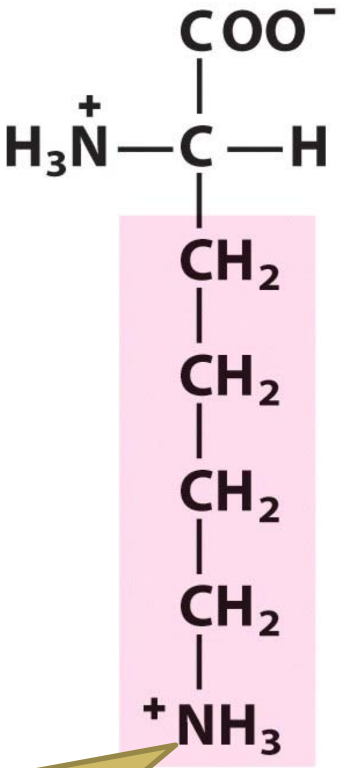
  lysine

  arginine

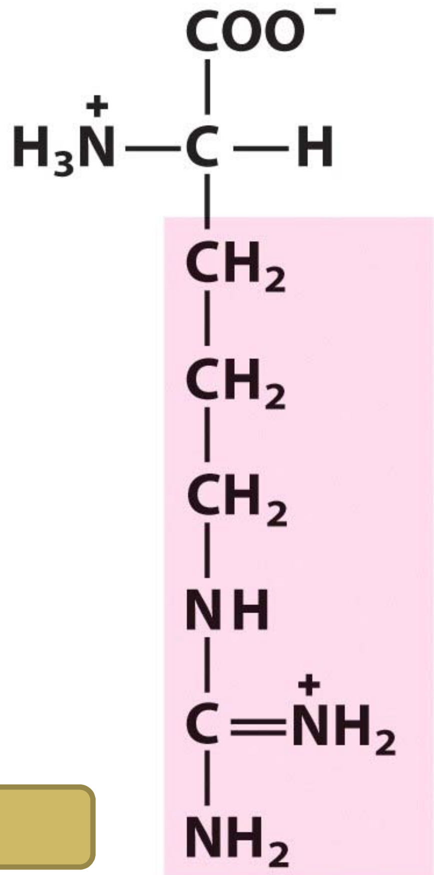
  histidine

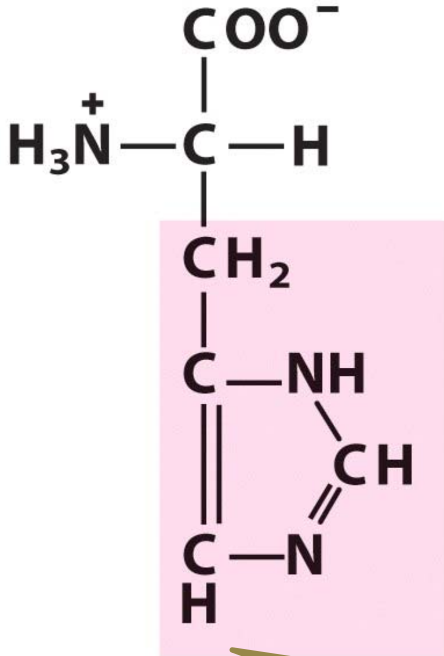
- Lysine has amino group on ε-position of its R-group.
- Arginine has positively charged guanidinium group in its R-group.
- Histidine has an aromatic imidazole group in its R-group.

Positively charged R groups



Name this function group? Amino





Name this group? imidazol

Lysine (K) **Arginine (R)** **Histidine (H)**

Name this function group? guanidino

If you want to give these three amino acids other name, what would you call them?????

A) Classification on the basis of their R-group (Cont.):

5) Amino acids with negatively charged (acidic) R-group:

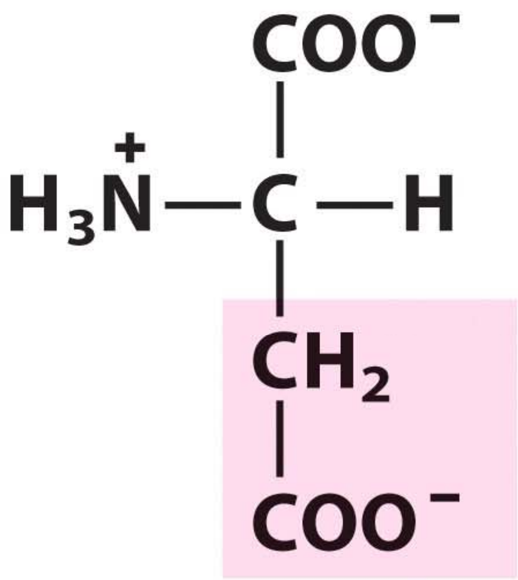
- The two amino acids having R-groups with a net negative charge at PH 7.0 are:

Aspartic acid (aspartate)

Glutamic acid (glutamate)

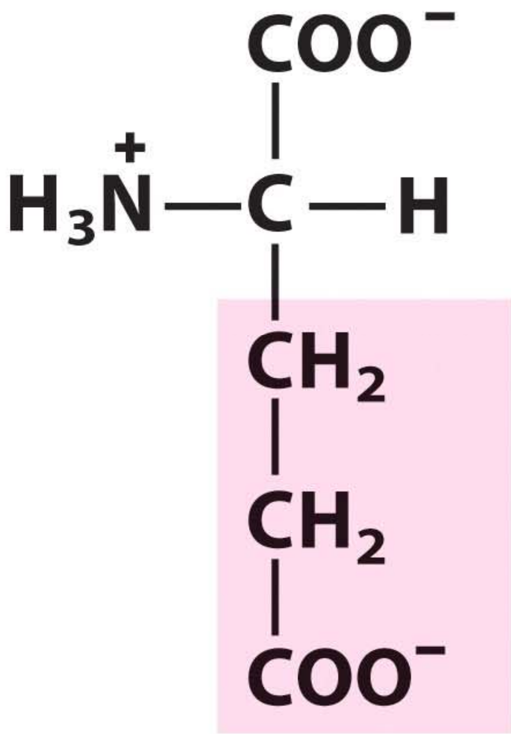
-These amino acids have carboxyl group in their side chains (R-groups).

Negatively charged R groups



Name this function group?

Aspartate (D)



Glutamate (E)

If you want to give these two amino acids other name (s), what would you call them?????

Trypotophan and **tyrosine** residues play special roles in "anchoring" membrane proteins within the cell membrane. In addition, tryptophan functions as a biochemical precursor for the following compounds:

- Serotonin (a neurotransmitter), synthesized by tryptophan hydroxylase
- Melatonin (a neurohormone) is in turn synthesized from serotonin, via N-acetyltransferase and 5-hydroxyindole-O-methyltransferase enzymes.
- Niacin, also known as vitamin B3, is synthesized from tryptophan via kynurenine and quinolinic acids.
- Auxins (a class of phytohormones) are synthesized from tryptophan

Uncommon amino acids

i) Rare amino acids of proteins:

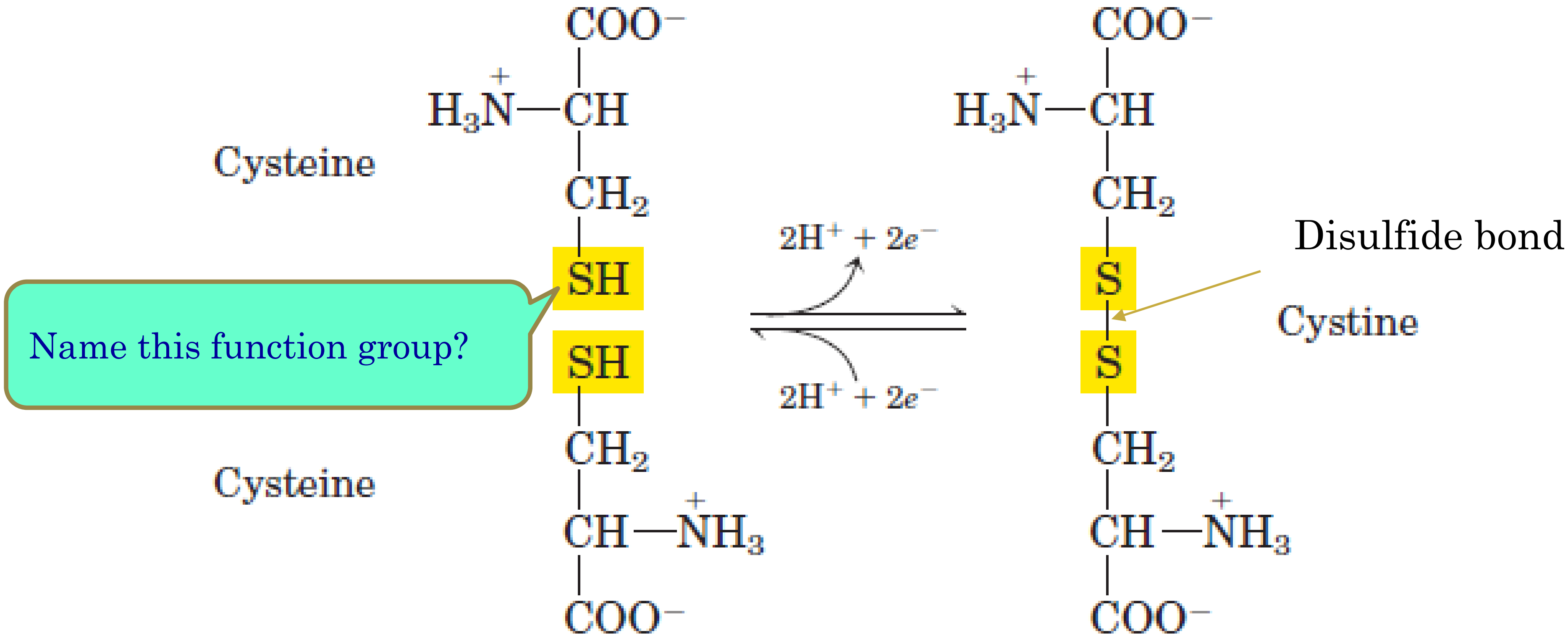
- uncommon amino acids also have important functions.
- In addition to the 20 common amino acids, proteins may contains uncommon amino acids which are derived from common (standard) amino acids.

Example:

4-hydroxyproline
5-hydroxylysine } **found in collagen.**

γ-carboxyglutamate } **found in the blood clotting protein prothrombin.**

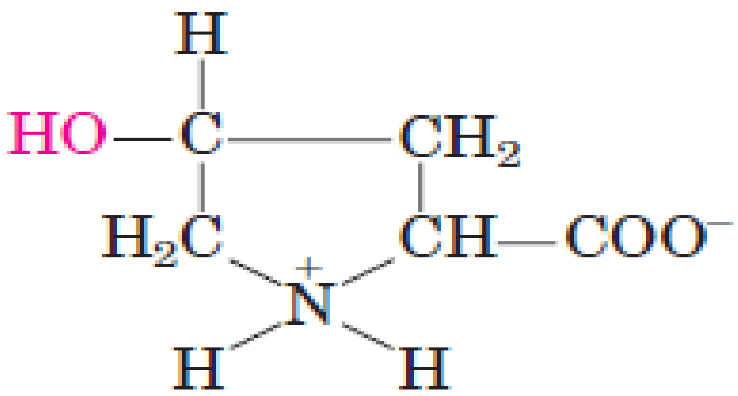
Cysteine can disulfide bond



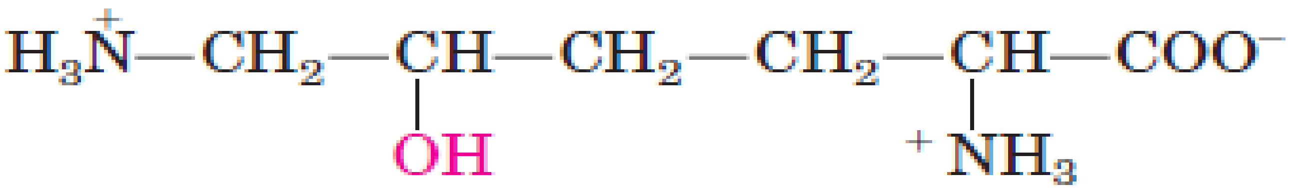
Two molecules of cysteines can bind to form cystine (Dicystein). Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine. Disulfide bonds between Cys residues stabilize the structures of many proteins.

Uncommon amino acids

Amino acids in Proteins Can be Reversibly Modified



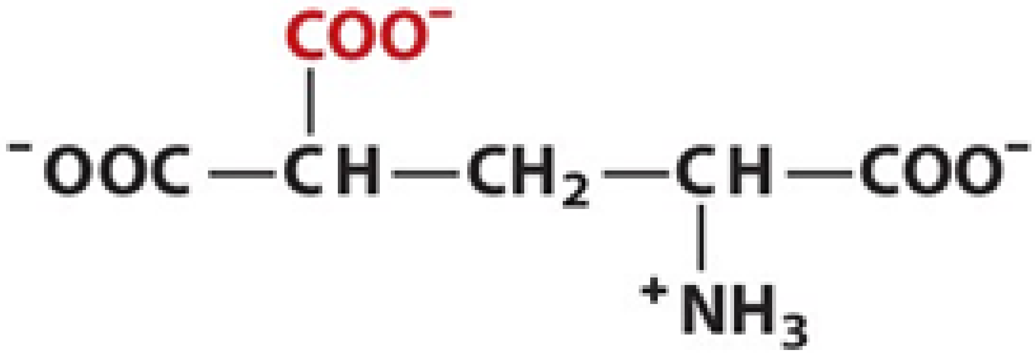
4-Hydroxyproline



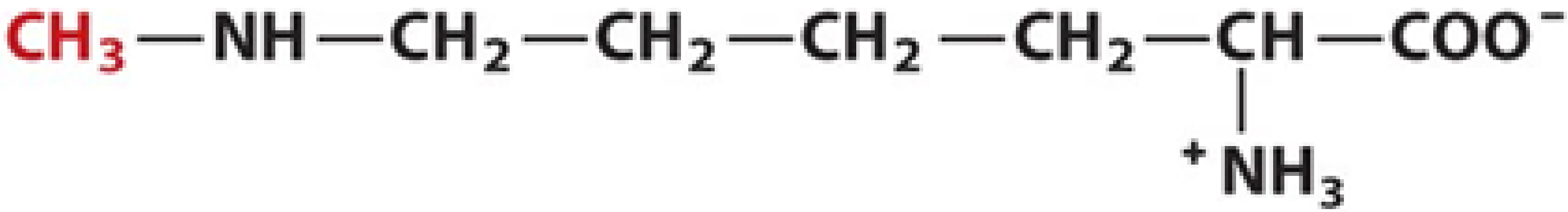
5-Hydroxylysine

Compare these amino acids with their precursors and explain what happened

Uncommon amino acids (Cont.)

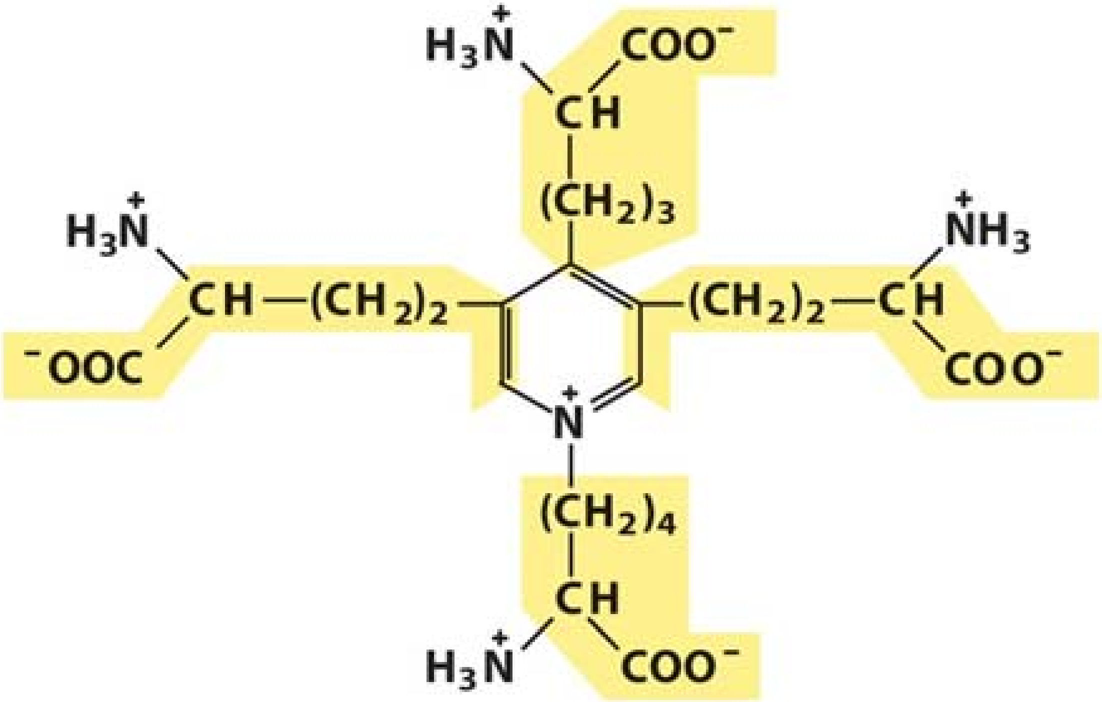


γ-Carboxyglutamate

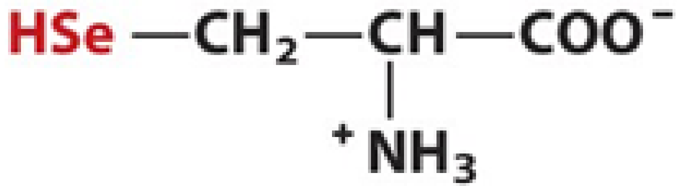


6-*N*-Methyllysine

Uncommon amino acids (Cont.)

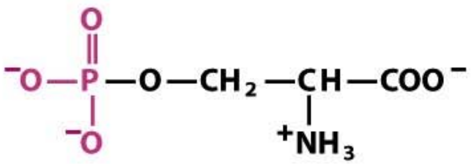


Desmosine

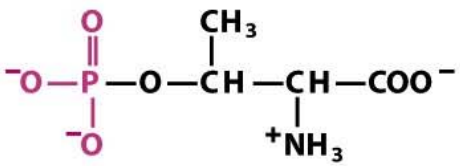


Selenocysteine

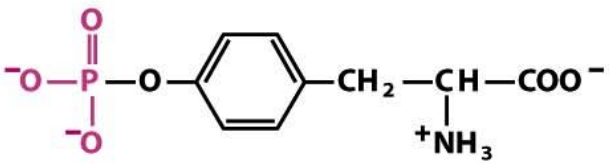
Write short essay about desmosine and selenocysteine



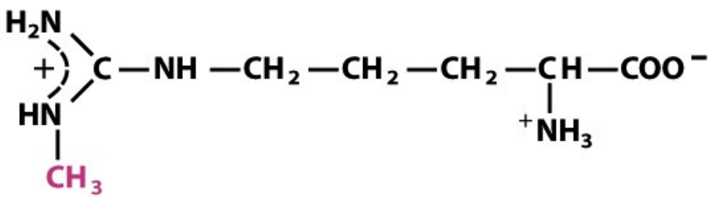
Phosphoserine



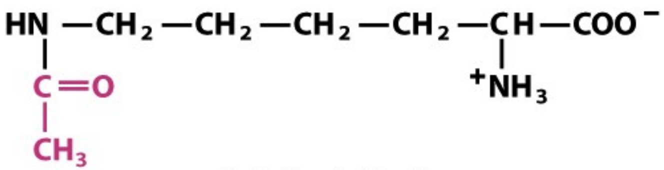
Phosphothreonine



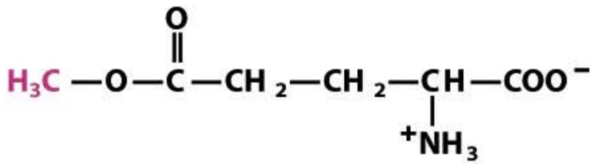
Phosphotyrosine



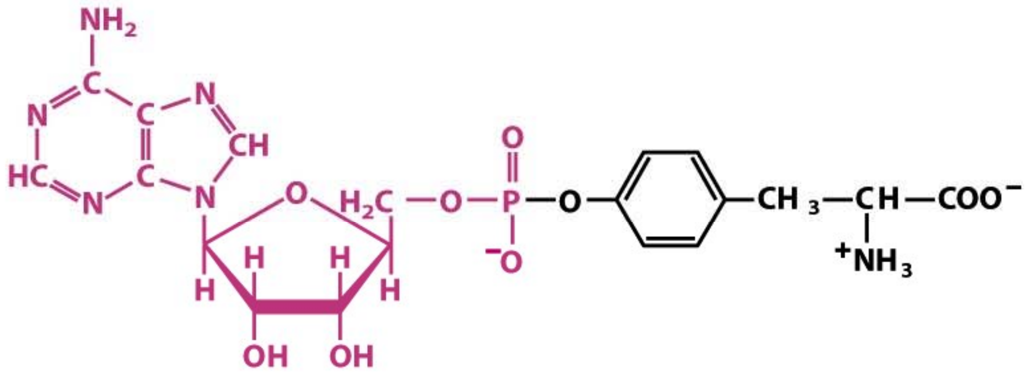
***σ-N*-Methylarginine**



6-*N*-Acetyllysine



Glutamate methyl ester



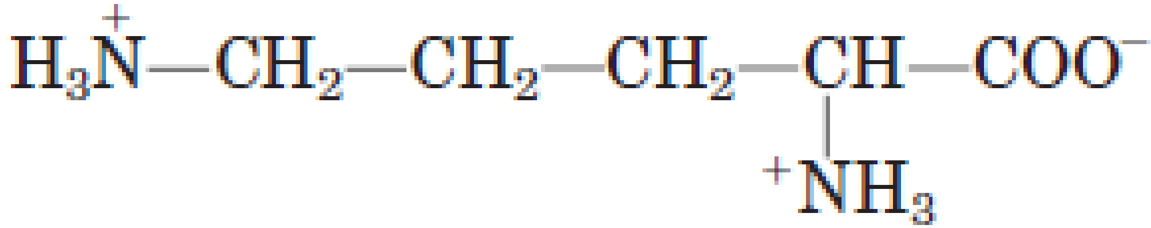
Adenylyltyrosine

Figure 3-8b
Lehninger Principles of Biochemistry, Fifth Edition

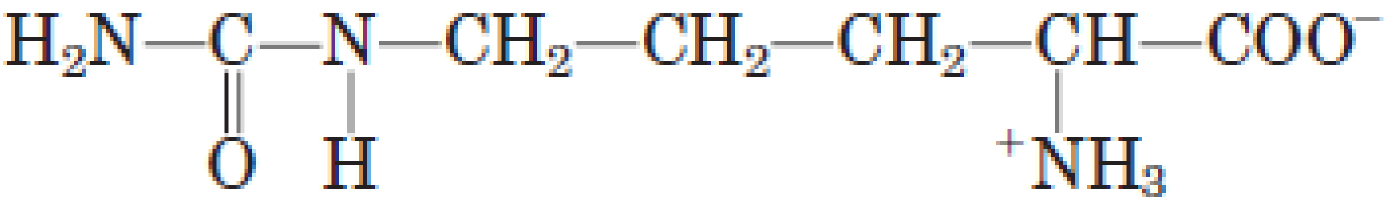
ii) Non-protein amino acids:

Ornithine and citrulline, are other two aminoacids but they are NOT found in proteins. They are intermediates in the biosynthesis of arginine in the urea cycle.

Do you remember arginine?
To which group it belongs?



Ornithine



Citrulline

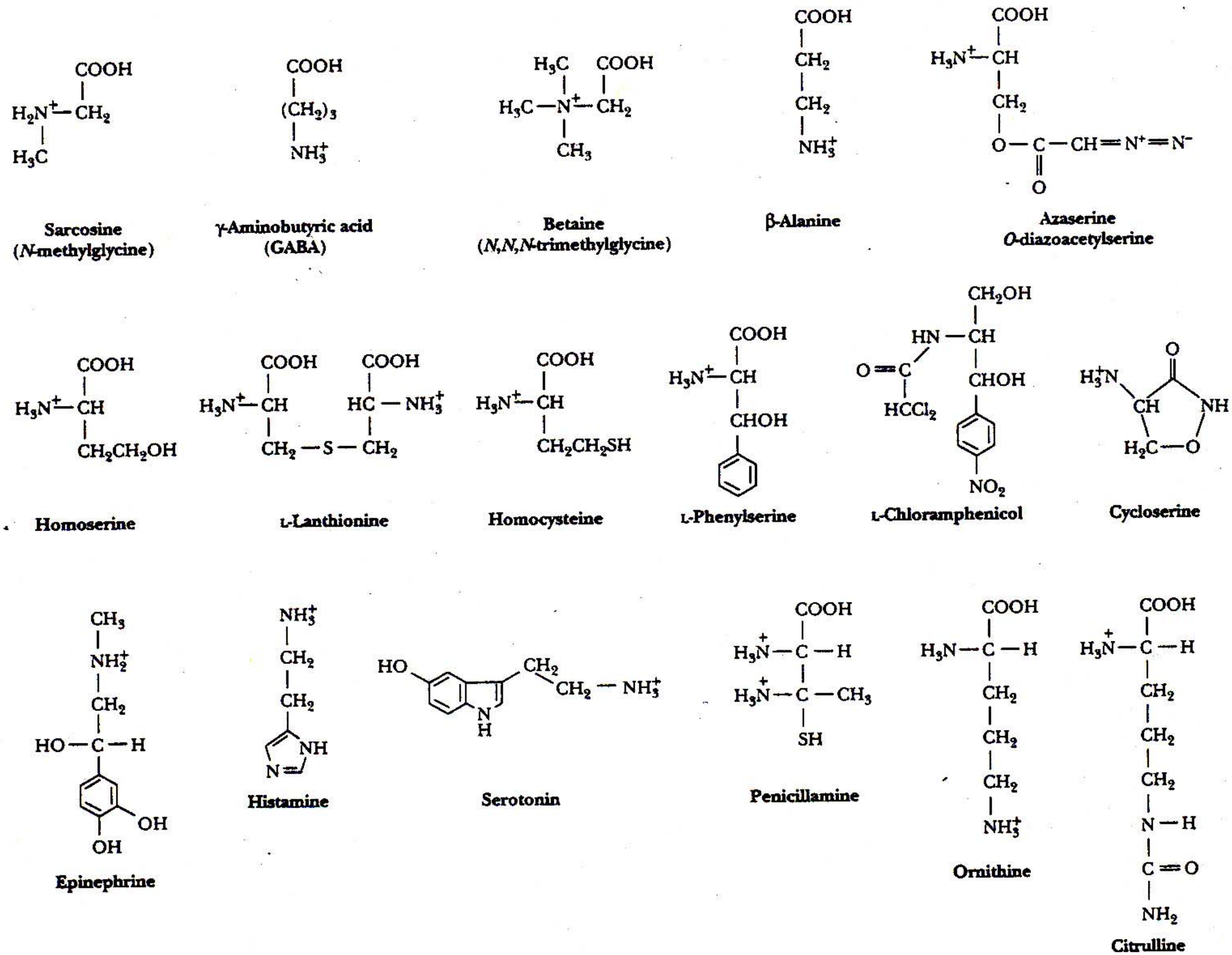
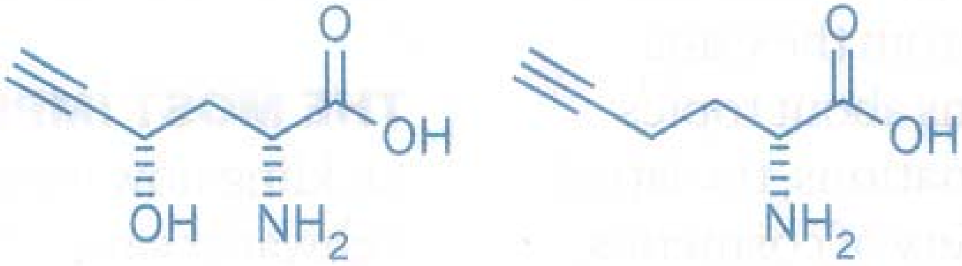
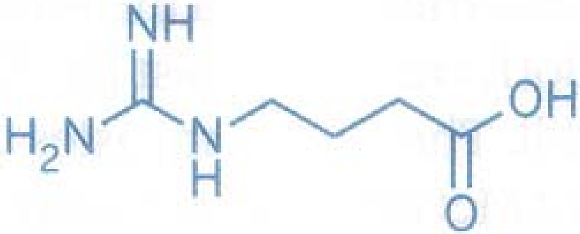


FIGURE 4.5 • The structures of some amino acids that are not normally found in proteins but that perform other important biological functions. Epinephrine, histamine, and serotonin, although not amino acids, are derived from and closely related to amino acids.

TOXIC AMINO ACIDS



New toxic amino acids



γ-Guanidinobutyric acid

A search for compounds producing Yunnan Sudden Unexplained Deaths found related to eating a mushroom.

Halford, B. C+E News Feb 13, 2012

Trogia venenata Zhu L

B) Nutritional classification:

i) Essential or indispensable amino acids*:

They can NOT be synthesized de novo (from scratch) in HUMAN body at a rate commensurate with its demand, and thus must be supplied in its diet.

Valine	Leucine	Isoleucine
Methionine	Phenylalanine	Tryptophan
Threonine	Lysine	Histidine
Arginine*		

ii) Non-essential (dispensable) amino acids:

They synthesized in human body.

Notice the R group of non-essential amino acids are simple and easy to be memorized

* Some books report a group of conditionally essential amino acids meaning their synthesis can be limited under special pathophysiological conditions, such as prematurity in the infant or individuals in severe catabolic distress (arginine, cysteine, glycine, glutamine, proline, and tyrosine)

C) Metabolic classification:

i) Glucogenic amino acids:

- which their metabolic derivatives can give glucose

  e.g., glycine, alanine, serine, proline, valine, arginine, glutamic acid, aparagine. Glutamine, aspartic acid, cysteine, histidine, methionine

ii) Ketogenic amino acids:

- That gives ketone bodies.

  e.g., leucine, lysine

iii) Both Glucogenic and ketogenic amino acids at the same time:

- give both glucose and ketone bodies.

  e.g., threonine, phenylalanine, tyrosine, tryptophan, isoleucine

Properties of amino acids

1) Optical activity of amino acids:

- All standard amino acids except glycine have an asymmetric carbon atom [α-carbon atom bound to four different substituent groups (i.e., a carboxyl group, amino group, R-group, and a hydrogen atom).

-The asymmetric α-carbon atom is thus a chiral center.

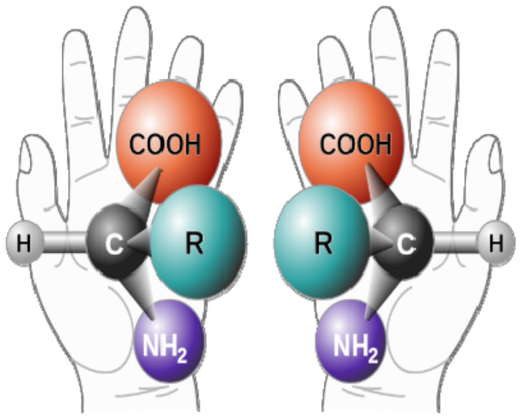
-Because amino acids that obtained from the hydrolysis of proteins have one or more asymmetric carbon atoms

⇒ so all amino acids except glycine are optically active.

⇒ **The α-carbon atom of all amino acids except glycine is asymmetric, and thus amino acids exist in two stereoisomeric forms: L-isomer, D-isomer.**

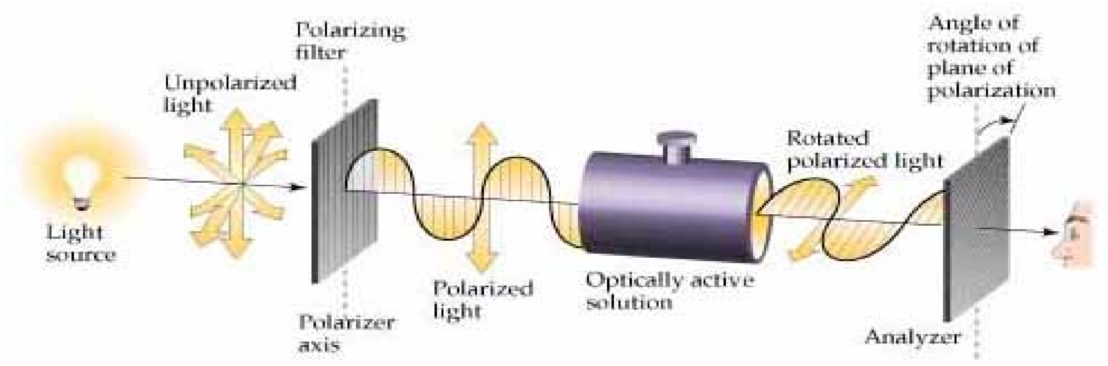
19 of the 20 natural amino acids are *optically active*

Compound with optical activity means it has chiral carbon and it can rotate the plane of polarized light as it travels through it to either right, *dextrorotatory* (clockwise) or to the left, *levorotatory* (anticlockwise)



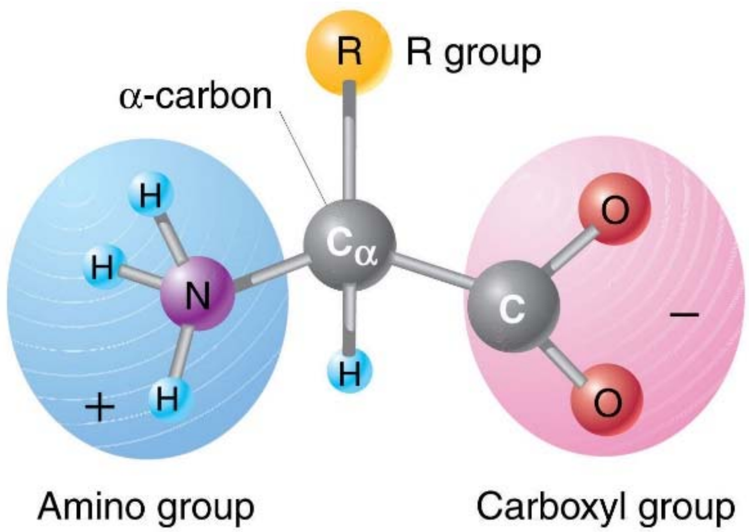
Do you know the meaning of polarized light and optical activity?

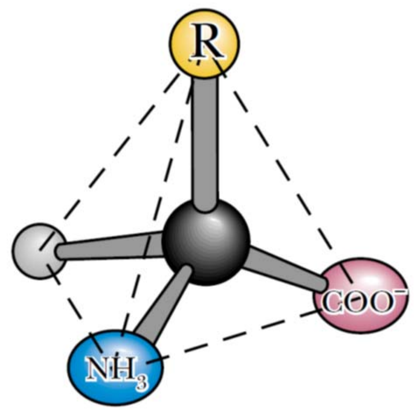
Which of the following amino acids is achiral?



All 20 of the natural amino acids are L α–amino acids.

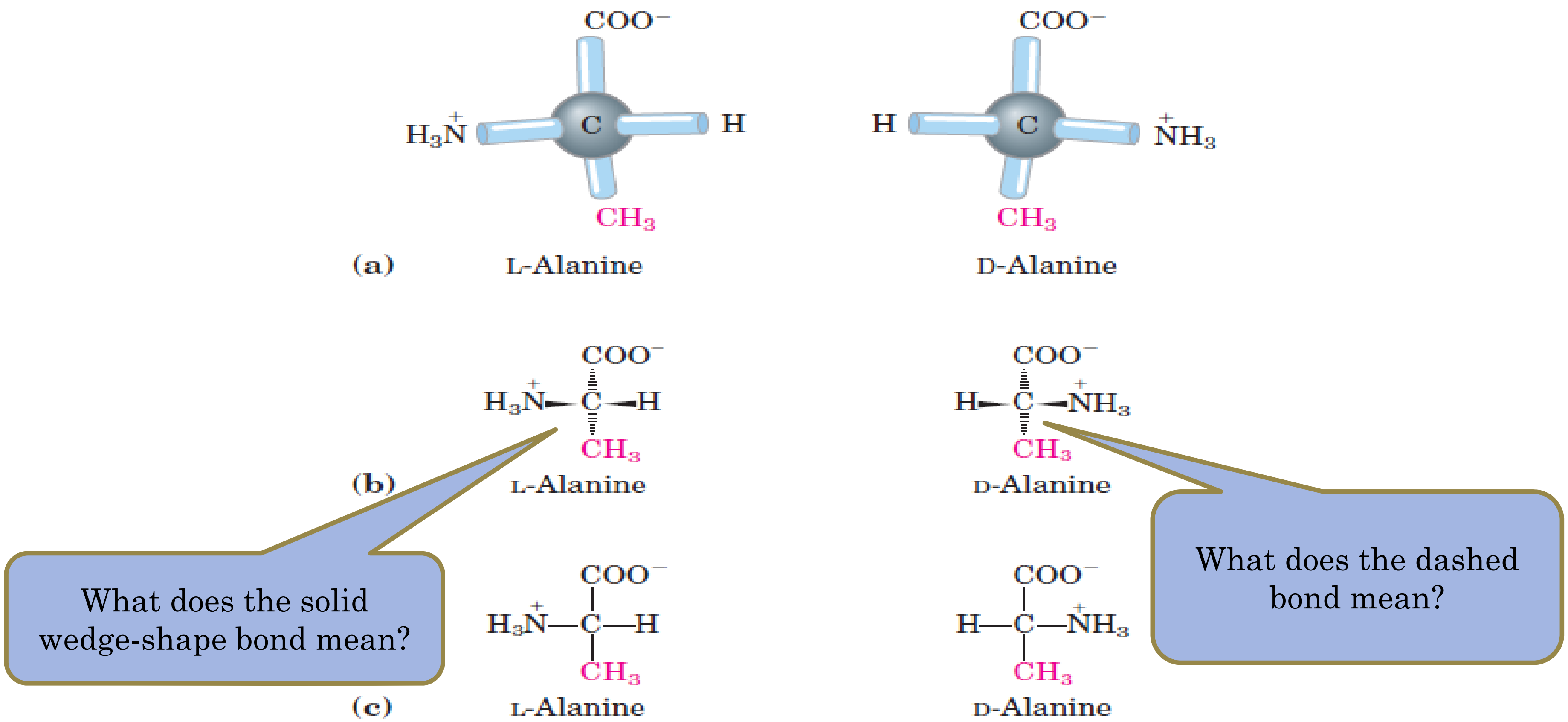
- Because of the tetrahedral arrangement of the bonding orbitals around the α-carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible nonsuperimposable mirror images stereoisomers called enantiomers (in Greek *opposite forms)*.





Amino acids are tetrahedral structures

Two stereoisomers (D and L) are found for each aminoacid:



(a) L- and D of alanine are nonsuper imposable mirror images of each other.
Two different conventions for showing the configurations in space.
(b) the solid wedge-shaped bonds project out of the plane of the paper, and the dashed bonds behind it.
(c) the horizontal bonds are assumed to project out of the plane of the paper, the vertical bonds behind.

ALL THE AMINO ACID RESIDUES IN PROTEINS ARE L-STEREOISOMERS

- Nearly all biological compounds with a chiral center occur naturally in only one stereoisomeric form, either D or L.
- The amino acid residues in protein molecules are **exclusively L-stereoisomers** and α–form.
- D-Amino acid residues have been found only in a few, generally small peptides, including some peptides of bacterial cell walls and certain peptide antibiotics.

Properties of amino acids (Cont.)

2) All amino acids contain α-carboxyl group and α-amino group (and functional groups in the side chain of some amino acids).

→ All these groups determine the physical and chemical properties of amino acids.

3) Amino acids are soluble in polar solvent (e.g., water).

Properties of amino acids (Cont.)

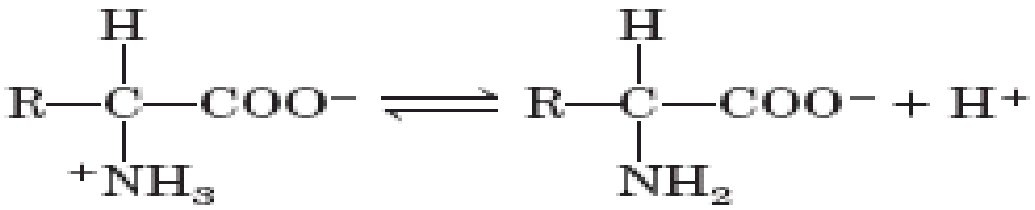
4) Amino acids are ionized in water solution and form dipolar ion or **zwitterion.**

FIGURE 3–9 Nonionic and zwitterionic forms of amino acids. The nonionic form does not occur in significant amounts in aqueous solutions. The zwitterion predominates at neutral pH.

Properties of amino acids (Cont.)

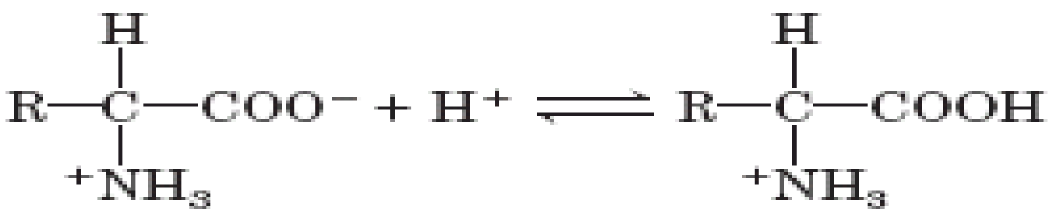
5) **Amino acids in aqueous solution are ionized and can act as acid or base.**

as either an acid (proton donor):



Zwitterion

or a base (proton acceptor):



Zwitterion

- Substances having two-way property are **amphoteric.**

In an electric field:

- The amino acid in acidic media carries positive charge
- So it moves towards → the negative cathode
- - The amino acid in basic media carries negative charge
- So it moves towards → the positive anode

Properties of amino acids (Cont.)

6) Isoelectric point or isoelectric pH (pI):

is the pH at which the net electric charge on amino acid is equal to zero.

→ an amino acid will not move in an electric field.

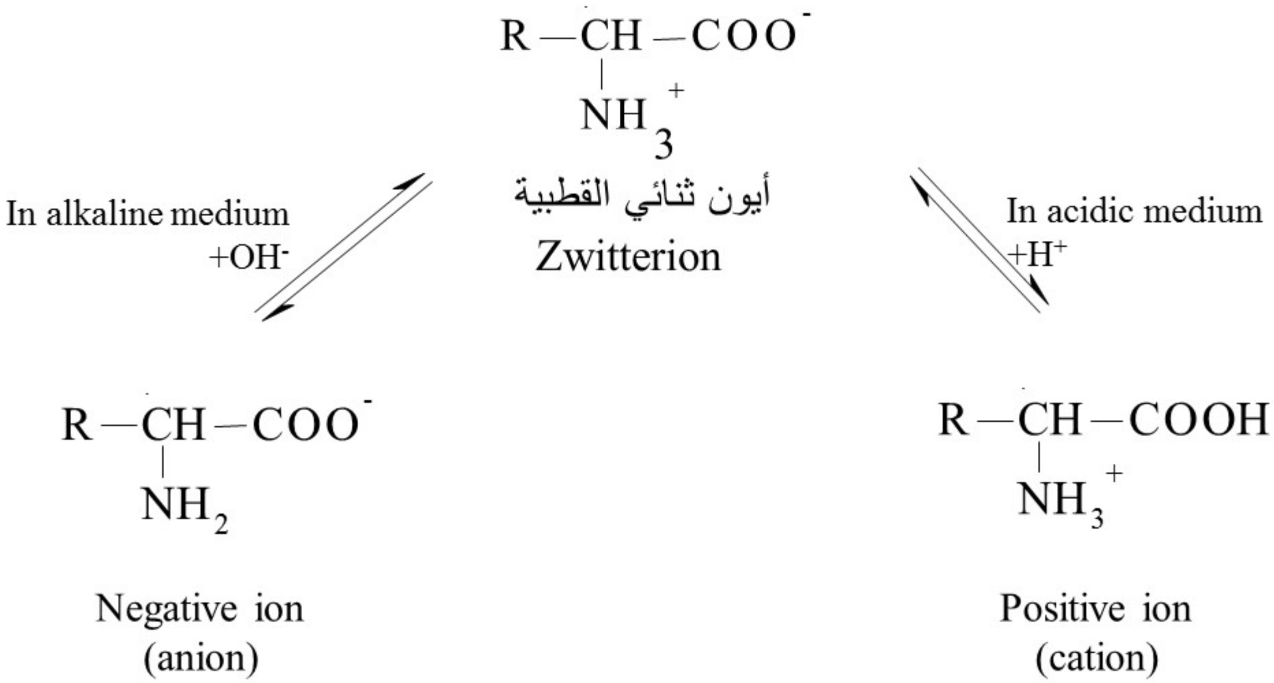
-Each amino acid has its specific pI.

e.g., glycine has pI = 5.97

AMINO ACIDS ARE AMPHOTERIC (IT CAN ACT AS ACID OR BASE)

- IN neutral solution (pH 7), the carboxyl group exists as $–COO^-$ and the amino group as $-NH_3^+$
- Because the resulting amino acid contains one positive and one negative charge, it is a neutral molecule called a **zwitterion.**

Each amino acid has characteristic isoelectric point (pI). The net charge is zero as the number of positive and negative charges are equal.



Remember

Amphoteric compound or Ampholyte is a molecule or ion that can react both as an acid as well as a base.

Amphipathic compound or Amphiphile is a term describing a chemical compound possessing both hydrophilic (*water-loving*, polar) and lipophilic (*fat-loving*) properties. ***In Greek*** *amphis means both and philia means love.*

Properties of amino acids (Cont.)

7) Titration curve of amino acids:

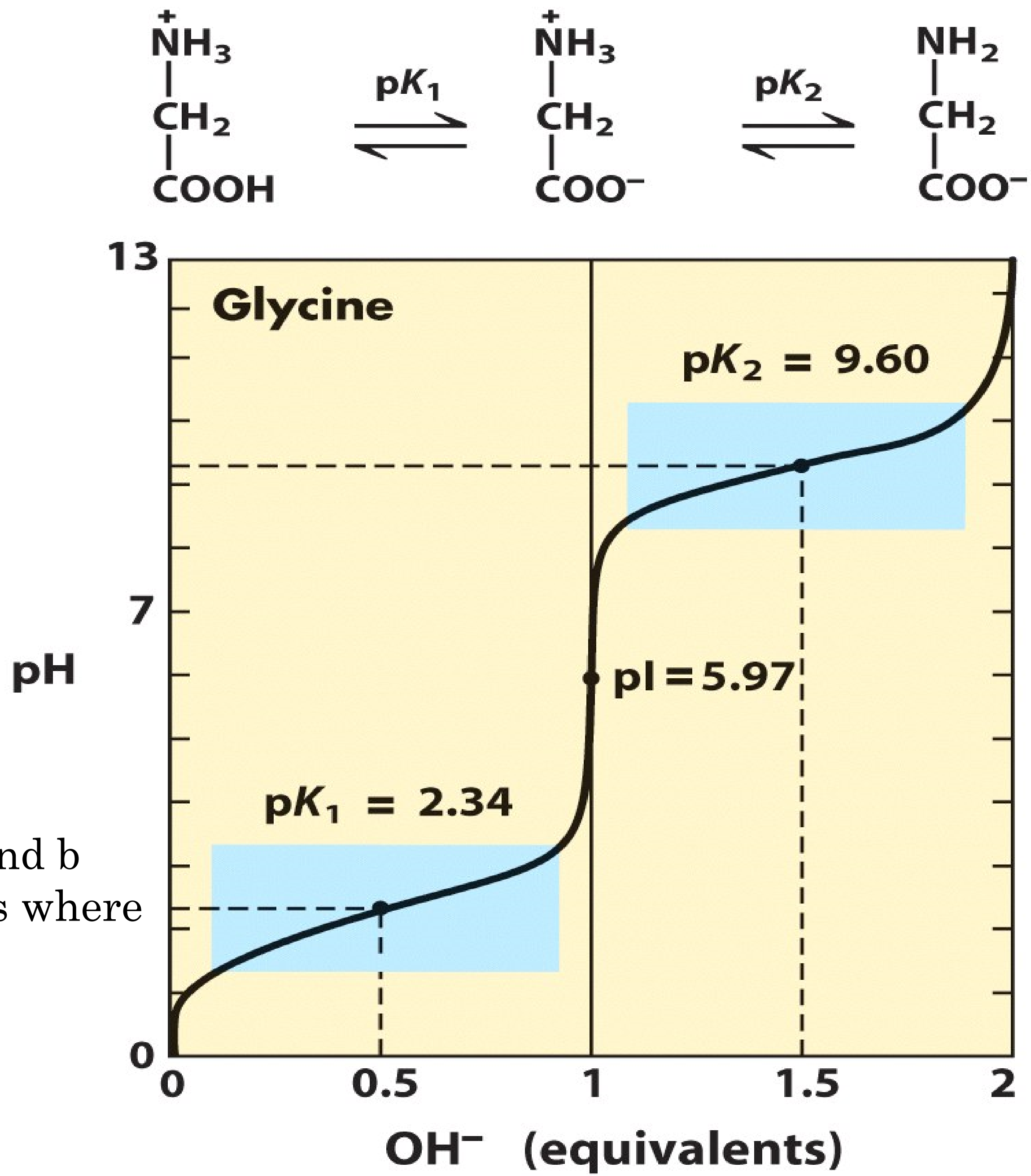
Titration curve:

is a plot of the pH versus the equivalents of base added during titration of an acid.

Titration curve of glycine:

- Glycine is non-polar amino acid.
- It has one carboxyl group (α-carboxyl group) and one amino group (α-amino group).
- Its titration curve consist of **two stages.**

https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(Bruice)/23%3A_The_Organic_Chemistry_of_Amino_Acids%2C_Peptides%2C_and_Proteins/23.03%3A_The_Acid-Base_Properties_of_Amino_Acids



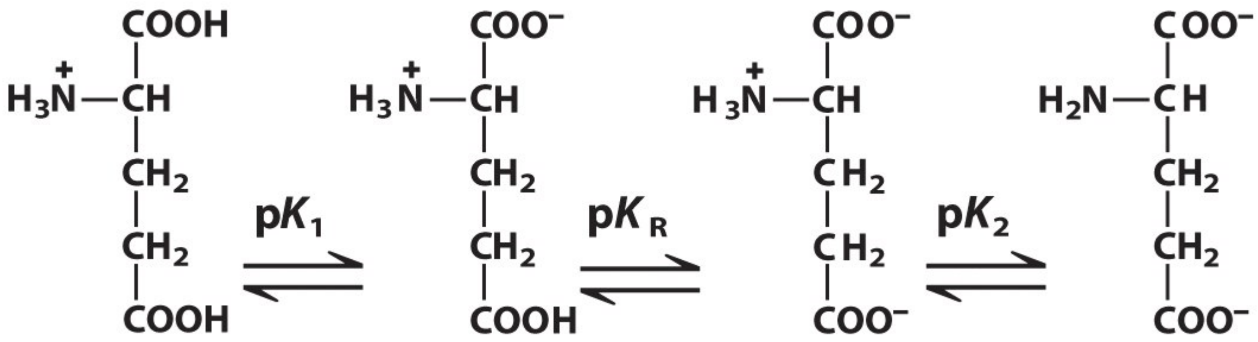
pI = (pK_a + pK_b) / 2.
Note that the subscripts a and b are between amino acid pK's where the net charge is zero.

<u>Titration curve of aspartic and glutamic acid:</u>

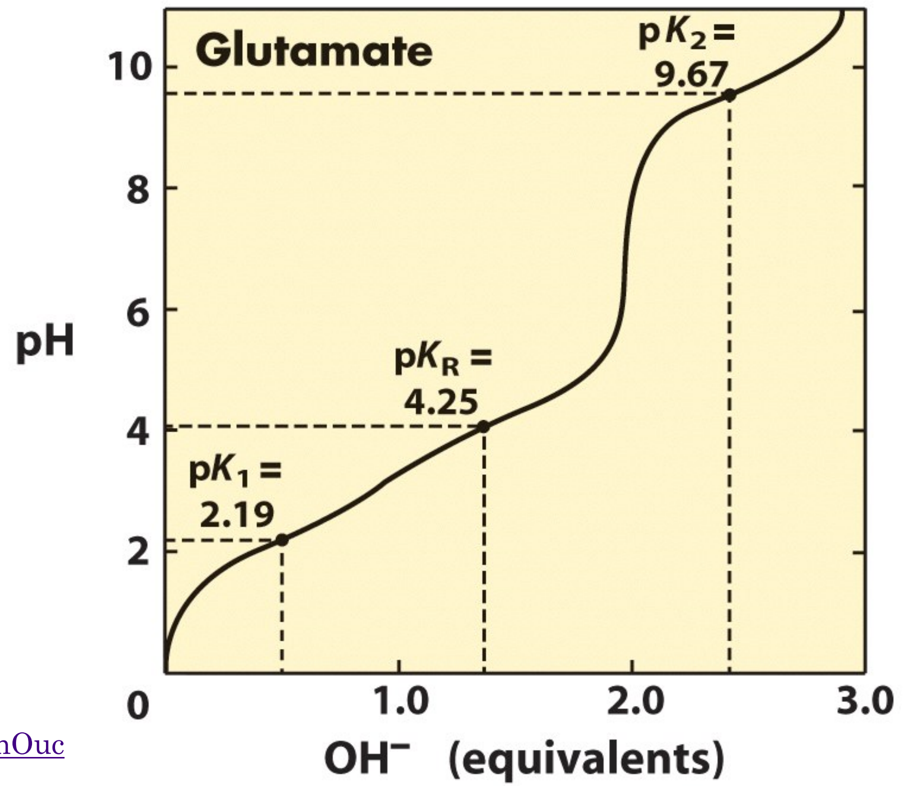
- Aspartic and glutamic acid are a negatively charged amino acid (acidic amino acid)

- They contain two carboxyl groups and one amino group.
- Their titration curve consist of **three stages.**

Glutamate has 3 pKa's



What is the pI ?
Is it at an equivalence point or pKa?
Think about it.



$pI = (pKa_1 + pKa_2)/2$
$= (2.19 + 4.25)/2 = 3.22$

https://www.youtube.com/watch?v=Gkb4it5nOuc

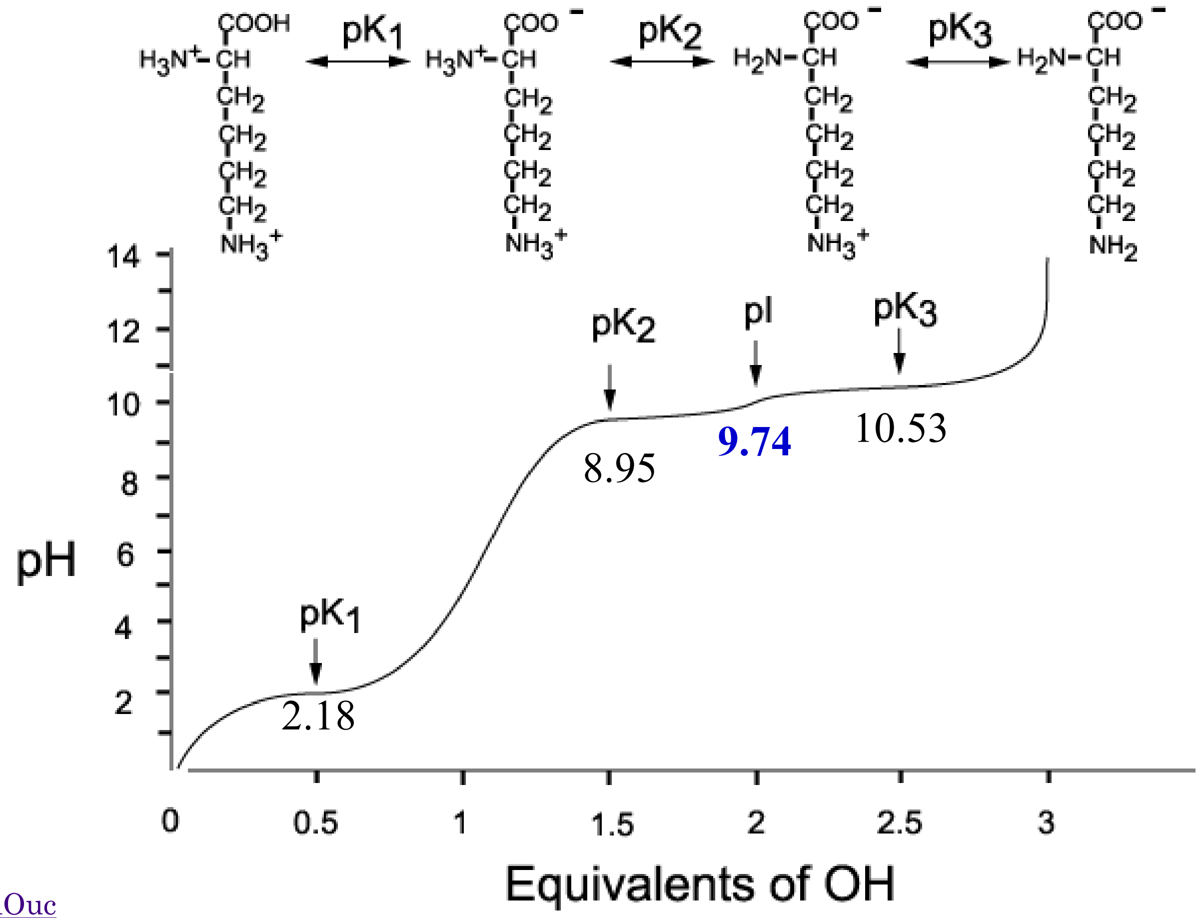
Titration curve of basic amino acids:

- Lysine, Arginine and Histidine are positively charged amino acid (basic amino acid).

- **Lysine** contains one carboxyl group and two amino groups.

  - Its titration curve consist of **three stages (3 pKa)**

$$pI = (pKa_2 + pKa_3)/2$$
$$= (8.95+10.53)/2$$
$$= 9.74$$

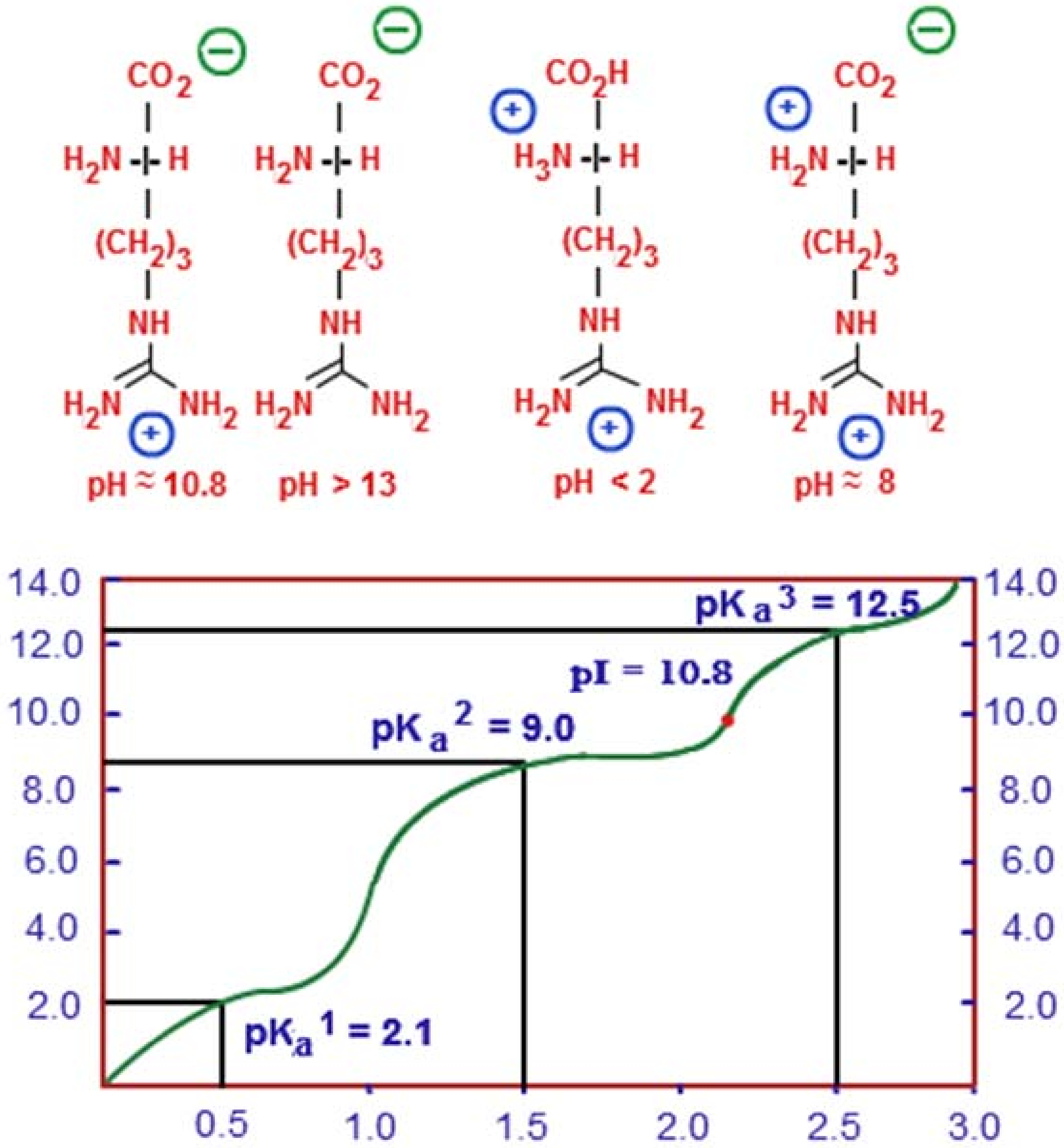


https://www.youtube.com/watch?v=Gkb4it5nOuc

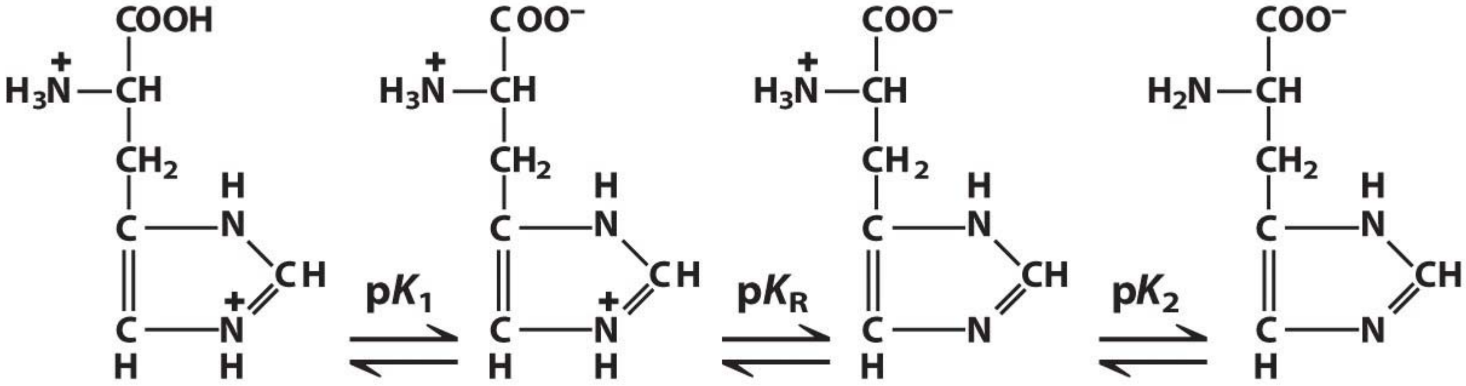
Titration curve of arginine

Arginine has 3 pKa

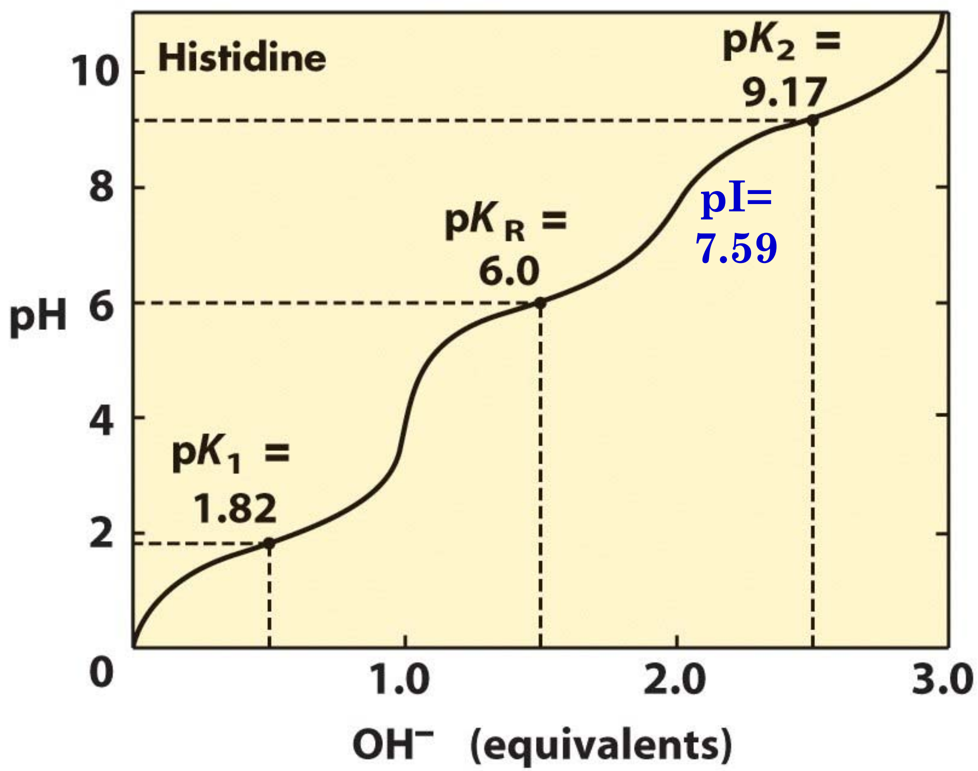
$pI = (pKa_2 + pKa_3)/2$
$= (9.0 + 12.5)/2 = 10.8$



Histidine has 3 pKa



$$pI = (pKa_2 + pKa_3)/2$$
$$= (6.0 + 9.17)/2 = 7.59$$



How to calculate the pI when the side chain is ionizable?

- Identify species that carries a net zero charge
- Identify pK_a value that defines the acid strength of this zwitterion: (pK_2)
- Identify pK_a value that defines the base strength of this zwitterion: (pK_1)
- Take the average of these two pK_a values

What is the pI of histidine?

pKa values of some amino acids

pKa values (25°C)

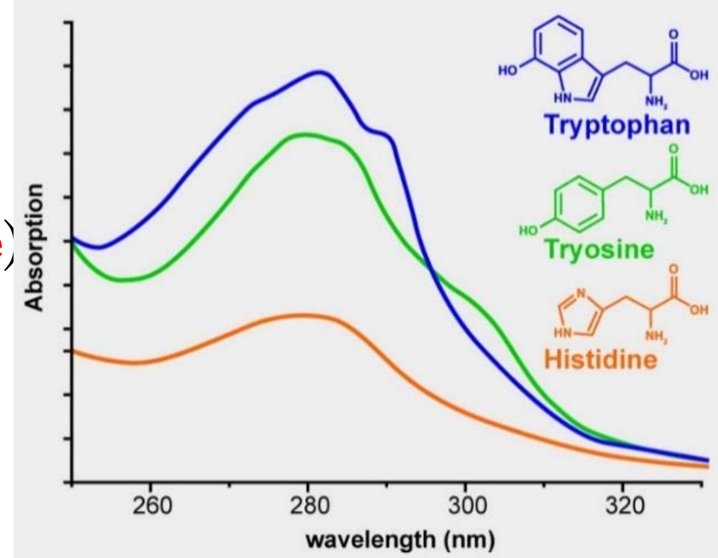
Amino acid	α-COOH group	α-NH_3^+ group	Side chain
Alanine	2.3	9.9	
Glycine	2.4	9.8	
Phenylalanine	1.8	9.1	
Serine	2.1	9.2	
Valine	2.3	9.6	
Aspartic acid	2.0	10.0	3.9
Glutamic acid	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Cysteine	1.8	10.8	8.3
Tyrosine	2.2	9.1	10.9
Lysine	2.2	9.2	10.8

Notice, Cysteine and tyrosine contain three stages titration curve

Stryer Biochemistry, 5th ed.

8) The spectroscopic properties of amino acids

- All amino acid do NOT absorb visible light of the electromagnetic spectrum.
- All amino acid absorb infrared light (IR).
- Several amino acids absorb ultraviolet radiation(UV).
- However, only the aromatic amino acids (tyrosine, tryptophan, and phenylalanine) exhibit significant UV absorption above 250 nm. The absorption of energy by electrons as they rise to higher-energy states.



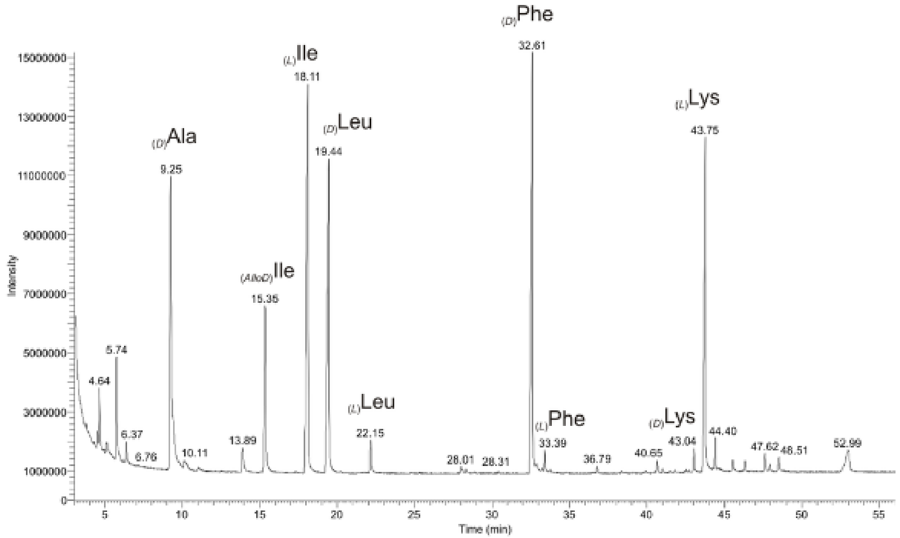
- Since most proteins contain tyrosine residues, measurement of light absorption at 280 nm in a spectrophotometer is an extremely rapid and convenient means of estimating the protein content of a solution.

2- Fluorescence

Aromatic amino acids also exhibit weak fluorescence that is used to determine the protein structure

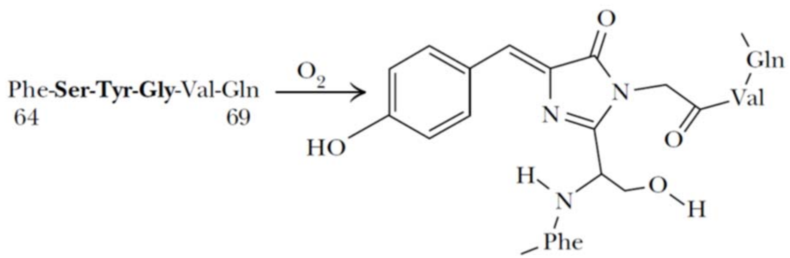
3- Nuclear Magnetic Resonance (NMR)

NMR is a spectroscopic technique that use the absorption of radio frequency energy by certain nuclei in the presence of magnetic field to explore the chemical characteristic s of amino acids and proteins.



GREEN FLUORESCENT PROTEIN—THE "LIGHT FANTASTIC" FROM JELLYFISH TO GENE EXPRESSION

- *Aquorea victoria,* a species of jellyfish found in the northwest Pacific Ocean, contains a **green fluorescent protein (GFP)** that works together with another protein, **aequorin,** to provide a defense mechanism for the jellyfish. When the jellyfish is attacked or shaken, aequorin produces a blue light. This light energy is captured by GFP, which then emits a bright green flash that presumably blinds or startles the attacker. Remarkably, the fluorescence of GFP occurs without the assistance of a **prosthetic group** —a "helper molecule" that would mediate GFP's fluorescence.
- Instead, the light-transducing capability of GFP is the result of a reaction between three amino acids in the protein itself. As shown below, adjacent **serine, tyrosine,** and **glycine** in the sequence of the protein react to form the pigment complex—termed a **chromophore.**
- No enzymes are required; the reaction is autocatalytic. Because the light-transducing talents of GFP depend only on the protein itself (upper photo, chromophore highlighted), GFP has quickly become a darling of genetic engineering laboratories.
- The promoter of any gene whose cellular expression is of interest can be fused to the DNA sequence coding for GFP. Telltale green fluorescence tells the researcher when this fused gene has been expressed (see lower photo and also Chapter 13).



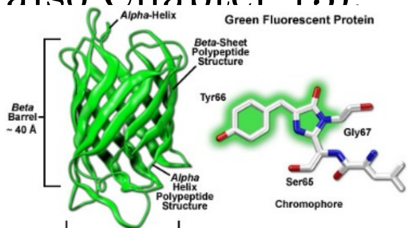


Figure 1

QUIZ

1-The smallest natural amino acid is ....

A- Glycine B- serine C- valine D- argenine

2-An example of sulfur containing amino acid is ....

A- Alanine B- threonine C- serine D- cysteine

3-Negatively charged amino acids contain R-group having ....

A-Hydroxyl group B- carboxyl group C- amino group D- all of the above

4-The amino acid become positive if it is present in .... medium

A-Acidic B- alkaline C- neutral D- water

5- The amino acids having –OH is ....

A-Serine B- Threonine C- Tyrosine D- All of the above

6- How many different natural amino acids are there?

A-10 B- 15 C- 20 D- 30

7-The isoelectric point of an amino acid is the pH at which it carries .... net charge

A-Zero B- positive C- negative D- non of above

QUIZ

8- We expect that aspartic and glutamic have pI ....

A- >7 B- < 7 C- =7 D- non of the above

9- We expect that lysine and histidine have pI ....

A- >7 B- < 7 C- =7 D- non of the above

10- Which of the following amino acids has an amide side chain?

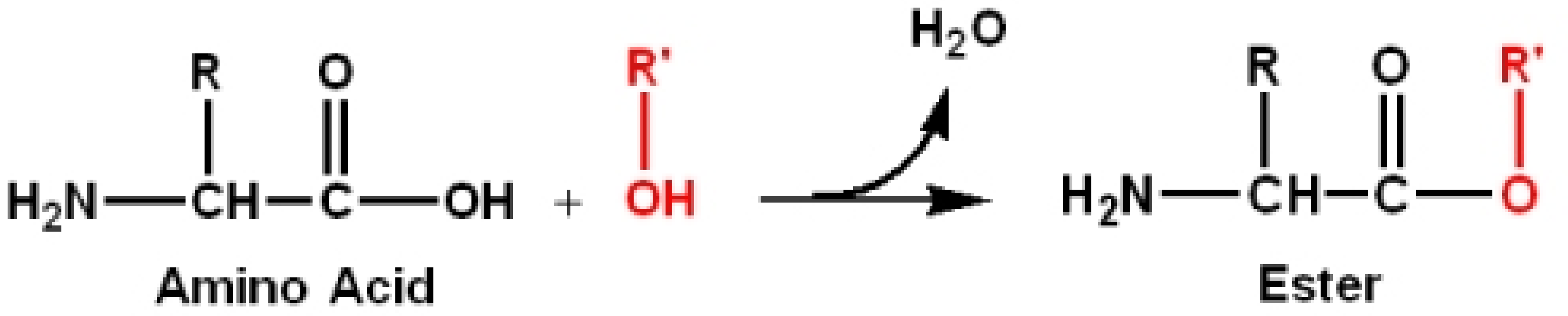
A- Aspartic acid B. Glutamic acid C. Asparagine D. Methionine

The chemical reactions of amino acids:

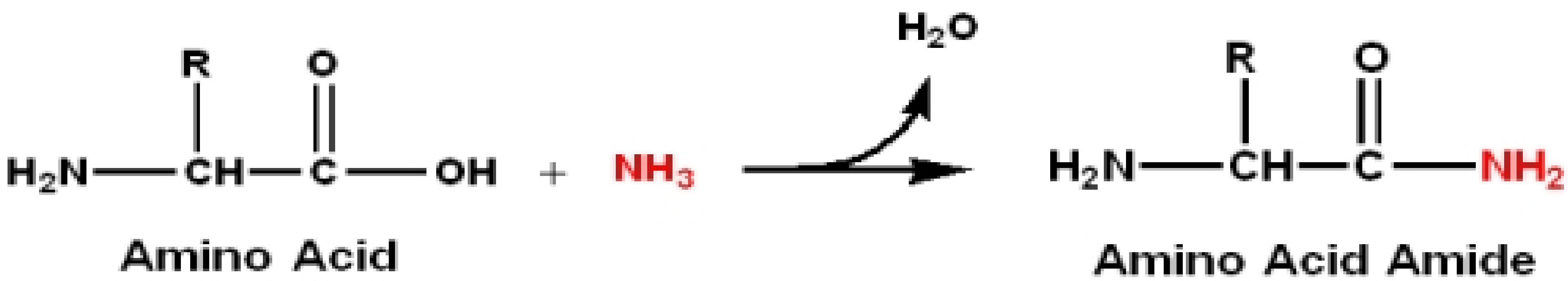
- As for all organic compounds, the chemical reactions of amino acids are those characteristic of their functional group. i.e.,
  - the α-carboxyl group
  - the α-amino group
  - and the functional groups present in the different side chains.

1) Reaction of carboxyl group:

a) Formation of ester:



b) Formation of amide:



c) Reduction of the α-COOH of amino acid with reducing agent lithium borohydride ($LiBH_4$) to yield the corresponding primary alcohol.

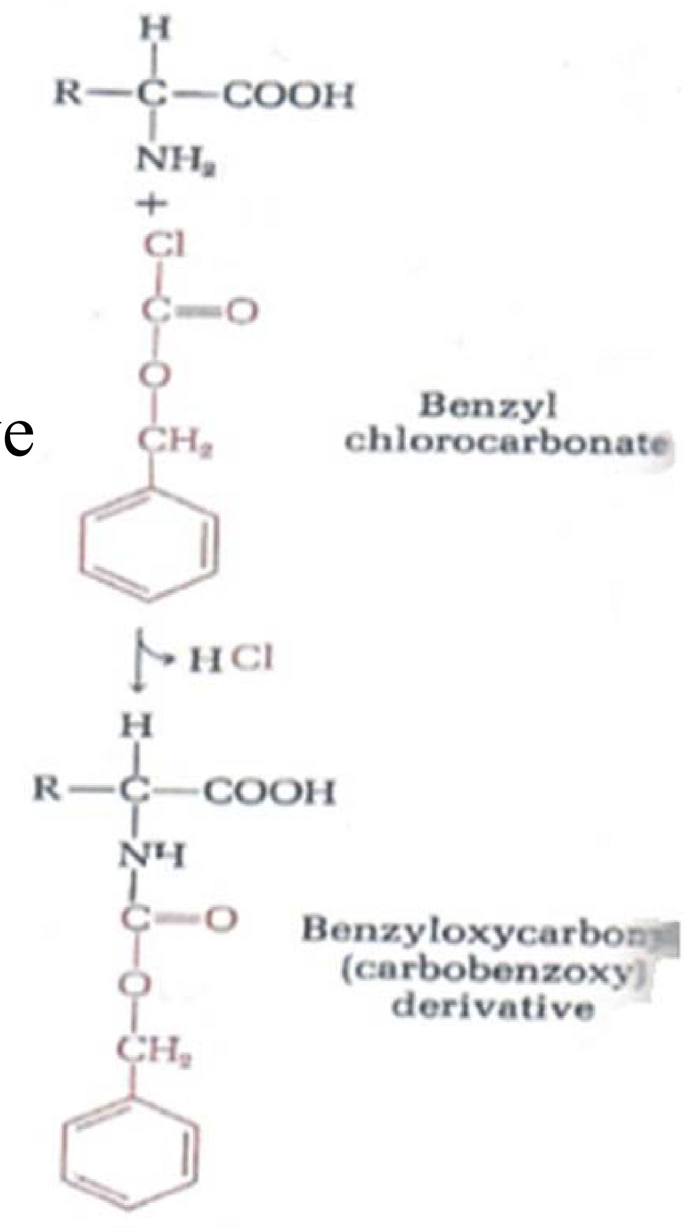
d) Decarboxylation

2) Reactions of amino group:

a) Acylated amino acid:

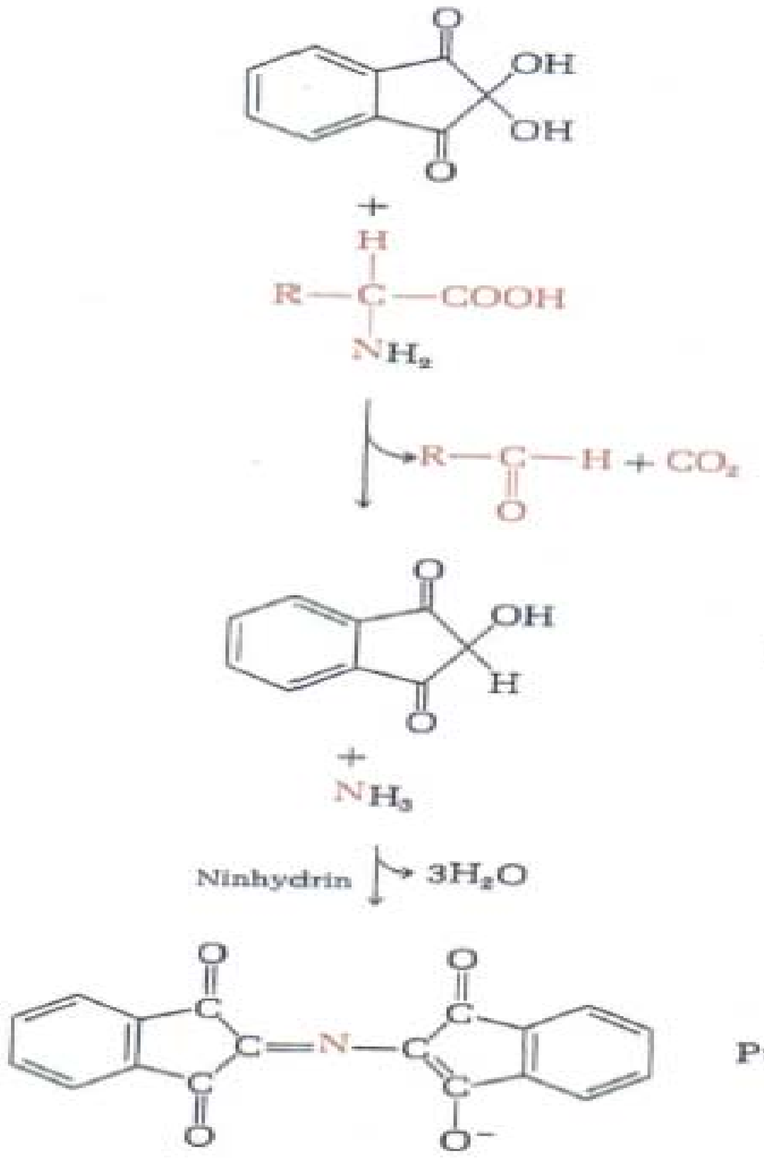
This is by treatment with acid halids.

e.g., formation of the benzyloxy carbomyl (carbobenzoxy) derivative of amino acids.



b) Ninhydrine reaction:

- can be utilized to estimate amino acids quantitatively in very small amounts.
- On heating, an α-amino acid reacts with two molecules of ninhydrine to yield an intensely coloured product.
- The amino groups of the 19 aminoacids and peptides having a free α-amino group give a purple colour in the ninhydrin reaction
- Proline and hydroxyproline, in which contains α-imino group gives yellow colour derivatives.



Ninhydrin

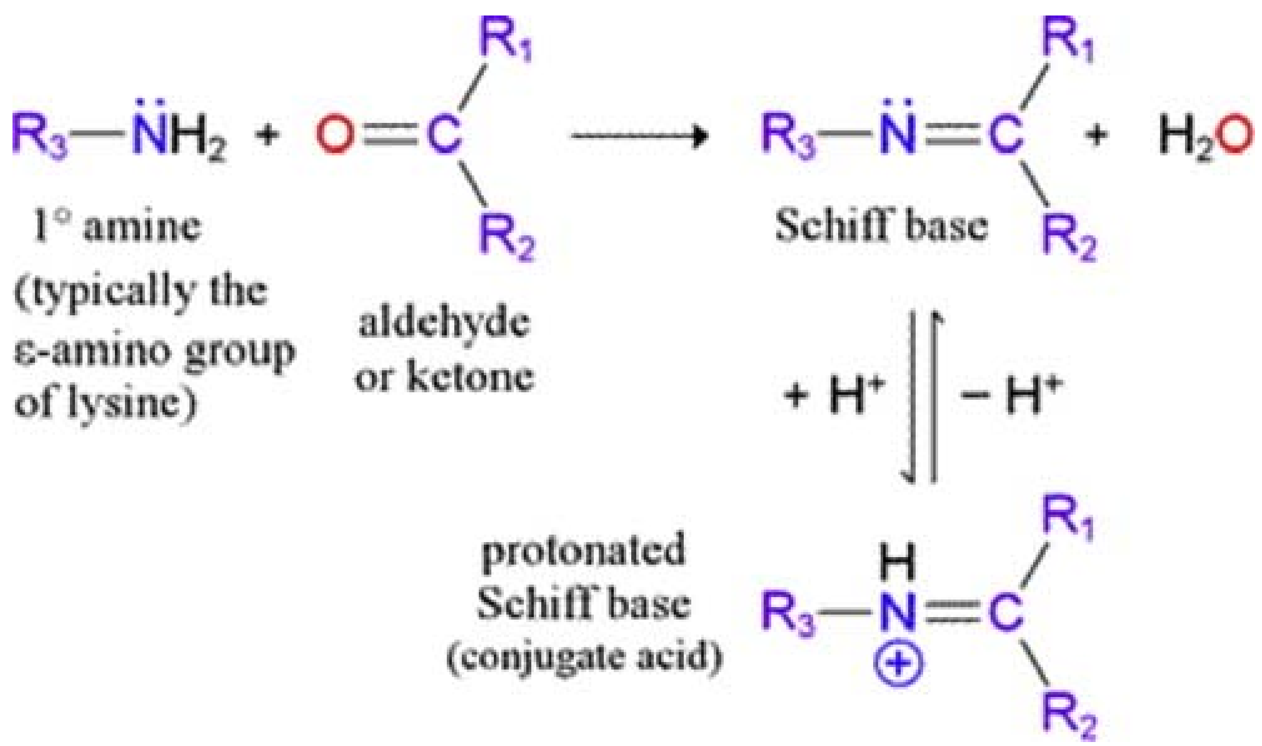
Amino acid

Hydrindantin

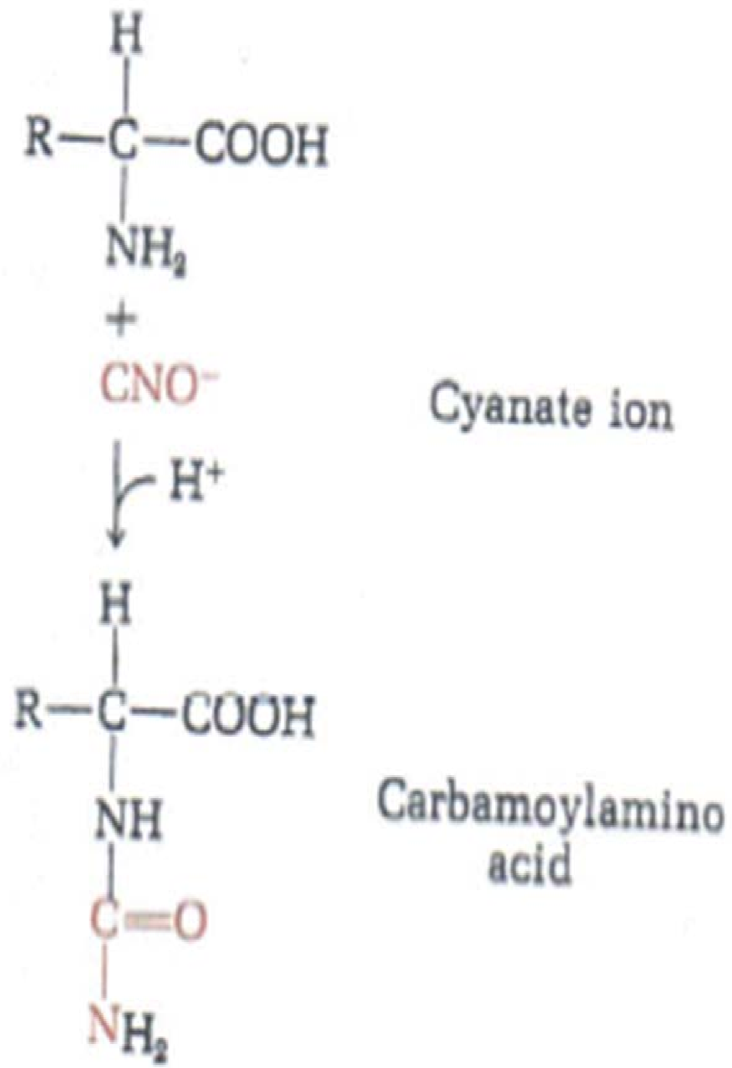
Purple pigment

c) Formation of a Schiff′s base:

- The α-amino groups of amino acids react reversibly with aldehydes to form compounds called Schiff′s base.
- Schiff′s bases appear to be intermediates in a number of enzymatic reaction.

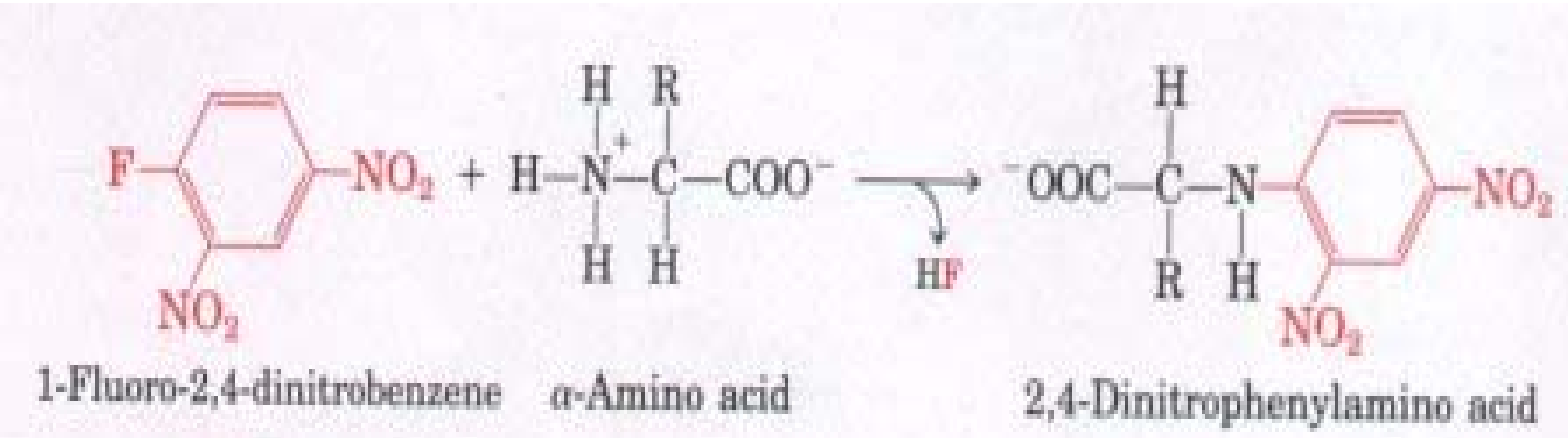


d) Reaction with cyanate:



e) Reaction of α-amino acid with reagent 1-fluoro 2,4 dinitrobenzen (FDNB):

- In mild alkaline solution FDNB reacts with α-amino acids to yield 2,4 dinitrophenyl derivatives, useful in identification of individual amino acids.



3) Reaction of the R-groups:

- Amino acids show qualitative colour reaction typical of certain functions present in their R-groups.

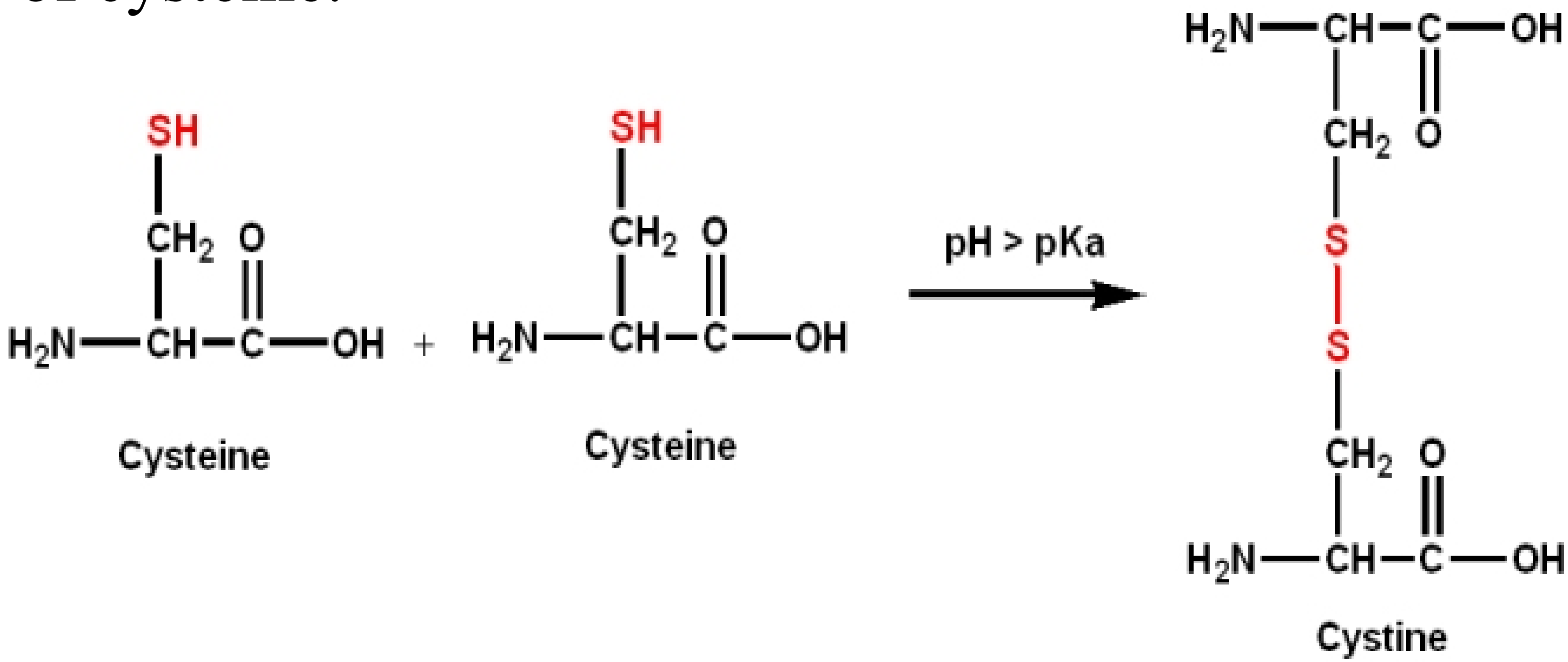
e.g., - The thiol group of cysteine

- The phenolic hydroxyl group of tyrosine
- The guanidinium group of arginine

A) Reaction of thiol group:

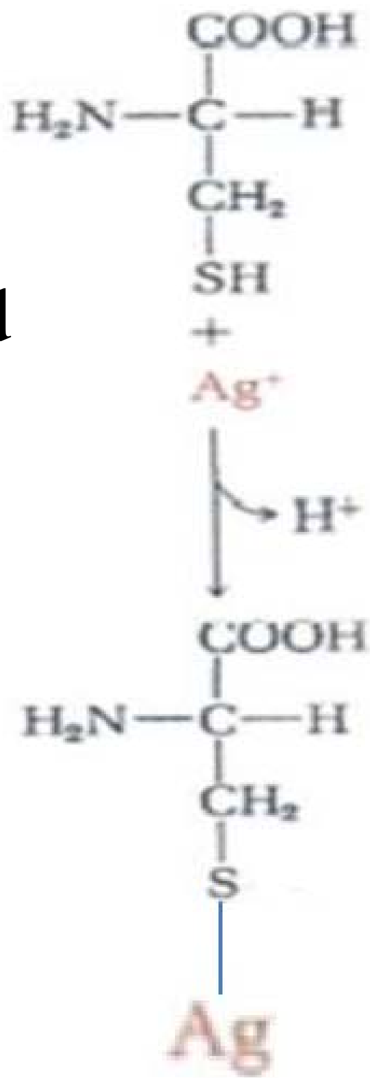
i) Oxidation of cysteine to cystine:

- Oxidation by atmospheric oxygen in the presence of iron salts or by other mild oxidizing agents.
- The oxidation product is cystine, in which the disulfide bond constitute a covalent bridge between two residues of cysteine.



ii) Reaction of cysteine with Ag^+:

-This is another reaction of thiol group of cysteine with heavy metals such as Hg^{+2} and Ag^+ which form mercaptides.



Cysteine

Cysteine silver mercaptide

iii) Reaction of cysteine with Elman′s reagent:

- The thiol groups of cysteine and cysteine residues in peptides and proteins can be measured by a quantitative reaction with Elman′s reagent, yielding a product that can be measured colorimetrically.

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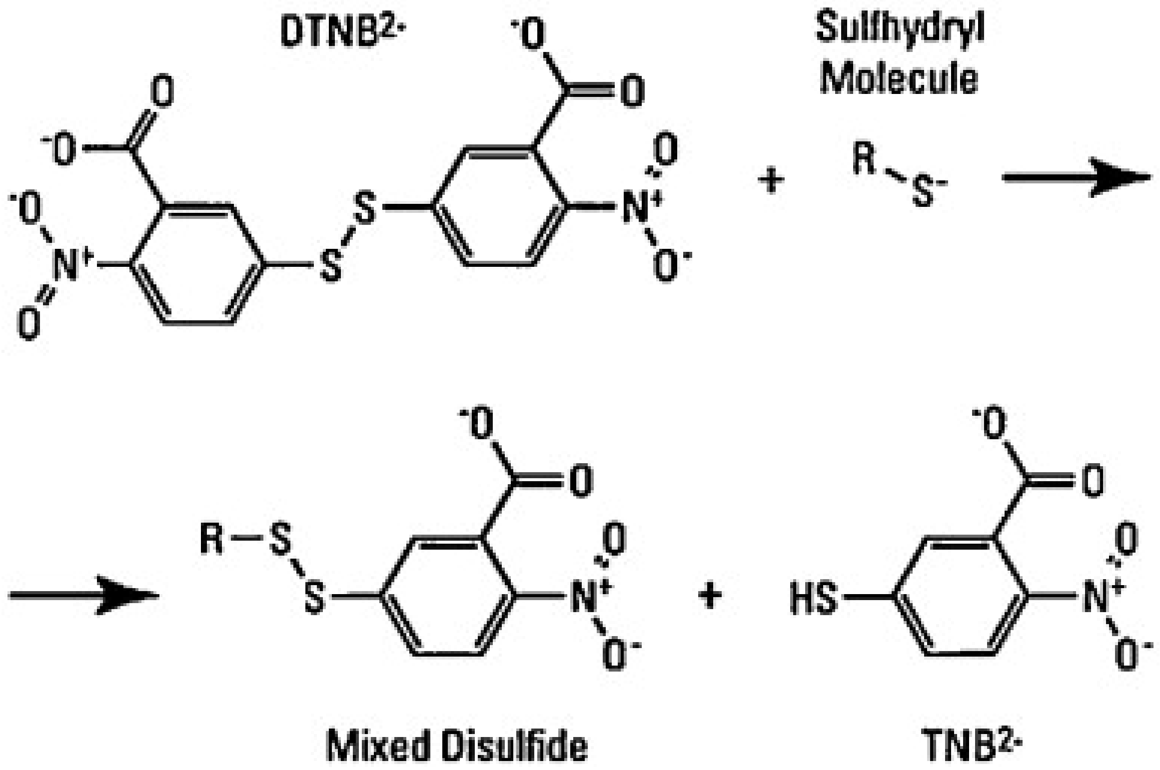
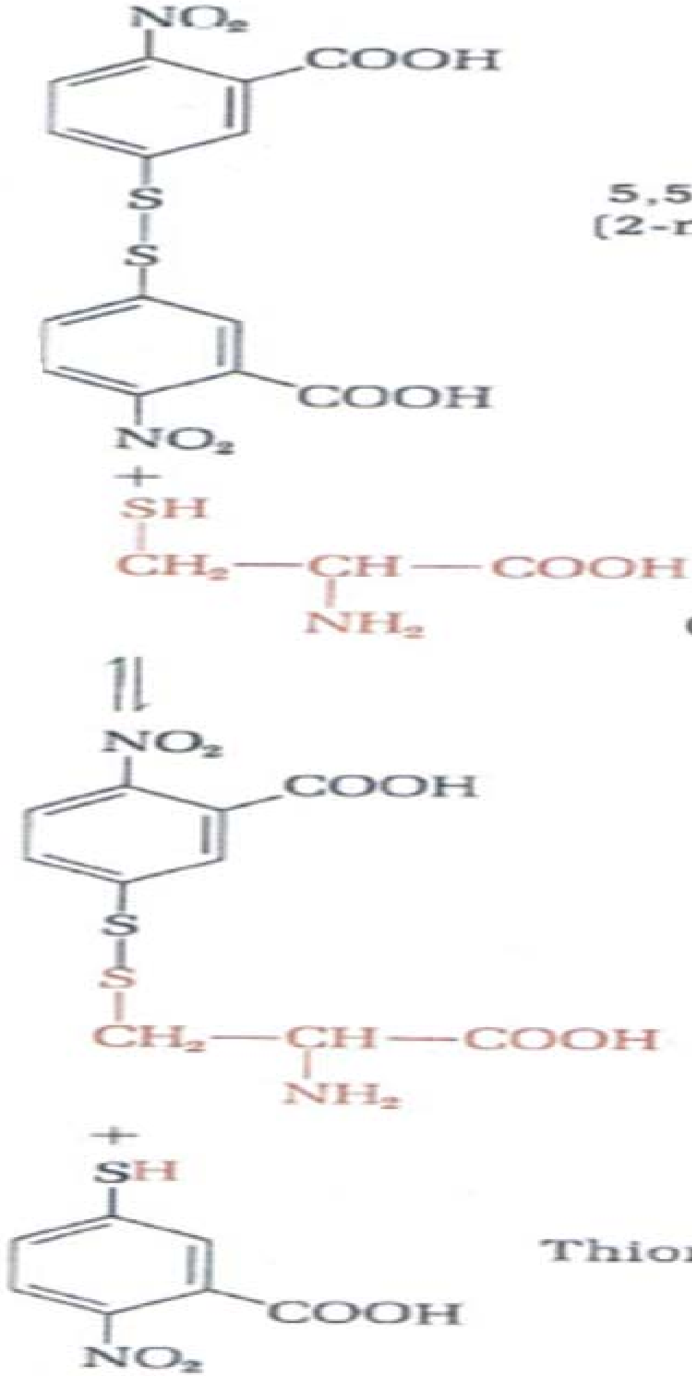
- The thiol groups of cysteine and cysteine residues in peptides and proteins can be measured by a quantitative reaction with Elman′s reagent, yielding a product that can be measured colorimetrically.



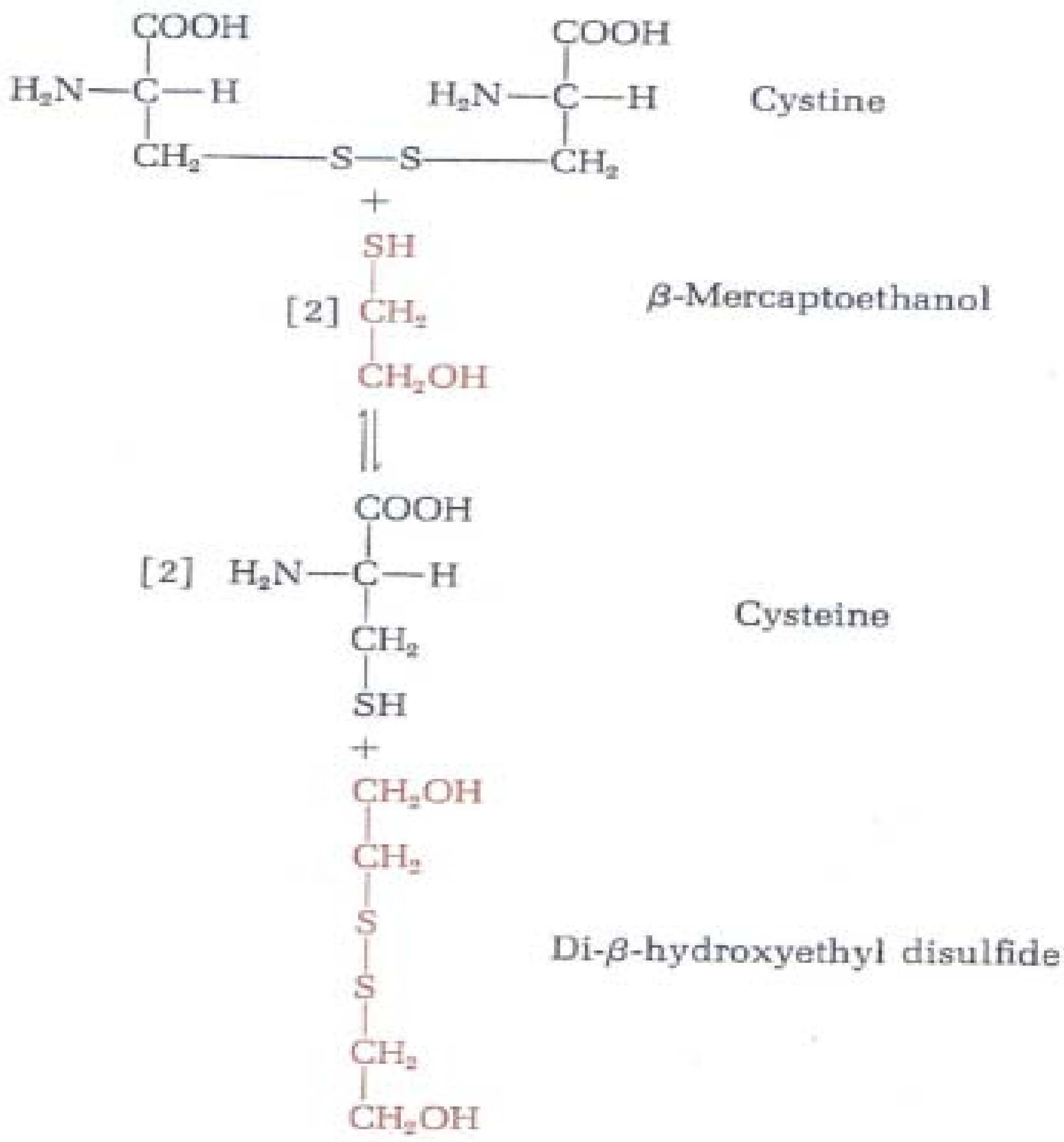


5,5'-Dithiobis-(2-nitrobenzoic acid)

Cysteine

Thionitrobenzoic acid

iv) Reduction of cystine to cysteine:



B) Reaction of phenolic hydroxyl group of tyrosine:

i) Millon reaction:

When tyrosine is heated with millon solution ($HgSO_4$ in H_2SO_4), a red colour is produced.

C) Reaction of guanidinium group of arginine:

i) Sakaguchie test:

The guanidinium group of arginine, react with α-naphthol and sodium hypochlorite to a red colour.

Separation of amino acids:

Complex mixtures of amino acids can be separated, identified and estimated by:

- Electrophoresis
- Ion exchange chromatography

Paper electrophoresis:

- The simplest method for separating amino acids is paper electrophoresis.

- A drop of an aqueous solution of the amino acid mixture is placed on a filter-paper strip moistened with a buffer at a given pH.
- A high-voltage electric field is applied to the strip.
- Because of their different pK values, the amino acids migrate in different directions and at different rates along the strip, depending on :
  - the pH of the buffer system
  - the voltage applied.

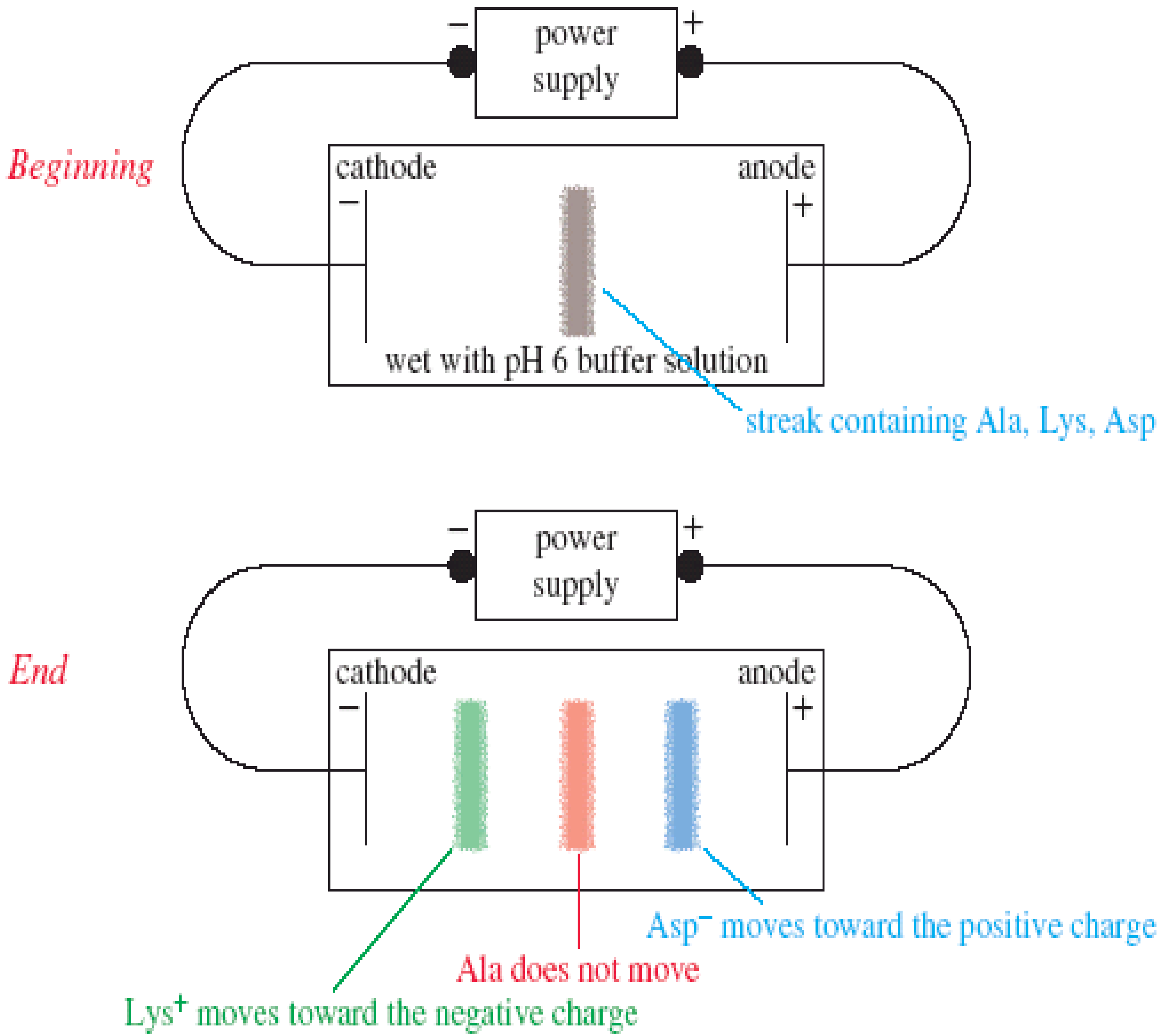
Example:

- **At pH 1.0,** histidine, arginine, and lysine have a charge of +2 and move more rapidly to the negatively charged cathode than the other amino acids, which have a charge of +1 .
- **At pH 6.0,** the positively charged amino acids (lysine, arginine, histidine) move to the cathode and the negatively charged amino acids (aspartic acid and glutamic acid) to the anode.
- **The other amino acids** will remain at or near the origin, since they have no ionizing groups other than their α-amino and α-carboxyl group and thus have about the same isoelectric point.

- To locate the amino acids on the paper, it is dried, sprayed with ninhydrin and heated.

Do you remember the purple and yellow colour?

- Blue or purple spots, each indicating the presence of an amino acid, will appear on the paper.



Beginning
power supply
cathode
anode
wet with pH 6 buffer solution
streak containing Ala, Lys, Asp
End
power supply
cathode
anode
Asp⁻ moves toward the positive charge
Ala does not move
Lys⁺ moves toward the negative charge

Ion exchange chromatography:

- It is the most widely used method for separating, identifying, and quantitating the amounts of each amino acid in a mixture.

- The chromatographic column consists of a long tube filled with the granules of a synthetic resin containing fixed charged groups.
- Resins with fixed anionic groups are called cation exchange resins.
- Resins with fixed cationic groups are anion exchange resins.

- In the simplest form of ion-exchange chromatography, amino acids can be separated on columns of cation-exchange resins, in which the fixed anionic groups, e.g sulfonic acid groups ($-SO_3^-$), are first charged with Na^+.

● an acid solution (pH 3) of the amino acid mixture to be analyzed is then poured on the column and allowed to percolate through slowly.

-At pH 3 , the amino acids are largely cations with net positive charges.

- As the mixture passes down the column, the positively charged amino acids will displace the bound Na^+ ions from the fixed ($-SO_3^-$) groups of the resin particles.

Example:

- At pH 3.0 , the amino acids with the largest positive charge (lysine, arginine, and histidine) will displace Na^+ from the resin first and will be bound to the resin most tightly.
- The amino acids with the least amount of positive charge at pH 3 (glutamic acid and aspartic acid) will be bound least.
- All the other amino acids will have intermediate amounts of positive charge.

- The different amino acids will therefore move down the resin column at different rates, which depend largely on their pK values.

→
- glutamic acid and aspartic acid will move down the column at the highest rates, since they bound least at pH 3.0
- whereas lysine, arginine, and histidine will move most slowly.

- small fractions of a few milliliters each collected from the bottom of the column and analyzed quantitatively.

TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_a values: pK_1 (—COOH)	pK_2 (—NH_3^+)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3