FDE 303
FOOD CHEMISTRY
WEEK-4

Amino Acids, Peptides and Proteins

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INTRODUCTION

- Amino acids, peptides and proteins are important constituents of food.
- They supply the required building blocks for protein biosynthesis.
- They directly contribute to the flavor of food and are precursors for aroma compounds and colors formed during thermal or enzymatic reactions in production, processing and storage of food.
- Proteins also contribute significantly to the physical properties of food through their ability to build or stabilize gels, foams, emulsions and fibrillar structures.
- The nutritional energy value of proteins (4 kcal/g) is as high as that of carbohydrates.

PROTEINS

- Highly complex polymers, made up of 20 (plus 2 new a.a.) different amino acids.

- At the elemental level, proteins contain on wt/wt basis

  - 50–55% carbon
  - 6–7% hydrogen
  - 20–23% oxygen
  - 12–19% nitrogen
  - 0.2–3.0% sulfur

- Protein synthesis occurs in ribosomes.
  - After synthesis, cytoplasmic enzymes modify some amino acid constituents.
  - This changes the elemental composition of some proteins.

PROTEINS-Classification

- ***Homoproteins***: Proteins that are not enzymatically modified in cells
- ***Prosthetic groups:*** The nonprotein components
- ***Conjugated proteins* or *heteroproteins:*** Proteins that are modified or complexed with nonprotein components

Examples of conjugated proteins:

- ***Nucleoproteins:*** noncovalent complexes containing nucleic acids (ribosomes)
- ***Glycoproteins:*** contain covalently linked carbohydrate (ovalbumin and *κ*-casein)
- ***Phosphoproteins:*** contain covalently linked phosphate groups (*α*- and *β*-caseins, kinases, and phosphorylases)
- ***Lipoproteins:*** noncovalent complexes containing lipids (proteins of egg yolk and several plasma proteins)
- ***Metalloproteins:*** noncovalent complexes containing metal ions (hemoglobin, myoglobin and several enzymes)

PROTEINS-Classification

Classification according to their gross structural organization:

- *Globular proteins*
  - exist in spherical or ellipsoidal shapes, resulting from folding of the polypeptide chain(s) on itself
  - A majority of enzymes are globular proteins
- *Fibrous proteins*
  - rod-shaped molecules containing twisted linear polypeptide chains
  - **Examples:** *tropomyosin, collagen, keratin, and elastin*
  - can also be formed as a result of linear aggregation of small globular proteins, for example, actin and fibrin
  - fibrous proteins invariably function as *structural proteins*

Classification according to their various biological functions:

- *Enzyme catalysts*
- *Structural proteins*
- *Contractile proteins* (myosin, actin, and tubulin)
- *Hormones* (insulin and growth hormone)
- *Transfer proteins* (serum albumin, transferrin, and hemoglobin)
- *Antibodies* (immunoglobulins)
- *Storage proteins* (egg albumen and seed proteins)

PROTEINS-Classification

Classification based on their solubility

- ***Albumins:*** soluble in water at pH 6.6
  - **Examples:**

    Serum albumin: blood

    Lactalbumin: milk

    Ovalbumin: egg

    Legumelin: lentil

    Leucocin: wheat

    Insulin: pancreatic hormone

- *Globulins:* soluble in dilute salt solutions at pH 7.0
  - **Examples:** glycinin, phaseolin, and β-lactoglobulin

- ***Glutelins:*** soluble only in acid (pH 2) and alkaline (pH 12) solutions
  - **Examples:** wheat glutelins

- *Prolamines:* soluble in 70% ethanol
  - **Examples:** zein and gliadins)

- Both prolamines and glutelins are highly hydrophobic proteins.

- The major storage protein of wheat is gluten.
  - Gluten is a heterogeneous mixture of proteins, mainly gliadins and glutenins, with limited solubility in water.

PROTEINS-Classification

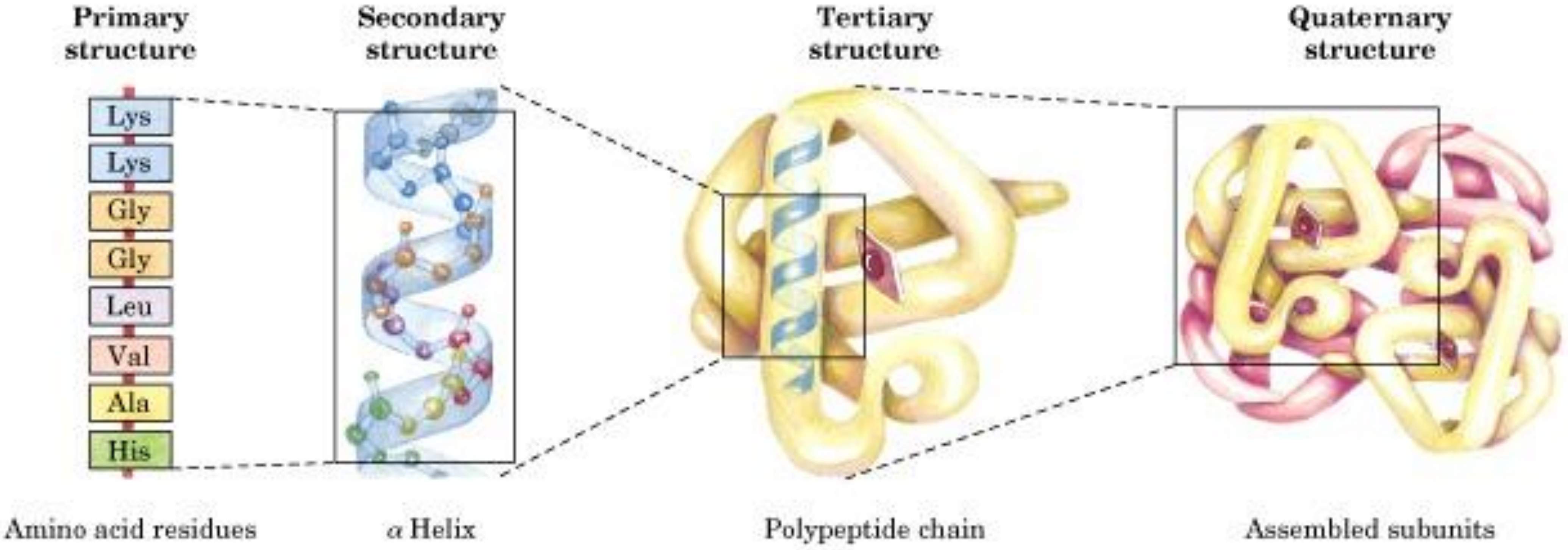
Osborne classification of proteins

- **Thomas Burr Osborne** classified the storage **proteins** into groups based on their extraction and solubility in water:
  - (albumins), dilute saline (globulins), alcohol ether mixtures (prolamins), and dilute acid or alkali (glutelins)

Fraction	Wheat	Rye	Oats	Barley	Corn	Rice	Millet
Albumins	Leukosin						
Globulins	Edestin		Avenalin				
Prolamins	Gliadin	Secalin	Avenin	Hordein	Zein	Oryzin	Cafirin
Glutelins	Glutenin	Secalinin		Hordenin	Zeanin	Oryzenin	

PROTEINS-structure

– The differences in structure and function arise from the sequence in which the amino acids are linked together via amide bonds.



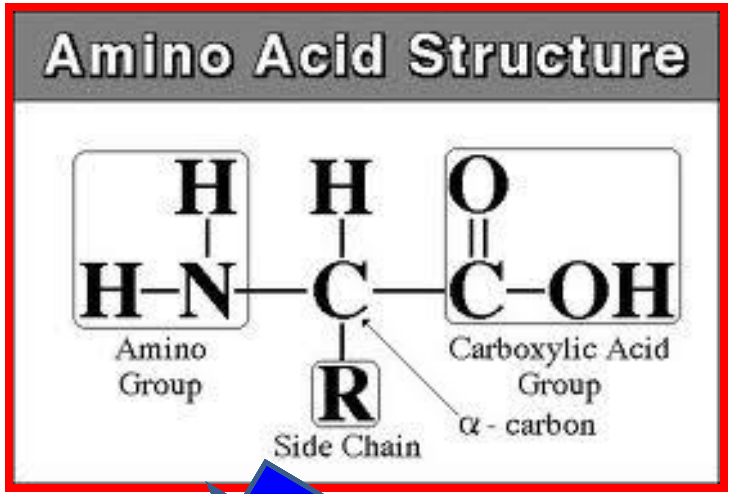
- There are 22 amino acids that are found in proteins.
  - only 20 are specified by the universal genetic code. The 21st and 22nd amino acids, selenocysteine and pyrrolysine are rare genetically encoded amino acids

PHYSICOCHEMICAL PROPERTIES OF AMINO ACIDS

- α-Amino acids: the basic structural units of proteins and consist of a α-carbon atom covalently attached to a hydrogen atom, an amino group, a carboxyl group, and a side chain R group.

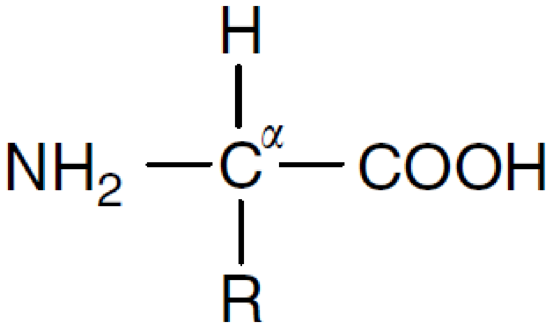
- These amino acids differ only in the chemical nature of the side chain R group.

- The physicochemical properties, such as net charge, solubility, chemical reactivity, and hydrogen bonding potential, of the amino acids are dependent on the chemical nature of the R group.



Side groups vary:

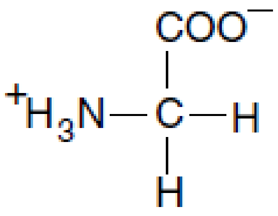
- ✓ **Size** (molar mass)
- ✓ **Composition** (C, O, H, S, N)
- ✓ **Structure** (linear, ring, branched)
- ✓ **Polarity** (polar, non-polar)
- ✓ **Charge** (neutral, positive, negative)
- ✓ **Chemical reactivity**



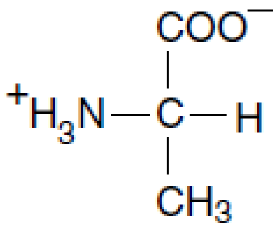
PRIMARY α-AMINO ACIDS IN PROTEINS

Aliphatic amino acids

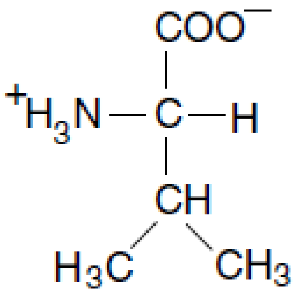
Glycine
(Gly, G)



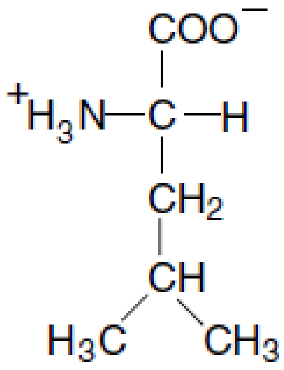
Alanine
(Ala, A)



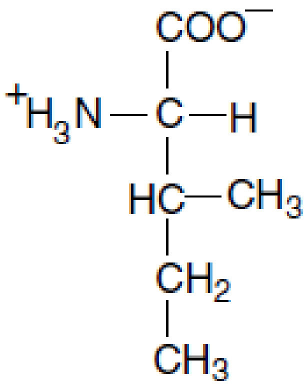
Valine
(Val, V)



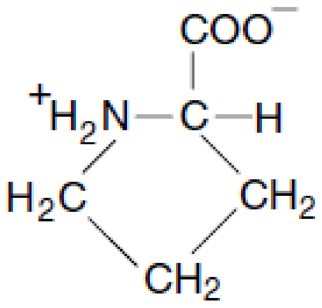
Leucine
(Leu, L)



Isoleucine
(Ile, I)

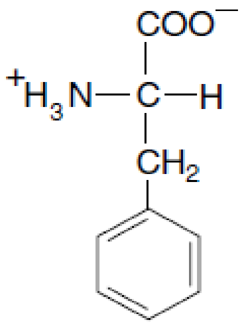


Proline
(Pro, P)

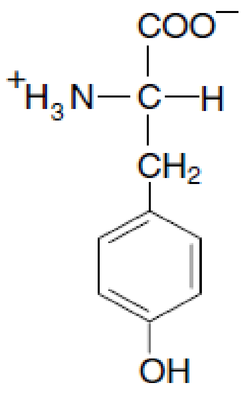


Aromatic amino acids

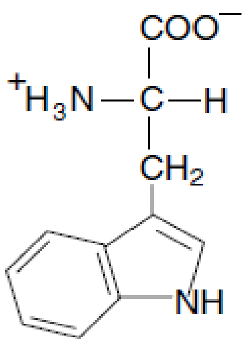
Phenylalanine
(Phe, F)



Tyrosine
(Tyr, Y)

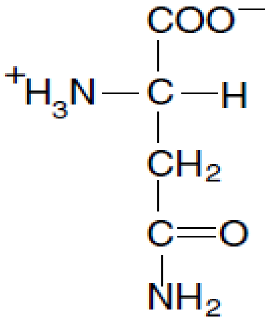


Tryptophan
(Trp, W)

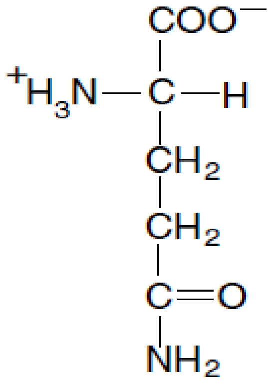


Amide amino acids

Asparagine
(Asn, N)



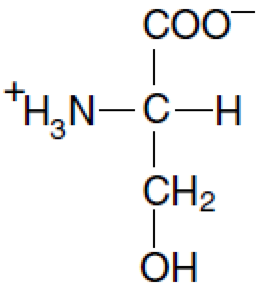
Glutamine
(Gln, Q)



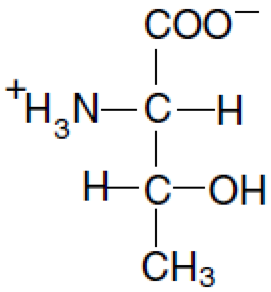
PRIMARY α-AMINO ACIDS IN PROTEINS

Hydroxy amino acids

Serine
(Ser, S)

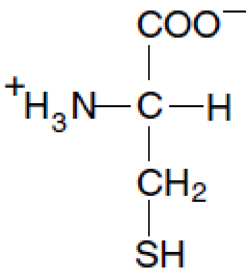


Threonine
(Thr, T)

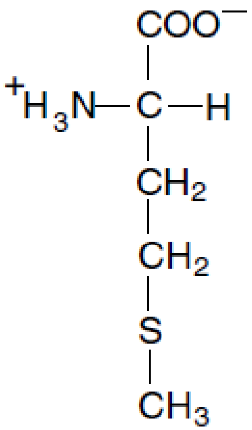


Sulfur amino acids

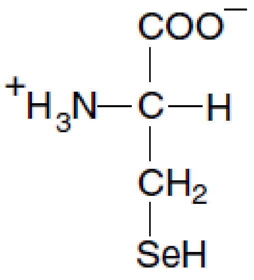
Cysteine
(Cys, C)



Methionine
(Met, M)

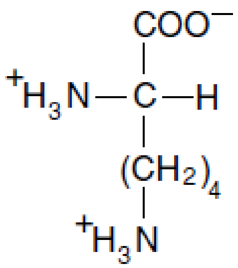


Selenocysteine
(SeCys)

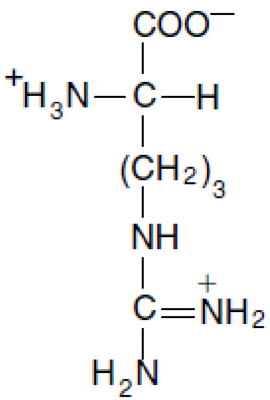


Basic amino acids

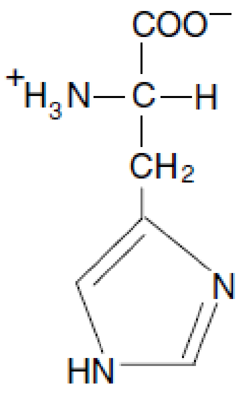
Lysine
(Lys, K)



Arginine
(Arg, R)

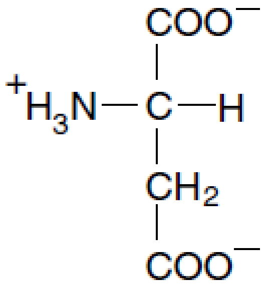


Histidine
(His, H)

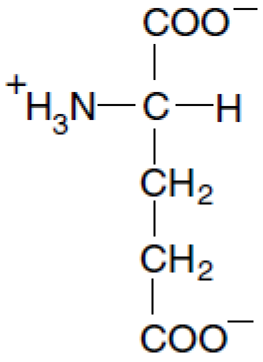


Acidic amino acids

Aspartic acid
(Asp, D)



Glutamic acid
(Glu, E)



Classification of amino acids based on the nature of interaction of the side chains with water

- Amino acids with aliphatic (Ala, Ile, Leu, Met, Pro, and Val) and aromatic side chains (Phe, Trp, and Tyr) are hydrophobic.
  - they exhibit limited solubility in water.

- Polar (hydrophilic) amino acids are quite soluble in water.
  - they are either charged (Arg, Asp, Glu, His, and Lys) or uncharged (Ser, Thr, Asn, Gln, and Cys).
  - the side chains of Arg and Lys contain guanidyl and amino groups, respectively, and thus are positively charged (basic) at neutral pH.
  - imidazole group of His is basic in nature. However, at neutral pH its net charge is only slightly positive.
  - the side chains of Asp and Glu acids contain a carboxyl group.
  - These amino acids carry a net negative charge at neutral pH.

- Both the basic and acidic amino acids are strongly hydrophilic.

- The net charge of a protein at physiological conditions is dependent on the relative numbers of basic and acidic amino acids residues in the protein.

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Stereochemistry of Amino Acids

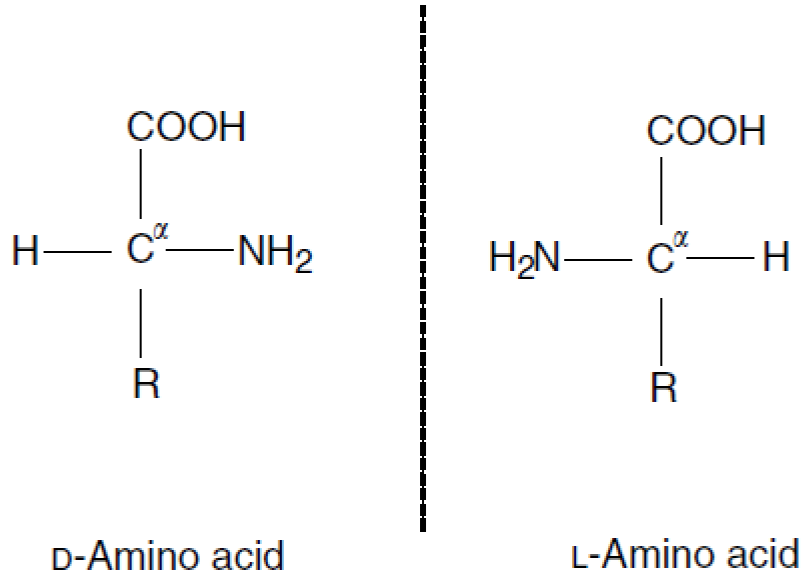
- With the exception of Gly, the α-carbon atom of all amino acids is asymmetric (four different groups are attached to it)

- Amino acids, except for glycine, have at least one chiral center and, hence, are optically active.
  - they rotate the plane of linearly polarized light.

- In addition to the asymmetric α-carbon atom, the β-carbon atoms of Ile and Thr are also asymmetric, and thus both Ile and Thr can exist in four enantiomeric forms (isomers).

- Among the derived amino acids, hydroxyproline and hydroxylysine also contain two asymmetric carbon centers.

- All proteins found in nature contain only L-amino acids.

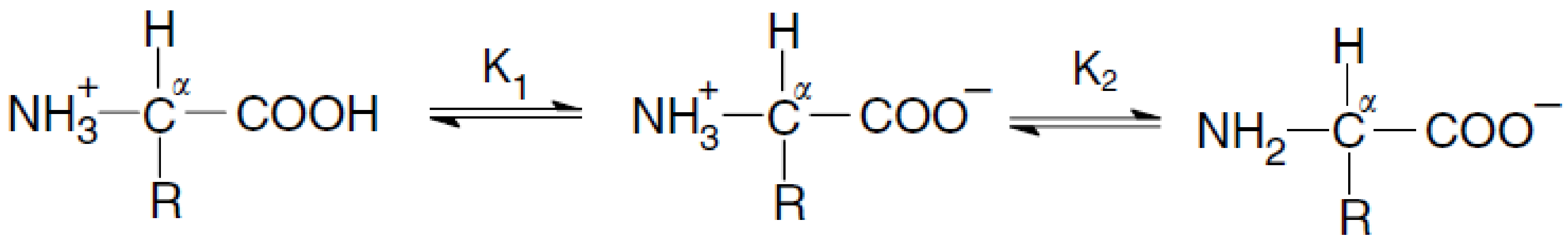


Conventional representation of the L- and D-enantiomers

Acid–Base Properties of Amino Acids

- Since amino acids contain a carboxyl group (acidic) and an amino group (basic), they behave both as acids and bases; that is, they are *ampholytes*.

- For example, Gly, the simplest of all amino acids, can exist in three different ionized states, depending on the pH of the solution.



Acidic **Neutral** **Basic**

- pKa_1 = the pH where the concentrations of COOH and COO^- are equal
- pKa_2 = the pH where the concentrations of NH_3^+ and HN_2 are equal
- Some amino acids also contain ionizable groups in side chains (pKa_3)

Acid–Base Properties of Amino Acids

- At around neutral pH, both the α-amino and α-carboxyl groups are ionized, and the molecule is a *dipolar* or a *zwitter ion*.

- The pH at which the dipolar ion is electrically neutral is called the *isoelectric point* (pI).

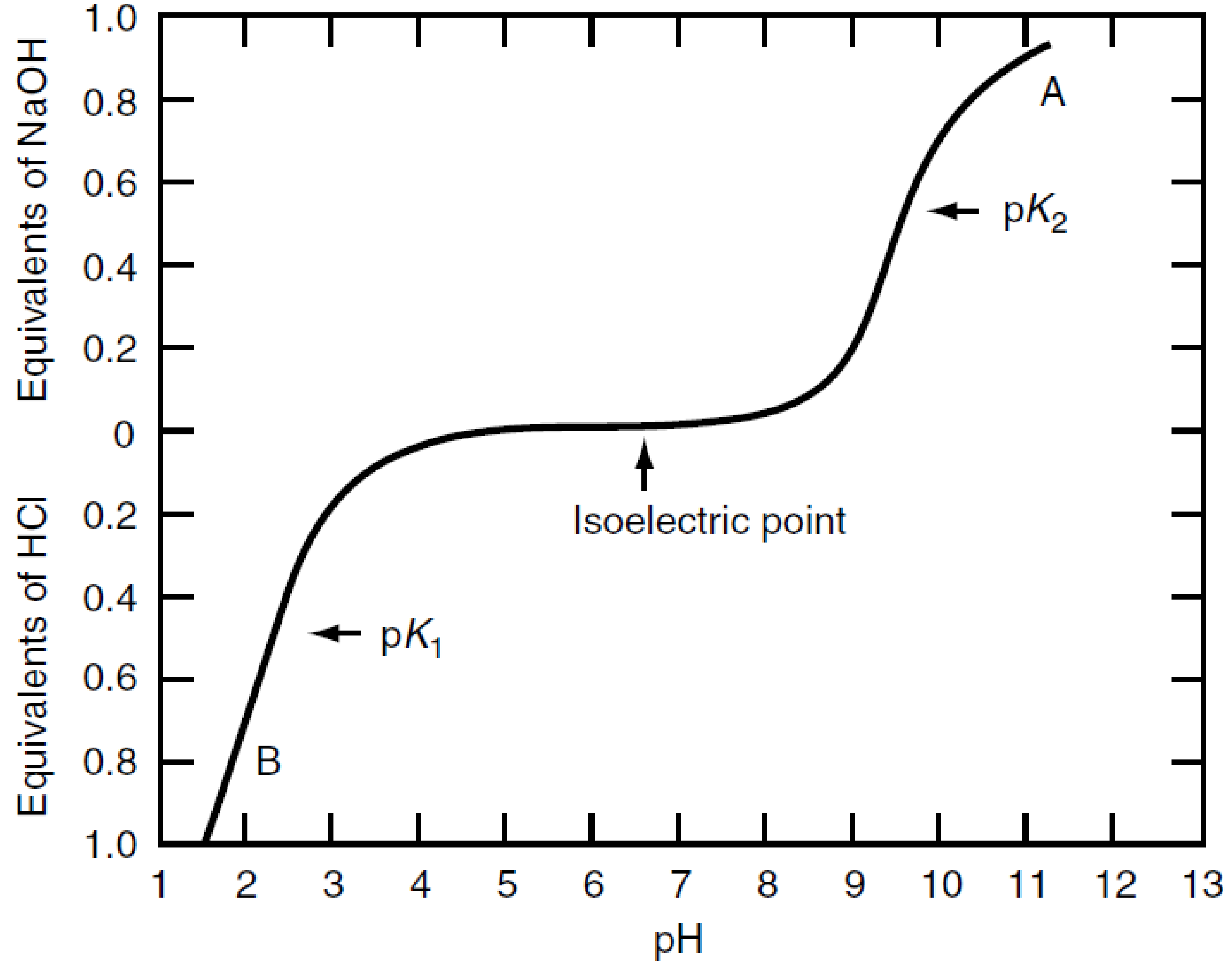
- When the zwitter ion is titrated with an acid, the COO^- group becomes protonated.

- The pH at which the concentrations of COO^- and COOH are equal is known as pK_{a1} (i.e., negative logarithm of the acid dissociation constant K_{a1}).

Acid–Base Properties of Amino Acids

- When the zwitter ion is titrated with a base, the NH^{+3} group becomes deprotonated.
- The pH at which $[NH^{+3}] = [NH_2]$ is known as pK_{a2}.
- In addition to the α-amino and α-carboxyl groups, the side chains of Lys, Arg, His, Asp, Glu, Cys, and Tyr also contain ionizable groups.

A typical electrometric titration curve for a dipolar amino acid



Acid–Base Properties of Amino Acids

- The isoelectric points of amino acids can be estimated from their pK_{a1}, pK_{a2}, and pK_{a3} values, using the following expressions:
  - For amino acids with no charged side chain, $pI = (pK_{a1} + pK_{a2})/2$
  - For acidic amino acids, $pI = (pK_{a1} + pK_{a3})/2$
  - For basic amino acids, $pI = (pK_{a2} + pK_{a3})/2$
- The subscripts 1, 2, and 3 refer to α-carboxyl, α-amino, and side chain ionizable groups, respectively.
- In proteins, the α-COOH of one amino acid is covalently coupled to the α-NH_2 of the next amino acid through an amide bondl
  - Thus, the only ionizable groups in proteins are the N-terminus amino group, the C-terminus carboxyl group, and ionizable groups on side chains.

Properties of ionizable groups in free amino acids at 25◦C

- The isoelectric points of amino acids can be estimated from their pKa1, pKa2, and pKa3 values

Amino Acid	pK_{a1} •(—COOH)	pK_{a2} •($—NH_3^+$)	pK_{a3} (Side Chain)	pI
Alanine	2.34	9.69	—	6.00
Arginine	2.17	9.04	12.48	10.76
Asparagine	2.02	8.80	—	5.41
Aspartic acid	1.88	9.60	3.65	2.77
Cysteine	1.96	10.28	8.18	5.07
Glutamine	2.17	9.13	—	5.65
Glutamic acid	2.19	9.67	4.25	3.22
Glycine	2.34	9.60	—	5.98
Histidine	1.82	9.17	6.00	7.59
Isoleucine	2.36	9.68	—	6.02
Leucine	2.30	9.60	—	5.98
Lysine	2.18	8.95	10.53	9.74
Methionine	2.28	9.21	—	5.74
Phenylalanine	1.83	9.13	—	5.48
Proline	1.94	10.60	—	6.30
Serine	2.20	9.15	—	5.68
Threonine	2.21	9.15	—	5.68
Tryptophan	2.38	9.39	—	5.89
Tyrosine	2.20	9.11	10.07	5.66
Valine	2.32	9.62	—	5.96

Acid–Base Properties of Amino Acids

- The significant shift in the p*K*a values in proteins as compared to free amino acids is related to altered electronic and dielectric environments of these groups in proteins.
  - This property is important in enzymes.
- The degree of ionization of a group at any given solution pH can be determined by using the Henderson–Hasselbach equation:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\text{conjugated base}]}{[\text{conjugated acid}]}$$

- The net charge of a protein at a given pH can be estimated by determining the degree of ionization of individual ionizable groups using this equation, and then adding up the total number of negative and positive charges.

Amino Acid	**pKa**
Asp (-COOH $\leftrightarrow$ -COO$^-$ + H$^+$)	3.9
Glu (-COOH $\leftrightarrow$ -COO$^-$ + H$^+$)	4.3
Arg (-NH3$^+$ $\leftrightarrow$ -NH2 + H$^+$)	12.0
Lys (-NH3$^+$ $\leftrightarrow$ -NH2 + H$^+$)	10.5
His (=NH$^+$ $\leftrightarrow$ =N + H$^+$)	6.08
Cys (-SH $\leftrightarrow$ -S$^-$+ H$^+$)	8.28
Tyr (-OH $\leftrightarrow$ -O$^-$+ H$^+$)	10.1

Hydrophobicity of Amino Acids

- One of the major factors affecting physicochemical properties, such as structure, solubility, fat binding

- Hydrophobicity can be defined as the excess free energy of a solute dissolved in water compared to that in an organic solvent under similar conditions.
  - **Hydrophobic Effect:** *Tendency to minimize contact area between polar and non-polar groups*

- Side chains may be polar (hydrophilic) or non-polar (hydrophobic)

- The most direct and simplest way to estimate hydrophobicities of amino acid side chains is experimental determination of free energy changes for dissolution of amino acid side chains in water and in an organic solvent, such as octanol or ethanol.

Optical Properties of Amino Acids

- The aromatic amino acids Trp, Tyr, and Phe absorb light in the near-ultraviolet region (250–300 nm).
  - In addition, Trp and Tyr also exhibit fluorescence in the ultraviolet region.

- These amino acid residues are responsible for ultraviolet absorption properties of proteins in the 250–300 nm range, ***with maximum absorption at about 280 nm*** for most proteins.

- Absorption readings at 280 nm are used for the determination of proteins and peptides.

- Histidine, cysteine and methionine absorb between 200 and 210 nm.

- Both absorption and fluorescence properties of these amino acids are influenced by the polarity of their environment.

  - thus, changes in the optical properties of proteins are often used as a means to monitor conformational changes in proteins.