

FOOD SCIENCE AND TECHNOLOGY

Lecture24

Lecture 24: Proteins and Polypeptides

Hello friends, Namaskar.



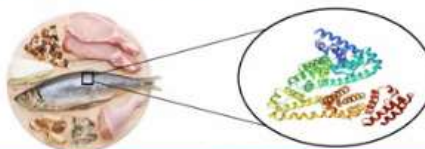
So now, in this lecture 24, we will talk about proteins and polypeptides. We are in module 5, where we are discussing food macronutrients, their structures, and functions. So, in today's lecture, the concepts that will be covered are what proteins are, their nature, and their classification.



Then we will talk about the structure and classification of amino acids, protein structure, and function. Functional properties of proteins like denaturation, gelation, and coagulation.

Proteins

- Proteins are the structurally most complex and functionally sophisticated molecules known.
- They direct virtually all activities of the cell by serving as enzyme catalysts, structural proteins, hormones, transfer proteins, antibodies, storage proteins, etc.
- High molecular weight compounds ($>10,000$ Da).
- Elementally, proteins contain 50–55% carbon, 6–7% hydrogen, 20–23% oxygen, 12–19% nitrogen, and 0.2–3.0% sulfur.



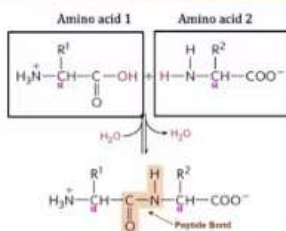
(Bink et al., 1998, www.alamy.com)

Dr. K. S. Narayana

So, what are proteins? They are the most structurally complex and functionally sophisticated molecules ever known. They drive virtually all cell activities by serving as enzyme catalysts. They are structural proteins, acting as hormones and playing roles in the transfer of proteins, antibodies, storage proteins, and many other functions. Almost all cellular functions are carried out by proteins. They are the building blocks of any cell in our body. Proteins are high molecular weight compounds, typically exceeding 10,000 Daltons. Elementally, they consist of approximately 50 to 55% carbon, 6 to 7% hydrogen, 20 to 23% oxygen, 12 to 19% nitrogen, and 0.2 to 0.3% sulfur. This represents the general composition of proteins. Proteins, like polysaccharides, are the building blocks of life, where the building blocks of polysaccharides are monosaccharides. Similarly, amino acids serve as the building blocks for proteins.

Formation of proteins

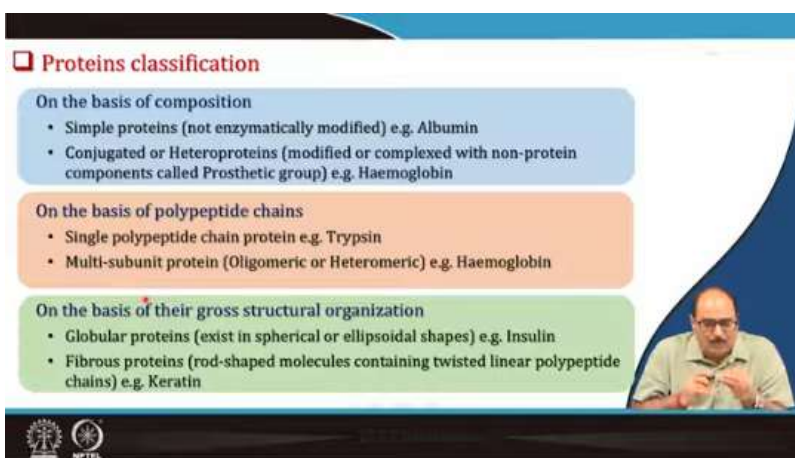
- All proteins are polymer of amino acids joined linearly.
- The shape of a protein is specified by its amino acid sequence and the location of each amino acid in the chain.
- The amino acids are linked together via an amide bond (peptide bond) to form a polypeptide typically made up of 300–500 amino acids.
- Proteins fold into only one or a few closely related three-dimensional shapes—called **Conformations**.



(Lehninger et al., 2017)

All proteins are polymers of amino acids. These amino acids contain a CONH group, an NH_3 group, and a COOH group. When two amino acids combine, the COOH group of one amino acid reacts with the NH_3 group of another. In this process, the OH from the COOH group and the H from the NH_2 group combine to release a water molecule, forming a

CONH linkage. This bond is known as a peptide linkage. When two amino acids join, they form a dipeptide. Similarly, three amino acids form a tripeptide, and four form a tetrapeptide. When an n number of amino acids are joined together, it becomes a polypeptide, which is generally referred to as a peptide linkage. Essentially, all proteins are peptides. The shape of a protein is determined by the nature and sequence of its amino acids, as well as the location and positioning of each amino acid in the peptide chain. Amino acids are linked together by an amide bond, forming a peptide. A polypeptide typically consists of 300 to 500 amino acid residues, which may be present in one peptide chain. These proteins then fold into one or a few types of highly organized three-dimensional structures known as their three-dimensional conformational structures.

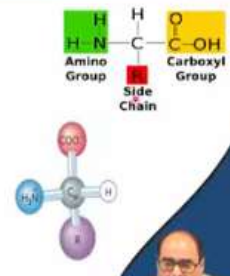


So, proteins can be classified in different ways, one of which is based on their composition. Simple proteins, such as non-enzymatically modified albumin, exist, or they may be conjugated proteins, also called heteroproteins. These proteins may be modified or complexed with non-protein elements known as prosthetic groups. For example, in haemoglobin, the heme is the prosthetic group, and globin is the protein. Proteins can also be classified based on their peptide chains. Some proteins have a single polypeptide chain, like trypsin, while others consist of multiple subunits, such as four or five polypeptides, which are referred to as individual subunits. These subunits may join to form oligomeric or heteromeric proteins, as seen in haemoglobin. Proteins can also be categorized based on their structural organization. For instance, globular proteins have spherical or ellipsoidal shapes, like insulin, while fibrous proteins consist of rod-shaped molecules with twisted linear polypeptides, as in keratin, which is found in hair. Another classification of proteins is based on their nutritional value. Proteins may be categorized as complete, partially complete, or incomplete depending on their essential amino acid content. A complete

protein contains all the essential amino acids required for the proper functioning of the body in adequate amounts.

Amino acids

- Amino acids are the fundamental units of protein
- Natural proteins contain up to 21 different primary amino acids.
- They consist of an α -carbon atom covalently attached to a hydrogen atom, an amino group, a carboxyl group, and a side chain R group. (Proline, a cyclic amino acid, is the exception).
- The α -carbon atom is a chiral center (except in Glycine, the R group is another hydrogen atom).
- All proteins found in nature contain only L-amino acids. However, most of the L-amino acids are Dextrorotatory.



(Lehninger et al., 2017; Lee, 2012)

Now, in an amino acid, the carbon has four vacancies - one occupied by a hydrogen atom, another by an amino group, a third by a carboxyl group, and the fourth by an alkyl or side chain. This combination constitutes an amino acid. These are the fundamental units of proteins, the building blocks of proteins. There are about 21 dietary primary amino acids, each consisting of an alpha carbon. This alpha carbon is bonded to an alpha carboxyl group, an amino group, a hydrogen atom, and an R-side chain. The alpha carbon atom serves as a chiral center, except in glycine. In glycine, the R group is another hydrogen atom. All proteins found in nature contain only L-amino acids. However, most L-amino acids are also dextrorotatory.

Derivatives of amino acids

- Amino acids differ from each other in their R-groups, which vary in structure, size, and electric charge.
- Proteins that contain derived amino acids are called conjugated proteins.

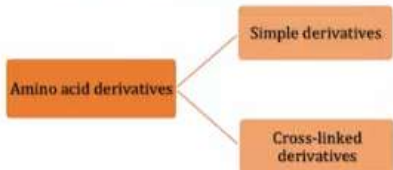
Amino acid derivatives

Simple derivatives

Cross-linked derivatives

e.g. 4-hydroxyproline and 5-hydroxylysine are found in *Collagen*.

e.g. Desmosine, Isodesmosine, and Di- & Tri-tyrosine, are found in *Elastin and Resilin*.



(Lehninger et al., 2017; Lee, 2012)

So, the proteins that are derivatives of amino acids may differ. There are different types of amino acids, and mainly, as seen in the earlier figure, all three components—Carboxyl, H, and NH_2 group will be common. It is only the R group, the side chain, that varies, making

one protein different from another. Depending upon the number and type of side chains, protein amino acids vary in their structure, size, shape, and electrical charge. Proteins that contain derived amino acids are called conjugated proteins, which may include simple derivatives or cross-linked derivatives. Simple derivatives, such as 4-hydroxyproline and 5-hydroxylysine, are found in collagen, while cross-linked derivatives like desmosine or di- and tri-tyrosine are found in elastin and resilin, etc.

□ Nature of amino acids

- Some amino acids have side chains with groups of positive or negative charges, while others have polar but uncharged groups.
- Charged amino acid side chains can form ionic bonds, and polar amino acids are capable of forming hydrogen bonds.
- Amino acids are ampholytes (they contain an acidic carboxyl group and a basic amino group), so behave both as acids and bases.
- 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 diagram illustrates the chemical structures of an amino acid in different states. At the top, the **Nonionic form** is shown as $\text{R}-\text{CH}(\text{NH}_2)\text{COOH}$ and the **Zwitterionic form** as $\text{R}-\text{CH}(\text{NH}_3^+)\text{COO}^-$. Below, the equilibrium between these forms is shown, involving the loss or gain of a proton (H^+) and the pK_{a} of the carboxyl group. Further down, the Zwitterion is shown acting as a **base** (losing a proton to form $\text{R}-\text{CH}(\text{NH}_2)\text{COO}^-$) or as an **acid** (gaining a proton to form $\text{R}-\text{CH}(\text{NH}_3^+)\text{COOH}$), with the pK_{a} of the amino group indicated.

Lehninger et al. 2017

As far as the nature of amino acids is concerned, they can be classified as acidic amino acids or basic amino acids, depending on their specific properties. Particularly, the overall charge on the amino acid depends on the R group present, which determines whether the charge is positive or negative. Amino acids have a side chain, as mentioned earlier, with groups that may carry positive or negative charges, while others are polar, and some are uncharged. Depending on the R group, an amino acid can be classified as neutral, acidic, or alkaline. The charged amino acid side chains can form ionic bonds with polar amino acids and are capable of forming hydrogen bonds. Amino acids are ampholytes, meaning they contain an acidic carboxyl group and a basic amino group, enabling them to behave as both acids and bases. At neutral pH, both the α -amino and α -carboxyl groups are ionized, resulting in the molecule forming a zwitterion. In this form, the COOH group loses a proton and becomes a COO minus ion, while the NH₂ group accepts a proton and becomes NH₃ plus. This dual charge, both positive and negative, is characteristic of a zwitterion.

Nature of amino acids (Contd.)

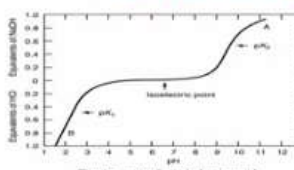
- The pH at which the dipolar ion is electrically neutral is called the isoelectric point (pI).
- When the zwitterion is titrated with an acid, the COO^- group becomes protonated.
- The pH at which $[\text{COO}^-] = [\text{COOH}]$ is known as pK_{a1} .
- Similarly, when titrated with a base, the NH_3^+ group becomes deprotonated.
- As before, the pH at which $[\text{NH}_3^+] = [\text{NH}_2]$ is known as pK_{a2} .

For amino acids with no charged side chain, $\text{pI} = (\text{pK}_{a1} + \text{pK}_{a2})/2$


For acidic amino acids, $\text{pI} = (\text{pK}_{a1} + \text{pK}_{a3})/2$, and

For basic amino acids, $\text{pI} = (\text{pK}_{a2} + \text{pK}_{a3})/2$

The subscripts 1, 2, and 3 refer to α -carboxyl, α -amino, and side chain ionizable groups, respectively.



Titration curve of a typical amino acid




[Srinivasan et al., 2000]

So, the pH at which the dipolar ion is electrically neutral is called its isoelectric pH, as you can see here, is called pI. When the zwitterion is titrated with an acid, the COO^- group becomes protonated. The pH at which the concentration of COO^- groups protonates to form COOH is referred to as pK_{a1} . Conversely, when titrated with a base like NH_3 , the NH_3^+ group becomes deprotonated. The pH at which the NH_3^+ concentration equals the combined concentration of its dissociated forms marks the dissociation point, known as pK_{a2} . For an amino acid without a charged side chain, its isoelectric point (pI) is calculated as the average of pK_{a1} and pK_{a2} : pI is equal to pK_{a1} plus pK_{a2} by 2. For acidic amino acids, the pI is determined as the average of pK_{a1} and the side chain pK_{a3} i.e. pI is equal to pK_{a1} plus pK_{a3} by 2. For basic amino acids, the pI is derived from the average of pK_{a2} , and the side chain pK_{a3} i.e. pI is equal to pK_{a2} plus pK_{a3} by 2. Here, pK_{a1} , pK_{a2} , and pK_{a3} correspond to the dissociation constants of the α -carboxyl, α -amino, and side chain ionizable groups, respectively.

Nature of amino acids (Contd.)

- The pK_a of the ionizable groups in proteins are different from those of free amino acids.
- The only ionizable groups in proteins are the N-terminus amino group, the C-terminus carboxyl group, and ionizable groups on side chains.
- The degree of ionization of a group at any given solution pH can be determined by using the Henderson-Hasselbach equation:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{conjugated base}]}{[\text{conjugated acid}]}$$


[Srinivasan et al., 2000]

The isoelectric point of the pH is an important characteristic and is utilized in the precipitation, fractionation, etc., of proteins. Additionally, the pK_a of the ionizable group

of the protein differs from that of the free amino group. The only ionizable groups of the protein are the N-terminus amino group, the C-terminus carboxyl group, and the ionizable groups of the side chain. So, pH is equal to pKa plus the log of the conjugate base divided by the conjugate acid.

Classification of amino acids

Classification of amino acids based on their side chains

| Group | Classification | Amino acids |
|-------|--------------------------------|--------------------|
| 1 | Acidic and their amides | Asp, Glu, Asn, Gln |
| 2 | Ring containing | Trp, Phe, Tyr, Pro |
| 3 | Non-aromatic hydroxyl R-groups | Ser, Thr |
| 4 | Basic groups | Arg, Lys, His |
| 5 | Sulfur groups | Cys, Met |
| 6 | Aliphatic groups | Ala, Val, Leu, Ile |

Smith & Shah, 2015

So, these amino acids can be classified based on their side chains, like acidic and their amides, such as asparagine, glutamine, aspartic acid, and glutamic acid, then ring-containing amino acids like tryptophan, phenylalanine, tyrosine, proline, etc. which contain an aromatic ring. Non-aromatic hydroxyl groups like serine and threonine, basic groups like arginine, lysine, and histidine, sulfur-containing groups like cysteine and methionine, aliphatic groups like alanine, valine, leucine, and isoleucine. So, these are the names of the amino acids and their classification based on the side chain R, which is the alkyl side chain they have. It may be acidic, non-acidic, basic, sulfur-containing, and so on. It may be a ring, an aromatic ring, or other structures.

Classification of amino acids (Contd...)

- Four main classes, based on the properties of their R groups, particularly their polarity or tendency to interact with water at biological pH (near pH 7.0) are

1. Amino acids with nonpolar side chains

- They are hydrophobic
- The side chains tend to cluster together in the interior of the protein.
- Help give the protein its three-dimensional shape.

(Cooper et al., 2019)

So, there are four main classes based on the properties of their R group. Particularly their polarity or tendency to interact with water at biological pH. Those four groups are number one, that is, amino acids with non-polar side chains, as they are hydrophobic. The side chains tend to cluster together in the interior of the protein, and they help give the protein its three-dimensional shape. They are glycine, alanine, valine, isoleucine, proline, cysteine, methionine, tryptophan, phenylalanine, and so on. These are the non-polar, that is, amino acids with the non-polar side chains.

Classification of amino acids (Contd...)

2. Amino acids with uncharged polar side chains

- These amino acids have zero net charge at physiologic pH.
- Quite soluble in water.
- The polarity is contributed by their hydroxyl groups, amide groups, or rarely by sulfhydryl group (Cysteine).

Polar amino acids

| | | | | |
|----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| $\begin{array}{c} \text{OH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Serine (Ser) S</p> | $\begin{array}{c} \text{CH}_2 \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Threonine (Thr) T</p> | $\begin{array}{c} \text{OH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Tyrosine (Tyr) Y</p> | $\begin{array}{c} \text{O} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Asparagine (Asn) N</p> | $\begin{array}{c} \text{O} \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Glutamine (Gln) Q</p> |
|----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|



(Cooper et al., 2019)

Then amino acids with uncharged polar side chains like serine, threonine, tyrosine, asparagine, etc. These amino acids have zero net charge at physiological pH. They are quite stable and soluble in water and the polarity is contributed by their hydroxyl group, amide groups, or rarely by sulfhydryl groups.

Classification of amino acids (Contd...)

3. Amino acids with acidic side chains

- The side chains of Asp and Glu acids contain a carboxyl group and act as proton donors.
- The fully ionized forms are called aspartate and glutamate.
- These amino acids carry a net negative charge at neutral pH.

4. Amino acids with basic side chains

- The side chains of Arg and Lys contain guanidyl and amino groups. The imidazole group of His is basic in nature.
- Proton acceptors.
- These amino acids carry a net positive charge at neutral pH.

Acidic amino acids

| | |
|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| $\begin{array}{c} \text{COO}^- \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Aspartate (Asp) D</p> | $\begin{array}{c} \text{COO}^- \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Glutamate (Glu) E</p> |
|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|

Basic amino acids

| | | |
|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| $\begin{array}{c} \text{CH}_2 \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Lysine (Lys) K</p> | $\begin{array}{c} \text{CH}_2 \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Arginine (Arg) R</p> | $\begin{array}{c} \text{CH}_2 \\ \\ \text{H}_2\text{N}-\text{C}-\text{COO}^- \\ \\ \text{H} \end{array}$ <p>Histidine (His) H</p> |
|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|

(Cooper et al., 2019)

Then the third category is the amino acids with acidic side chains like those found in aspartic acid and glutamic acid. So, these side chains of aspartic acid and glutamic acid also contain a carboxylic group and act as proton donors. The fully ionized forms are called

aspartate or glutamate. These amino acids carry a net negative charge at neutral pH. The fourth category is amino acids with basic side chains like those in lysine, arginine, and histidine. The side chains of arginine and lysine contain guanidyl and amino groups, and the imidazole group of histidine is basic in nature. They are proton acceptors, and these amino acids carry a net positive charge at neutral pH.

Structure of proteins

- Proteins in any of their functional, folded conformations are called native proteins.
- Protein structures are not static.
- The conformations existing under a given set of conditions are usually the ones that are thermodynamically most stable—that is, having the lowest Gibbs free energy (G).
- The three-dimensional structure of proteins is most frequently analyzed by X-ray crystallography.

Random Polypeptide → Disordered Polypeptide → Native Polypeptide

(Cooper et al., 2019)

Now, let us talk about the structure of protein. This is how the three-dimensional conformation of the structure is. These proteins have very ordered structures. The structures of proteins are not very stable, although each protein has a definite three-dimensional ordered configuration. It changes depending upon various conditions, etcetera.

Structure of proteins (Contd...)

- The structure of proteins can be described at the levels of complexity.
- Four levels of protein structure are defined.

Amino acids → Primary protein structure (sequence of a chain of amino acids)

Pleated sheet → Alpha helix → Secondary protein structure (hydrogen bonding of the protein backbone causes the amino acids to fold into a repeating pattern)

Tertiary protein structure (three-dimensional folding pattern of a protein due to side chain interactions)

Quaternary protein structure (protein consisting of more than one amino acid chain)

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So, now this three-dimensional structure of protein is most frequently analyzed by X-ray crystallography. This is a structure when you talk about their folded functional conformation, etcetera, of the native protein, and it is understood in four levels. The first is the primary structure, which refers to the linear sequence of amino acids. For instance, in a peptide chain with 50 amino acids, identifying the amino acids at positions 35 or 49 is

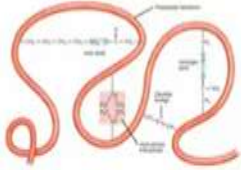
part of the primary structure. This is crucial because even a single change in the position of an amino acid can significantly influence the protein's final characteristics. Next is the secondary structure, where proteins are stabilized in their native conformation through hydrogen bonding in the peptide backbone. This bonding causes the amino acids to fold into repeating patterns, such as pleated sheets or alpha helices. The tertiary structure refers to the protein's three-dimensional conformational folding pattern, which results from side-chain interactions. This is key to the overall functionality of protein. Thus, for a single chain, the primary structure is its linear sequence, while the secondary structure involves the coiling of the amino acids into alpha helices or beta-pleated sheets. This explanation highlights the various levels of protein structure while maintaining their relationship to one another. The tertiary structure involves the folding of the protein, creating its three-dimensional conformational pattern. Following this, the quaternary structure is characterized by the interaction of multiple subunits within an individual peptide chain. For instance, if there are three or four subunits, their interaction determines the overall characteristics of the protein, forming a three-dimensional regular pattern of polypeptide structures. These organized three-dimensional structures, however, are not very stable and may change under varying conditions. This change in the organized structure, while preserving the peptide bonds, is referred to as denaturation of the protein. We will address this concept in more detail later.

Proteins are typically stabilized by their native conformation, which depends on the type of amino acids present. For example, in the coiling, the CONH group interacts with positively charged groups that come into bonding distance with negatively charged groups from other amino acids. There may be amide linkages, hydrostatic interactions, hydrogen bonding, or van der Waals forces. Proteins are held together by both covalent interactions, such as disulfide bonds, and non-covalent interactions, such as hydrophobic interactions, electrostatic forces, hydrogen bonding, and van der Waals interactions. These bonds play



Structure of proteins (Contd.)

Proteins are stabilized in their native conformation by

- Covalent interactions
 - ✓ Disulfide bonding
- Non-covalent interactions
 - ✓ Hydrophobic interactions (Predominates)
 - ✓ Electrostatic interactions
 - ✓ Hydrogen bonding
 - ✓ Van der Waal interactions



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a vital role in maintaining the protein's primary, secondary, tertiary, and quaternary structures. During denaturation, while the peptide bonds remain intact, these structural interactions may be disrupted. During denaturation, the three-dimensional conformation of the protein is altered, but the peptide bond—and thus the primary structure—remains unchanged. Any alteration to the primary structure would result in proteolysis, not denaturation. In denaturation, the integrity of the primary structure is preserved.

Now, let us explore the details of these structures step by step. Starting with the primary structure, as mentioned, it pertains to the nature of the amino acids and their linear sequence

Primary structure Structure of proteins (Contd...)

- It refers to the nature of amino acids and their linear sequence in the polypeptide chain.
- The chain length and the sequence determines the physicochemical, structural, biological properties, and functions of a protein.
- **Important structural implications of primary structure**
 - ✓ It is a resonance structure.
 - ✓ Peptide conformation is defined by three dihedral angles/ torsion angles $\{\phi, \psi, \omega\}$.
 - ✓ Due to the partial double bond character, the rotation of the CO—NH bond (ω -angle) is restricted.
 - ✓ Each six-atom segment of the peptide backbone lies in a single plane.

(Srivastava et al., 2008)

within the polypeptide chain. This sequence is fundamental, as it dictates the protein's overall structure and function. The chain length and sequence of amino acids determine the physical, chemical, structural, biological, and functional properties of the protein. As mentioned, even the precise position of each amino acid in a polypeptide chain—whether it contains 50, 60, or 70 amino acids—is crucial for defining the protein's biological and functional characteristics. The primary structure plays a significant role in structural applications, and its resonance structure is particularly noteworthy. The peptide conformation is characterized by three-dimensional dihedral angles or torsion angles. Due to the partial double-bond character of the CONH bond, the rotation, particularly around the omega angle, is restricted. Moreover, each six-atom segment within the peptide backbone lies in a single plane, contributing to the rigidity and defined geometry of the structure.

Now, the secondary structure describes the local spatial arrangement of its main chain atoms. It occurs when the dihedral angle remains the same or nearly the same throughout the segment. The types of secondary structures are the alpha helix, beta conformations or beta sheets, and sometimes also beta turns.

Structure of proteins (Contd...)

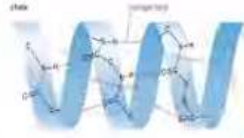
❖ Secondary Structure

- It describes the local spatial arrangement of its main-chain atoms.
- Occurs when each dihedral angle, ϕ and ψ , remains the same or nearly the same throughout the segment.
- The types of secondary structure are the α -helix, β conformations (β -sheet) and β -turns.

▪ α -helix

- ✓ Most common form of secondary structure
- ✓ It forms a rigid, right-handed spiral, while right-handed α -helix is the common form.
- ✓ Each helical turn includes 3.6 amino acid residues (5.4 Å).
- ✓ The form is stabilized by a hydrogen bond.
- ✓ Amino acids that disrupt an α -helix are proline and glycine.

Example- Keratin (fibrous) and Myoglobin (fibrous).



(Cooper et al., 2019)

So, the alpha helix is the most common form of secondary structure, it forms a rigid right-handed spatial arrangement, while the right-handed alpha helix is the common form. Each helical turn, that is here, you see that this comes, and this is called one turn. So, each turn may include 3.6 amino acid residues, and this form is stabilized by hydrogen bonds. There are intensive hydrogen bonds, you can see here, which keep this secondary structure in place. The amino acids that disrupt the alpha helix are proline and glycine, like in the case of creatine and myoglobin. Myoglobin is fibrous, and Keratin is also a fibrous structure.

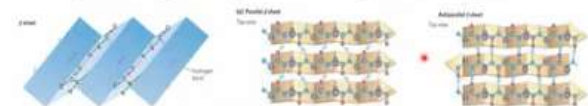
Then, the beta conformation or beta sheet, you can see here in the figure, is a more extended conformation. The beta sheet is formed when two parts of a polypeptide chain lie side by side.

Secondary structure of proteins (Contd...)

▪ β -Conformation/ β -Sheet

- ✓ It is a more extended conformation
- ✓ β -sheet is formed when two parts of a polypeptide chain lie side by side.
- ✓ The backbone of the polypeptide chain is extended into a zigzag structure.
- ✓ Due to the pleated appearance of the overall sheet, also called as β -pleated sheets.
- ✓ Hydrogen bonds form between adjacent/distant segments of polypeptide chain within the sheet.
- ✓ The adjacent polypeptide chains in a β -sheet can be either parallel or antiparallel.

Example, Immunoglobulins, green fluorescent proteins, SH3 domains, etc.



(Lehninger et al., 2017)

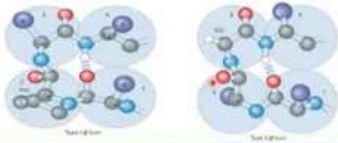
The backbone of the polypeptide chain is extended into a zigzag structure. And due to the pleated appearance of the overall sheet, they are also called the beta-pleated sheet. The hydrogen bonds form between adjacent or distant segments of the polypeptide chain within the sheet and the adjacent polypeptide chains in a beta sheet. They can be either parallel or

anti-parallel, as in the case of immunoglobulins, green fluorescent proteins, SH3 domains, and so on.

Secondary structure of proteins (Contd...)

❖ **β-Turns/ β-Bends/ Reverse turns**

- ✓ Connecting elements that link successive runs of α-helix or β-conformation (commonly antiparallel β-sheet).
- ✓ β-Bends reverse the direction of a polypeptide chain, helping it form a compact, globular shape.
- ✓ The structure is a 180° turn involving four amino acid residues.
- ✓ The amino acid residues Asp, Cys, Asn, Gly, Tyr, and Pro are common in β-bends.
- ✓ Often found near the surface of a protein.



(Lehninger et al., 2017)

NPTEL


Then beta turns, beta bends, reverse turns, etc. You can see here in these figures: it is the type 1 beta turn, type 2 beta turn. And these connecting elements that link successive runs of alpha helix or beta conformation are commonly anti-parallel beta sheets. So, beta bends reverse the direction of a polypeptide chain, helping it form a compact globular shape. The structure is a 180-degree turn involving four amino acid residues. The amino acid residues are aspartate, cysteine, asparagine, glycine, threonine, and proline. These are common in beta bends. Others, which are found near the surface of the proteins, are also there.

Structure of proteins (Contd...)


❖ **Tertiary structure**

- Spatial arrangement is attained when a linear protein chain with secondary structure segments folds further into a compact three-dimensional form.
- The tertiary structures of several single polypeptide proteins are made up of domains (or Mini-proteins of usually 50-200 amino acids).
- Two general classes of proteins based on tertiary structure are fibrous and globular.
- The most important rearrangement during tertiary structure formation involves:
 - ✓ Relocation of most hydrophobic residues at the interior of the protein structure.
 - ✓ Relocation of most hydrophilic residues (especially charged residues) at the protein-water interface.

Fibrous Protein



Globular Protein



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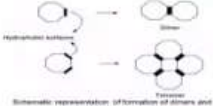
NPTEL

Then, after this, the tertiary structure. Tertiary structure means the folding of the coiled chain. The spatial arrangement is attained when the linear protein chain with secondary structure segments folds further into a compact 3-dimensional conformation as you can see whether it is getting a fibrous structure or a globular structure. In the globular structure, there are interior wide spaces. So, the water can easily penetrate into it. Sometimes, they

are water-soluble; these fibrous structures are very compact. There is no interior space, so the water cannot penetrate into them; they are mostly water-insoluble proteins. Though the tertiary structure of several single polypeptide proteins is made up of domains There are mini proteins of usually 50 to 200 amino acids. Two general classes of proteins based on tertiary structure are the discussed fibrous and globular proteins. The most important rearrangement during tertiary structure formation involves the relocation of the most hydrophobic residues at the interior of the protein structure and the relocation of the most hydrophilic residues that is, especially charged residues at the protein-water interface. So, that is the part and also the part organization of the R group, that is, the polar R groups, that is, the formation of the 3-dimensional structure. If all the polar R groups of the amino acids are extended towards the exterior of the protein molecule, then it will be a water-soluble protein. If these R groups, polar R groups, are extended towards the interior, then it may be a water-insoluble protein and all those things. So, even the protein's characteristics, functionality, and other properties are all regulated by the manner in which the three-dimensional conformation is decided.


❖ Quaternary structure

- It refers to spatial arrangement of a protein when it contains more than one polypeptide chain.
- The quaternary structure exists as dimers, trimers, tetramers, and so forth.
- These quaternary complexes (also referred to as oligomers) can be made up of protein subunits (monomers).
- These monomers can be same (homogeneous) or different (heterogeneous).
- Formation of oligomeric structures is the result of specific protein-protein interactions.



Schematic representation of formation of dimers and oligomers in proteins.


(Srinivasan et al., 2008)





Three-dimensional structure of hemoglobin (PDB ID: 1HHO) showing the four polypeptide chains and heme groups.

(Lehninger et al., 2017)

Structure of proteins (Contd...)



Then comes the quaternary structure. It refers to a spatial arrangement of a protein when it contains more than one polypeptide chain. The quaternary structure can exist as dimers, trimers, tetramers, and so forth. These quaternary complexes, also referred to as oligomers, can be made up of protein subunits that are monomers, like different ones. And these monomers can be the same, like homogeneous, or different, heterogeneous. The formation of oligomer structures is the result of specific protein-protein interactions, and different interactions are there. So, you get a three-dimensional protein conformation, which is the quaternary structure.

Functional properties of proteins

| Techno-functional property | Mode of action | Food system |
|------------------------------|-------------------------------------------------------------|----------------------------------------------------|
| Solubility | Protein solvation | Beverages |
| Water absorption and binding | Hydrogen bonding of water, Entrapment of water (no drip) | Meat, sausages Breads, cakes |
| Viscosity | Thickening, water binding | Soups, gravies |
| Gelation | Protein matrix formation and setting | Meats, curds, cheese |
| Cohesion-adhesion | Protein act as adhesive material | Meats, sausages, baked goods, pasta |
| Elasticity | Hydrophobic binding in gluten, Disulfide links in gels | Meats, bakery |
| Emulsification | Formation and stabilization of fat emulsions | Sausages, bologna, soups, cakes |
| Fat absorption | Binding of free fat | Meats, sausages, doughnuts |
| Flavor-binding | Absorption, entrapment, release | Simulated meats, bakery etc. |
| Foaming | Form stable film to entrap gas | Whipped toppings, chiffon desserts, angel cakes |

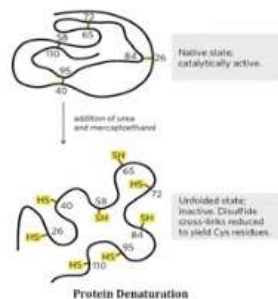
Rural et al., 2013



NITT Tiruchirappalli

So, all these structures, are actually very important as far as the functionality of the protein is concerned. Functional properties of the protein, like solubility, where protein solvation is the major mechanism, are very important in foods like beverages, etc. Similarly, the viscosity or gelation viscosity, the mode of action, is like thickening and water binding. And it is again important in soups and gravy. The gelation involves the formation of the gel in the protein matrix, setting in meats, curd, cheese, etc. So, all these properties, like techno-functional properties, elasticity, emulsification, fat absorption, flavor bonding, foaming, etc. And there is a mode of action. Or food systems where these functional properties are important and that is very important, as provided here in this table. And it is very important; one should accordingly understand how this protein takes its structure, how its structure is changed during processing, etcetera, as it will influence various characteristics.

Denaturation



Protein Denaturation

(Lehninger et al., 2017)

- It is a phenomenon wherein a well-defined native state of protein formed under physiological conditions is transformed into an ill-defined final state under non-physiological conditions using a denaturing agent.
- Under most conditions, denatured proteins exist in a set of partially folded states.
- Denaturation is usually reversible.
- The process in which proteins regain their native structure is called renaturation.

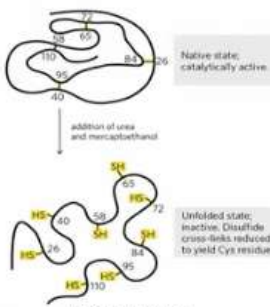


NITT Tiruchirappalli

Then comes denaturation. It is a phenomenon wherein a well-defined native state of protein, formed under physiological conditions, is transformed into an ill-defined final state under non-physiological conditions using a denaturing agent. So, this is the native state,

crystalline and active, and then it is the addition of urea or such other denaturing agents, etcetera. Then you can see here the organized structure is broken. It becomes an open structure, etcetera, or the three-dimensional conformation. Under most conditions, the denatured protein exists as a set of partially folded states; there will be an unfolding of the structure. Denaturation is usually, however, reversible; the process in which proteins regain their native structure is called renaturation. So, denaturation brings about many changes in a protein. Most rapidly available for hydrolysis, denatured protein decreases, and solubility decreases. For example, we discussed that while forming the three-dimensional protein. In the tertiary structure, all the polar R groups are towards the interior. It may happen when denaturation takes place, and then the organized structure changes.

Denaturation (Contd...)



Native state; catalytically active

addition of urea and mercaptoethanol

Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

Denaturation brings about many changes in a protein

- ✓ More readily available for hydrolysis,
- ✓ Decreased solubility,
- ✓ Increased viscosity and optical rotation, and
- ✓ Loses biological properties.

Protein Denaturation
(Lehninger et al., 2017)

NTT Knowledge

Then, maybe most of that is some of the polar R groups that were initially there on the exterior. They may go to the interior, and this may result in a decrease in solubility. There may be increased viscosity and optical rotation, also there may be a loss of biological properties.

Denaturation (Contd...)

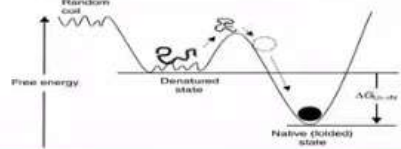
Denaturing Agents

Physical

Chemical

e.g. Heat, Stirring, Shaking, High pressure, and Ultraviolet radiations.

e.g. pH, 6-8M Urea & Guanidine hydrochloride and Synthetic detergents (SDS).



Free energy

Random coil

Denatured state

Native (folded) state

ΔG_{denat}

Schematic representation of the energy of a protein molecule as a function of its conformation.

[Srinivasan et al., 2000]

NTT Knowledge

So, the various denaturing agents may be physical agents like heat, stirring, shaking, high pressure, ultraviolet radiation, etcetera. Chemical agents like pH 6 to 8 molar urea and guanidine hydrochloride and synthetic detergents like sodium dodecyl sulphate, SDS, etcetera are the denaturing agents. Then comes coagulation. Another important property of the protein is when the protein coagulates. You can see that it precipitates in the milk. That is when the milk is converted into curd or cheese, etcetera, the protein precipitates or the protein coagulates.

Coagulation

- Coagulation is defined as the transformation of proteins from a liquid state to a solid form.
- Coagulation often begins around 38°C, and the process is complete between 71°C and 82°C.
- The natural structures of the ingredients are altered irreversibly by a series of physical, chemical, and biochemical interactions.

The diagram illustrates the process in three stages: 1. **Native proteins**: A cluster of small, interconnected spheres representing a folded protein structure. 2. **Denaturation**: The spheres are shown as a loose, disorganized chain, indicating the loss of the native structure. 3. **Coagulation**: The disorganized chains are shown aggregating into a larger, more solid mass, representing the formation of a precipitate or curd.


Source: <https://opencartuaksharam.com/proteins-an-introduction/>

So, coagulation is defined as the transformation of protein from a liquid state to a solid form. Coagulation often begins around 38 degrees, and the process is complete between 71 to 82 degrees Celsius, and the natural structure of the ingredients is altered irreversibly by a series of physical, chemical, and biochemical alterations. As you can see here in the native protein, has an organized three-dimensional structure. In denaturation, these organized structures may change; that is denaturation. And then again, in coagulation, it means that there might be some rearrangement of this, and then it may again get into depending upon the SPI and other changes, etc. It may get precipitated or coagulated, like when you boil an egg or curdle milk, etc.

Coagulation (Contd.)

❖ The main types of protein that cause coagulation

Egg proteins



- The white, or albumen, contains approximately 40 different proteins, ovalbumin (54%) and ovotransferrin (12%).
- The yolk contains mostly lipids (fats), but also lipoproteins.
- The white of an egg coagulates between 60°C and 65°C and the yolk between 62°C and 70°C.
- Which is why a cooked egg can have a fully set white but still runny yolk.

Dr. Khuram


So, the main type of protein that coagulation equals is one good example is egg protein, which is when you heat the egg, and boil the egg. The white of the egg, or albumin, contains approximately 40 different proteins. Ova albumin is 54 per cent, and ovotransferrin is 12 per cent. The yolk contains mostly lipids, which are fat, but it also contains lipoproteins. So, the white of an egg coagulates between 60 to 65 degrees Celsius, whereas the yolk coagulates between 62 and 70 degrees Celsius. This is why a cooked egg can have a fully set white but still have a runny yolk. So, depending upon the temperature, etcetera, to what it has been heated or boiled, okay.

Coagulation (Contd.)

❖ The main types of protein that cause coagulation

Egg proteins

Dairy & soy proteins



- Casein, a semi-solid substance formed by the coagulation of milk.
- Used primarily in cheese.
- Rennet is used to coagulate, or thicken, milk during the cheese-making process.
- Similarly, tofu is made from soybean milk with the use of either salt, acid, or enzyme-based coagulants.


Dr. Khuram

Then also the dairy and soya proteins, the casein, they told you. It is a semi-solid substance formed by the coagulation of milk. It is used primarily in cheese or curd, etc. That is, in cheese preparation, rennet, an enzyme, is used to coagulate the proteins or to thicken the milk during the cheese-making process. Similarly, tofu is made from soybean milk using either salt, acid, enzyme-based coagulation, etc. So, protein is basically precipitated and coagulates, becoming into the solid form.

Coagulation (Contd...)

❖ The main types of protein that cause coagulation

- Egg proteins
- Dairy & soy proteins
- Wheat proteins (Gluten)



- Wheat flour contains two main proteins, glutenin and gliadin, which form gluten when mixed with liquid.
- The coagulation of gluten is what happens when bread bakes.
- It is the firming or hardening of these gluten proteins, usually caused by heat, which solidify to form a firm structure.

Dr. Khuram

Similarly, the wheat protein gluten is formed from two main proteins found in wheat flour: glutenin and gliadin. When water is added to the wheat flour and mechanical force is applied through kneading, these proteins combine to create a coherent, three-dimensional network structure known as gluten. During baking, the coagulation of gluten occurs, which helps in forming the final structure of bread and other baked products in the oven. The gluten coagulates, and it is a soft dough that is converted into thick or thin or soft or little hard bakery products, depending upon the condition of baking and other agents present in the mixture. So, it is the forming or hardening of these gluten proteins, which is usually caused by heat and which solidifies to form a firm structure of the bakery products in breads, cakes, biscuits, cookies, pastries, etc.

❑ Gelation

- Protein gelation refers to the transformation of a protein from the "sol" state to a "gel-like" state.
- Facilitated by heat, enzymes, or divalent cations under appropriate conditions.
- Proteins that form gels have structures with a high degree of asymmetry.
- Examples of gel-forming proteins - gelatin and casein (coagulated by the action of enzyme rennin).

Involves hydrophobic, hydrogen bonds, and electrostatic interactions.

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graph LR
    Sol[Sol] -- Irreversible --> Pro-Gel[Pro-Gel]
    Pro-Gel --> Network[Network Formation]
  
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Dr. Khuram

Another very important property of the protein is gelling or gelation. Protein gelation refers to the transformation of a protein from the solid state to a gel-like state. It is facilitated again by heat, enzymes, or divalent cations under appropriate conditions. Proteins that form gels have structures with a high degree of asymmetry. Examples of gel-forming proteins include gelatin and casein, which are coagulated by the action of the enzyme rennet. So,

this is basically the salt. It is irreversible. It gives pro-gel that involves hydrophobic hydrogen bonds and electrostatic interactions. The network formation takes place, and protein is actually converted into a gel-like substance; there is a difference between the gel and a coagulant, etc. So, that is the gel.

Gelation (Contd.)

- Proteins form two types of gels, that is, coagulum (opaque) gels and translucent gels.
- The type of gel formed depends upon its molecular properties and solution conditions.
- Limited proteolysis can help in the formation of protein gels e.g. cheese.
- Enzymatic cross-linking at room temperature can result in the formation of a gel network.
- Divalent cations, such as Ca^{2+} and Mg^{2+} can also be used to form protein gels. e.g. Tofu from soy proteins.

PN is a native state, PD is an unfolded state, and n is the number of protein molecules taking part in cross-linking.

[Grierson et al., 2008]

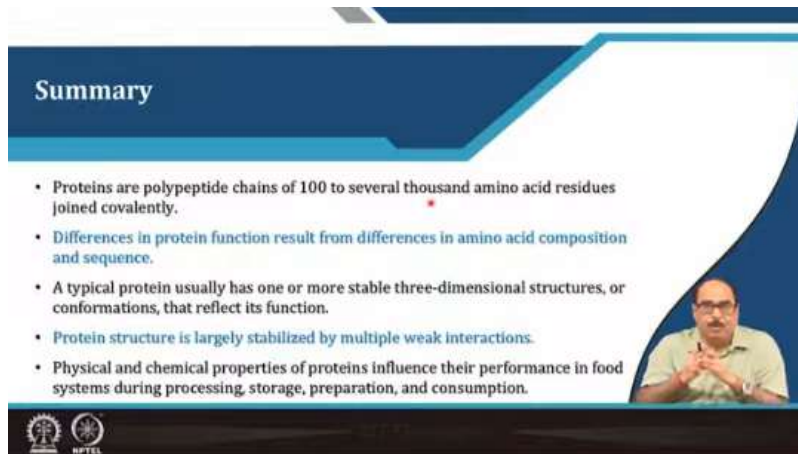
So, the proteins basically form two types of gel: that is coagulum, which is an opaque gel, and a translucent gel. So, the type of gel formed depends upon its molecular properties and the cooling conditions. Limited proteolysis can help in the formation of protein gels, like in the case of cheese, etc. Enzymatic cross-linking at room temperature can result in the formation of a gel network and divalent cations such as calcium ions or magnesium ions can also be used to form protein gels, like tofu from soy protein or even if calcium, etc., is present, it gives a firm gel, etc., if there are calcium ions or calcium caseinate, etc., as it is there. So, it is shown here that nPN is the protein in its native state when it is given heat, then it becomes nPD, meaning denatured protein in an unfolded state, and it is the number of protein molecules taking part. Then it may be an aggregation coagulation type gel or, if you cool it, it becomes a translucent gel, different types of gels.

❖ **Factors affecting gelation**

- Number and types of cross-links formed per monomer chain.
- Intrinsic (such as the size, net charge, etc.) and extrinsic factors (such as pH, temperature, ionic strength, etc.).
- Protein concentration.
- Several environmental factors, such as pH, salts, and other additives.

www.citgrierson.com

So, basically, the factors which affect gelation include the number of cross-linked polymers per monomer chain. The intrinsic factors such as size, net charge, etc., as well as extrinsic factors like pH, temperature, and ionic strength, all influence gelation. Gelation, gelling property, okay, and even the protein concentration, like what is the case with soy proteins, which form better gels at 12% concentration also. So, several environmental factors like pH, salt, and other additives, all will influence the gelling behaviour of the protein.



Summary

- Proteins are polypeptide chains of 100 to several thousand amino acid residues joined covalently.
- Differences in protein function result from differences in amino acid composition and sequence.
- A typical protein usually has one or more stable three-dimensional structures, or conformations, that reflect its function.
- Protein structure is largely stabilized by multiple weak interactions.
- Physical and chemical properties of proteins influence their performance in food systems during processing, storage, preparation, and consumption.

In summary, proteins are polypeptide chains consisting of 100 to several thousand amino acid residues covalently linked. Their functions vary based on differences in amino acid composition and sequence. Typically, a protein adopts one or more stable three-dimensional structures or conformations, which determine its functionality and properties. This functionality is largely derived from the organized structure of the protein. However, during processing or handling, factors such as heat, acid, physical and chemical influences, or radiation can alter this organized structure, a process known as denaturation. The protein structure is largely stabilized by multiple weak interactions. Properties such as gelation, denaturation, renaturation, and coagulation play a significant role in determining the functionality, characteristics, and nutritional value of proteins in food. Among the various amino acids present in proteins, there are about 8 to 10 essential amino acids that our bodies cannot synthesize. Hence, it is crucial to obtain these essential amino acids through dietary protein. Consuming a balanced form of protein ensures that we receive all the essential amino acids in the appropriate amounts for optimal health.

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These are the references that were used in this lecture.

So, with this, thank you very much.

Thank you for your patient hearing.