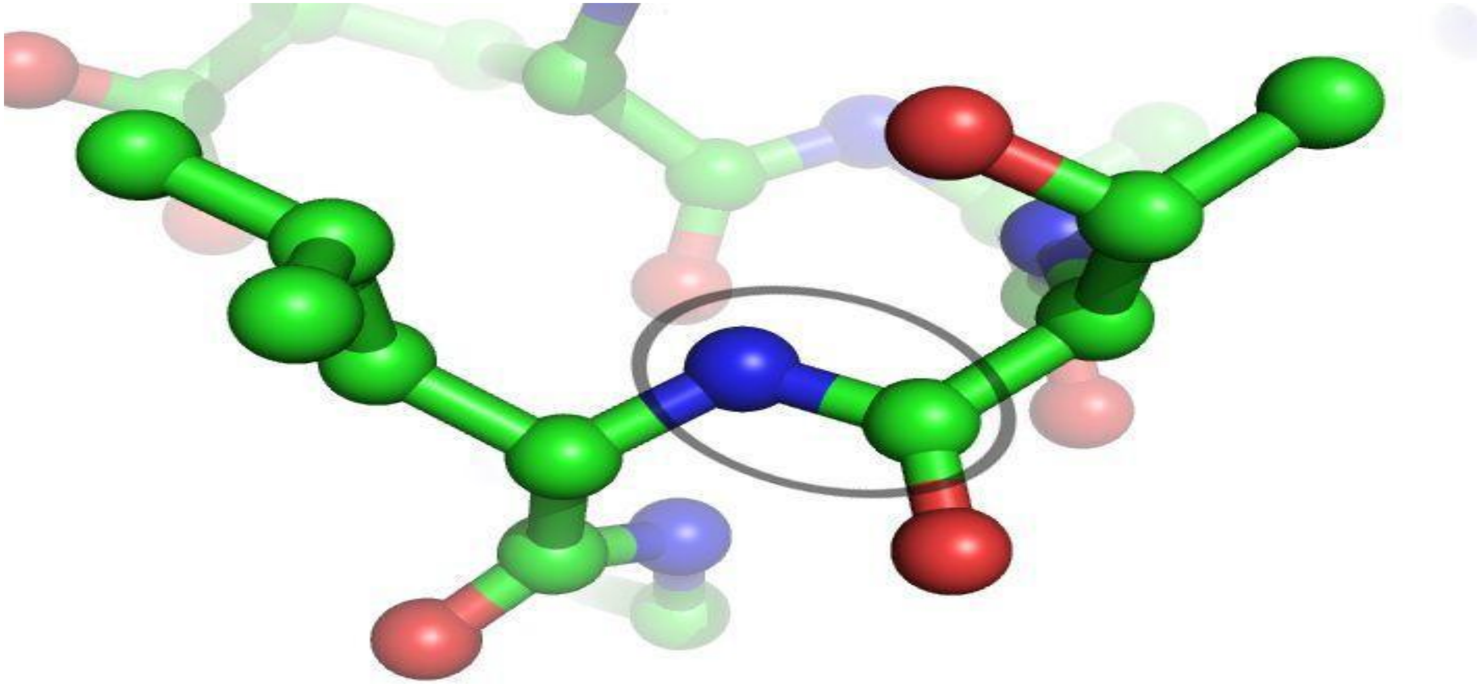
Peptides



Dr. Amel Taha Yaseen

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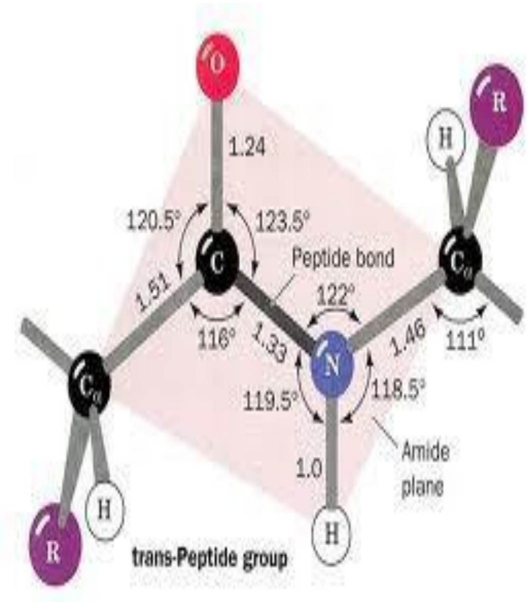


-Peptides (from the Greek word means "digested") are short polymers of amino acid (monomers) linked by peptide bonds, the covalent chemical bonds formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule.

-Peptides are distinguished from proteins on the basis of size, typically containing fewer than 50 monomer (AA) units.

-The shortest peptides are dipeptides, consisting of two amino acids joined by a single peptide bond. There are also tripeptides, tetrpeptides, etc.

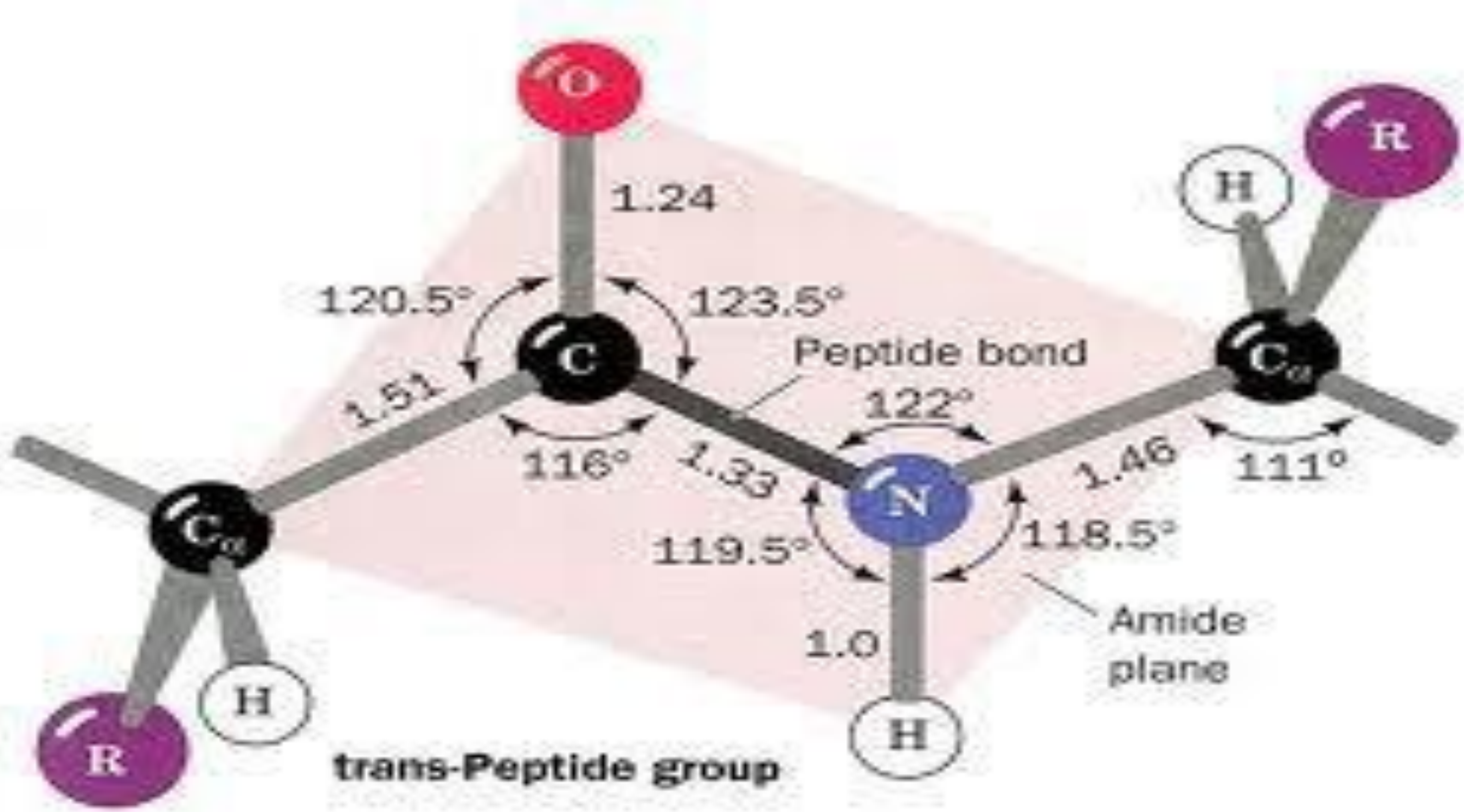
-Amino acids which have been incorporated into a peptide are termed "residues"; every peptide has a N-terminus and C-terminus residue on the other ends of the peptide.

-A polypeptide is a long, continuous, and unbranched peptide.

-Proteins consist of one or more polypeptides arranged in a biologically functional way and are often bound to cofactors, or other proteins.

-Long peptides such as amyloid beta can be considered proteins, whereas small proteins such as insulin can be considered peptides.

Peptide Bond



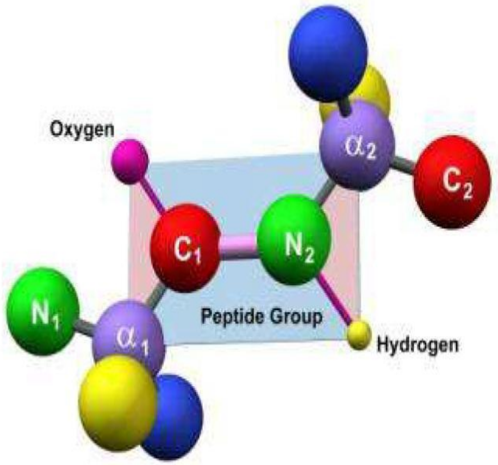
Peptide Bond Formation

-Amino acids are linked together by condensation reaction between carboxylic and amino groups from two different amino acids (with elimination of water).

-The amide bond formed is called peptide bond.

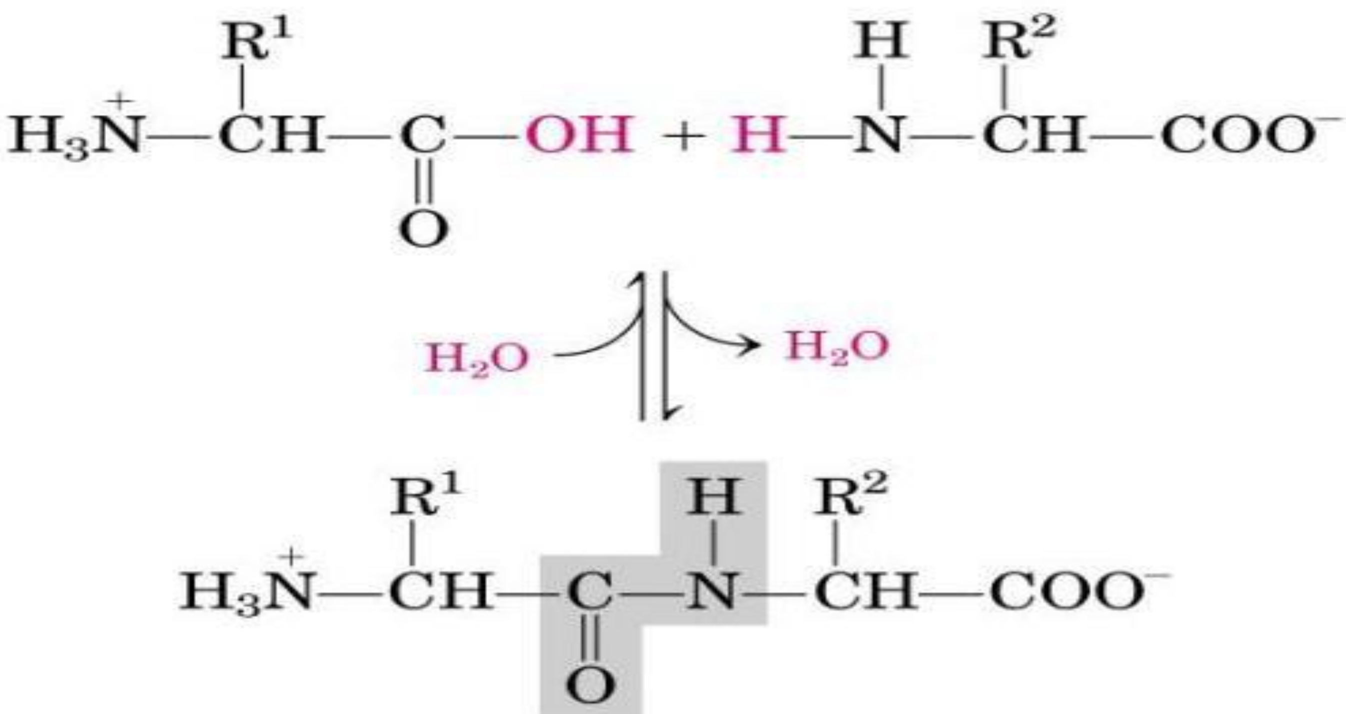
-The product is called a peptide, and named according to the number of amino acids involved: e.g. dipeptide (2), tripeptide (3), decapeptide (10).

-Big peptides (> 50 amino acids) are called polypeptides.



Peptide bonds

-Peptide bonds are formed by a condensation reaction of carboxylic group of an amino acid and amino group of another amino acid with removal of water molecule.



Characteristics of Peptide Bonds

- Peptide bonds are strong with partial double bond character:
  - They are not broken by usual denaturing agents like heating or high salt concentration.
  - They can be broken by:
    - Prolonged exposure to strong acid or base at elevated temperatures.
    - Specific enzymes such as digestive enzymes.
- Peptide bonds are rigid and planner resisting free rotation, therefore they stabilize protein structure

Characteristics of Peptide Bonds

- **Peptide bonds are uncharged but polar:**
  - **Peptide bonds contain polar hydrogen atoms of amino groups (with a partial positive charge) and polar oxygen atoms of carboxyl groups (with a partial negative charge).**
  - **This allows hydrogen bonds to form between peptide bonds in different parts of the chain.**

-Peptides are formed by binding amino acids together through an amide linkage. On the other hand, peptide hydrolysis results in free amino acids.

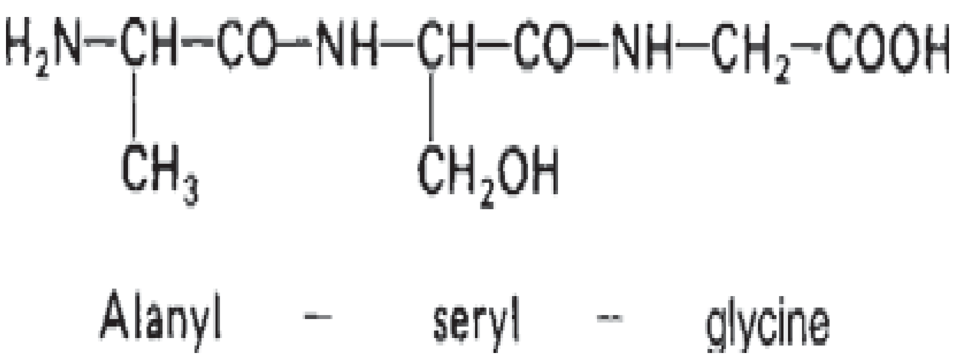
-Functional groups not involved in the peptide synthesis reaction should be blocked. The protecting or blocking groups must be removed after synthesis under conditions which retain the stability of the newly formed peptide bonds.

of amino acid by the number denoted are -Peptides and the term tetrapeptides di-, tri-, as residues "oligopeptides" is used for those with 10 or less amino acid residues.

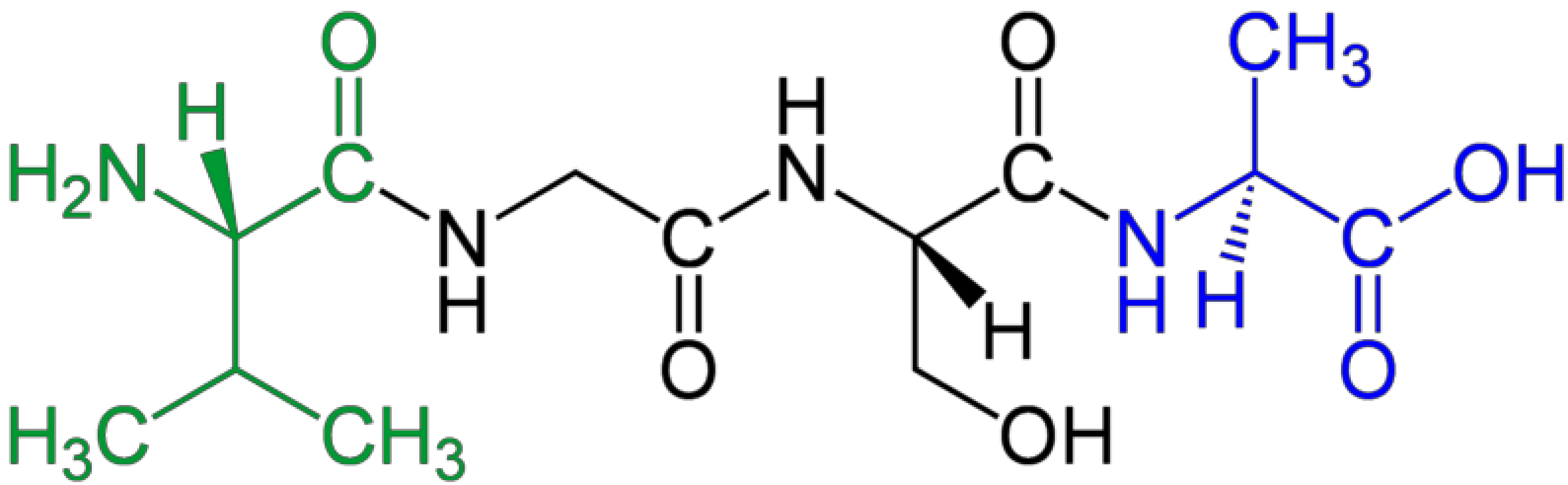
-Higher molecular weight peptides are called polypeptides.

-The transition of "polypeptide" to "protein" is rather undefined, but the limit is commonly assumed to be at a molecular weight of about 10 kdal, i.e., about 100 amino acid residues are needed in the chain for it to be called a protein.

-The first three letters of the amino acids are used as symbols to simplify designation of peptides. Thus, the peptide shown can also be given as:



Ala Ser Gly or ASG

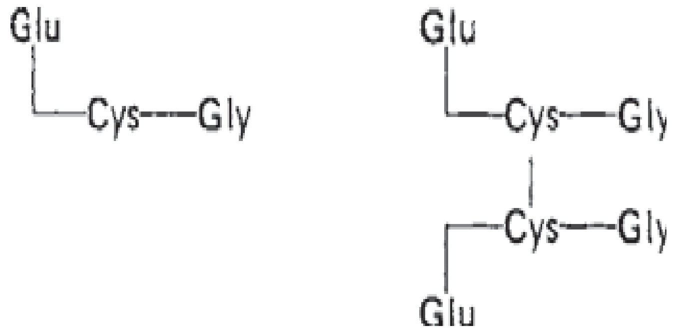


A tetrapeptide (example Val-Gly-Ser-Ala) with green marked amino end (L-Valine) and blue marked carboxyl end (L-Alanine).

-One-letter symbols are used for amino acid sequences of long peptide chains.

-In compounds in which a functional group of the side chain is involved, the bond is indicated by a perpendicular line.

-The tripeptide glutathione (glutamyl-cysteinyl-glycine) is given as an illustration along with its corresponding disulfide, oxidized glutathione.



-The amino acid residue with the free amino group is always placed on the left. The amino acids of the chain ends are denoted as N-terminal and C-terminal amino acid residues.

-The peptide linkage direction in cyclic peptides is indicated by an arrow, i.e., CO → NH-

Peptide classes

-Peptides are divided into several classes, depending on how they are produced:

1- Milk peptides

Milk peptides are formed from milk proteins by enzymatic breakdown by digestive enzymes or by the proteinases formed by *lactobacilli* during the fermentation of milk.

2-Ribosomal peptides

-Ribosomal peptides are synthesized by translation of mRNA. They are often subjected to proteolysis to generate the mature form. These function, typically in higher organisms, as hormones and signaling molecules.

-Some organisms produce peptides as antibiotics, such as microcins. Since they are translated, the amino acid residues involved are restricted to those utilized by the ribosome. However, these peptides frequently have post-translational modifications, such as hydroxylation, sulfonation, and disulfide formation.

Peptide classes

3- Nonribosomal peptides

-These peptides are assembled by enzymes that are specific to each peptide, rather than by the ribosome.

-The most common non-ribosomal peptide is glutathione, which is a component of the antioxidant defenses of most aerobic organisms.

-Other non-ribosomal peptides are most common in plants, and fungi and are synthesized by enzyme complexes called *nonribosomal peptide synthetases*.

-These peptides are often cyclic and can have highly-complex cyclic structures, although linear non-ribosomal peptides are also common.

Peptide classes

4-Peptones

-Peptones are derived from animal milk or meat digested by proteolytic digestion.

-In addition to containing small peptides, the resulting spray-dried material includes fats, metals, salts, vitamins and many other biological compounds.

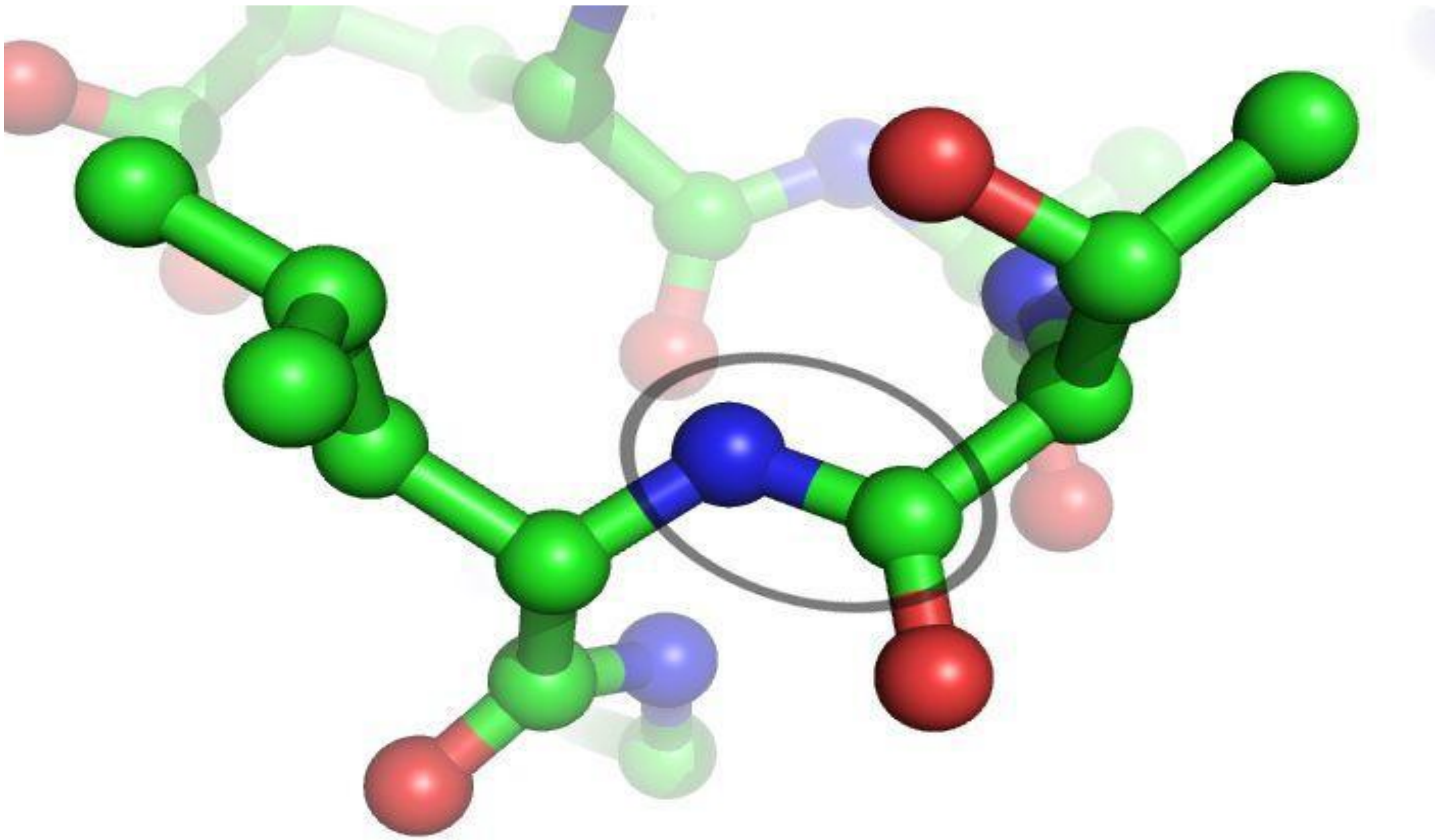
-Peptone is used in nutrient media for growing bacteria and fungi.

5-Peptide fragments

-Peptide fragments refer to fragments of proteins that are used to identify or quantify the source protein.

-Often these are the products of enzymatic degradation performed in the laboratory on a controlled sample, but can also be samples that have been degraded by natural effects.

Physical Properties of Peptides



1- Dissociation

-The isoelectric points (pI) for some peptides are listed in the table.

-The acidity of the free carboxyl groups and the basicity of the free amino groups are lower in peptides than in the corresponding free amino acids.

-The amino acid sequence also has an influence (e. g., Gly- Asp/Asp-Gly).

Peptide	pI
Gly-Gly	5.65
Gly-Gly-Gly	5.59
Ala-Ala	5.72
Gly-Asp	3.63
Asp-Gly	3.31
Asp-Asp	3.04
Lys-Ala	9.16
Ala-Lys-Ala	8.98
Lys-Lys	10.53
Lys-Lys-Lys	10.93
Lys-Glu	6.10
His-His	7.30

2- Sensory Properties

Taste threshold values of various peptides (tested in aqueous solution at pH 6–7);

bi -bitter

Peptide[a]	Taste	
	Quality	Intensity[b]
Gly-Leu	bi	19–23
Gly-D-Leu	bi	20–23
Gly-Phe	bi	15–17
Gly-D-Phe	bi	15–17
Leu-Leu	bi	4–5
Leu-D-Leu	bi	5–6
D-Leu-D-Leu	bi	5–6
Ala-Leu	bi	18–22
Leu-Ala	bi	18–21
Gly-Leu	bi	19–23
Leu-Gly	bi	18–21
Ala-Val	bi	60–80
Val-Ala	bi	65–75
Phe-Gly	bi	16–18
Gly-Phe	bi	15–17
Phe-Gly-Phe-Gly	bi	1.0–1.5
Phe-Gly-Gly-Phe	bi	1.0–1.5

[a] L-Configuration if not otherwise designated.
[b] Recognition threshold value in mmol/l .

2- Sensory Properties

-While the taste quality of amino acids does depend on configuration, peptides, except for the sweet dipeptide esters of aspartic acid, are neutral or bitter in taste with no relationship to configuration.

-Bitter tasting peptides can occur in food after proteolytic reactions. For example, the bitter taste of cheese is a consequence of ripening.

-Therefore, the wide use of proteolytic enzymes to achieve well-defined modifications of food proteins, without producing a bitter taste, causes some problems.

2- Sensory Properties

Bitter taste of dipeptide A–B: dependence of recognition threshold value (mmol/l) on side chain hydrophobicity (0: sweet or neutral taste)

A/B	Asp	Glu	Asn	Gln	Ser	Thr	Gly	Ala	Lys	Pro	Val	Leu	Ile	Phe	Tyr	Trp	
	0	0	0	0	0	0	0	0	85	26	21	12	11	6	5	5	
Gly	0[a]	–	–	–	–	–	–	0	0	–	45	75	21	20	16	17	13
Ala	0	–	–	–	–	–	–	0	0	–	–	70	20	–	–	–	–
Pro	26	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–
Val	21	–	–	–	–	–	–	65	70	–	–	20	10	–	–	–	–
Leu	12	–	–	–	–	–	–	20	20	–	–	–	4.5	–	–	3.5	0.4
Ile	11	43	43	33	33	33	33	21	21	23	4	9	5.5	5.5	–	–	0.9
Phe	6	–	–	–	–	–	–	17	–	–	2	–	1.4	–	0.8	0.8	–
Tyr	5	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–
Trp	5	–	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Threshold of the amino acid (cf. Table 1.12).

-As with amino acids, the taste intensity is influenced by the hydrophobicity of the side chains.

-The taste intensity does not appear to be dependent on amino acid sequence.

2- Sensory Properties

Peptides with a salty taste

Peptide[a]	Taste	
	Threshold (mmol/l)	Quality[b]
Orn-βAla.HCl	1.25	3
Orn-γAbu.HCl	1.40	3
Orn-Tau.HCl	3.68	4
Lys-Tau.HCl	5.18	4
NaCl	3.12	3

[a] Abbreviations: Orn, ornithine; β-Ala, β-alanine, γ-Abu, γ-aminobutyric acid; Tau, taurine.
[b] The quality of the salty taste was evaluated by rating it from 0 to 5 on a scale in comparison with a 6.4 mmol/L NaCl solution (rated 3); 4 is slightly better, 5 clearly better than the control solution.

-Some peptides exhibit a salty taste, e.g. ornithyl-β-alanine hydrochloride and may be used as substitutes for sodium chloride.

Sensory Properties

Effect of HCl on the salty taste of Orn-(3-Aia

Equivalents HCl	pH	Taste	
		saltyb	so11.1r
0	8.9	0	
0.79	7.0	0	
0.97	6.0	1	
1.00	5.5	2	
Jl.lO	4.7	3	+/–
1.20	4.3	3.5	+
1.30	4.2	3	++

a Peptide solution: 30 n1n'l.ol/L.

b Tbe values 13 and 5 co:r1Fespond in intensity to 0.5o/o 0.25o/o and 0.1 acm somut ons respectively.

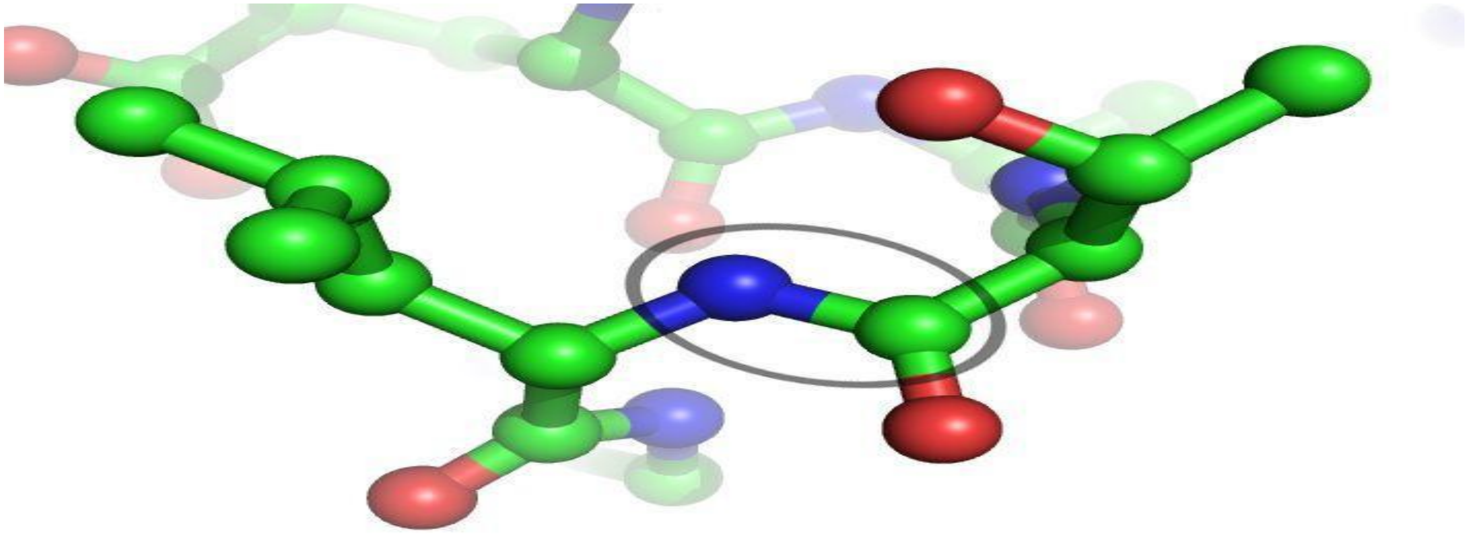
c Very weak (+) and slightly soul" (++).

-The intensity of the salty taste of Orn-P-Aia depends on the pH.

Individual Peptides

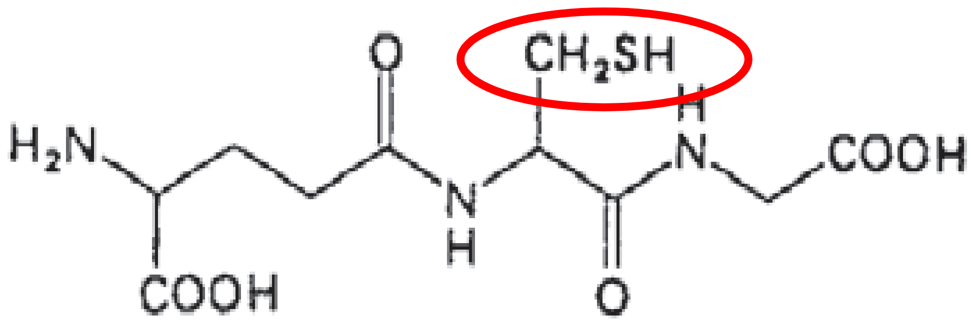
Peptides are widespread in nature.

They are often involved in specific biological activities (peptide hormones, peptide toxins, peptide antibiotics).



1-Glutathione

-Glutathione (γ-glutamyl-L-cysteinyl-glycine) is widespread in animals, plants and microorganisms.



-Beef (200), broccoli (140), spinach (120), chicken (95), potatoes (71), paprika (49), tomatoes (49) and oranges (40) are especially rich in glutathione (mg/kg).

-A noteworthy feature is the binding of glutamic acid through its carboxyl group.

-The peptide is the coenzyme of glyoxalase.

-It is involved in active transport of amino acids and, due to its ready oxidation, is also involved in many redox-type reactions.

-It influences the rheological properties of wheat flour dough through thiol-disulfide interchange with wheat gluten.

-High concentrations of reduced glutathione in flour bring about reduction of protein disulfide bonds and a corresponding decrease in molecular weight of some of the protein constituents of dough gluten.

The tripeptide glutathione (*γ*-glutamyl-L-cysteinyl glycine) contains an unusual *γ*-amide bond. (Note that the *γ*-carboxyl group of the glutamic acid residue, not the *α*-carboxyl group, contributes to the peptide bond.) Found in almost all organ- isms, glutathione (GSH) is involved in protein and DNA synthesis, drug and envi- ronmental toxin metabolism, amino acid transport, and other important biological processes. One group of glutathione's functions exploits its effectiveness as a reducing agent. Glutathione protects cells from the destructive effects of oxida- tion by reacting with substances such as peroxides (R–O–O–R), by-products of O2 metabolism. For example, in red blood cells,

hydrogen peroxide (H_2O_2) oxidizes the iron of hemoglobin to its ferric form (Fe^{3+}). Methemoglobin, the product of this reaction, is incapable of binding O_2. Glutathione protects against the formation of methemoglobin by reducing H_2O_2 in a reaction catalyzed by the enzyme glutathione peroxidase. In the oxidized product GSSG, two tripep- tides are linked by a disulfide bond:

$$2\,GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

2-Vasopressin

also called antidiuretic hormone, contains nine amino acid residues. It is synthesized in the hypothalamus, a small structure in the brain that regulates a wide variety of functions including water balance, appetite, body temperature, and sleep. In response to low blood pressure or a high blood Na^+ concentration, osmoreceptors in the hypothalamus trigger vasopressin secretion. Vasopressin stimulates water reabsorption in the kidneys by initiating a signal transduction mechanism that inserts aquaporins (water channels) into kidney tubule membrane. Blood pressure rises as water then flows down its concentration gradient through the tubule

cells and back into the blood. The structure of vasopressin is remark- ably similar to that of another peptide produced in the hypothalamus called oxytocin .

2. Oxytocin

the signal molecule that stimulates the ejection of milk by mammary glands during lactation. Oxytocin produced in the uterus stimulates the contraction of uterine muscle during childbirth. Because vasopressin and oxytocin have simi- lar structures, it is not surprising that the functions of the two molecules over- lap, has mild antidiuretic activity and vasopressin has some oxytocin-like activity.

4. Atrial natriuretic factor (ANF)

Atrial natriuretic factor (ANF), a peptide produced by specialized cells in the heart in response to stretching and in the nervous system, stim- ulates the production of a dilute urine, an effect opposite to that of vasopressin. ANF exerts its effect, in part, by increasing the excretion of Na+, a process that causes increased excretion of water, and by inhibiting the secretion of renin by the kidney. (Renin is an enzyme that catalyzes the formation of angiotensin, a hor- mone that constricts blood vessels).

Antioxidant peptides

-Antioxidant properties that prevent enzymatic (lipoxygenase) and non-enzymatic peroxidation of essential fatty acids have been found in peptides derived from milk proteins.

-Most of the peptides identified are encoded in the sequence of α-casein.

-The addition of a leucine or proline residue to the Nterminus of a His-His dipeptide, for example, can enhance antioxidant activity and facilitate further synergy with non-peptide antioxidants like BHT or BHA (synthetic antioxidants).

Food Applications of Bioactive Peptides

-A large number of the bioactive peptides found naturally in traditional foods that have been consumed long before the term 'bioactive' was established.

-Many of these peptides are released from the host proteins by fermentation of milk, including cheese ripening.

-Many peptides are generated by enzymatic reactions in the gut after ingestion of foods containing precursor proteins (e.g. after drinking a glass of milk).

Food Applications

-Bioactive peptides are fundamental constituents of many products or ingredients marketed as 'Functional Foods' or 'Nutraceuticals'.

-In these products the bioactive peptides are either added or enriched by modification of the usual manufacturing process (e.g. by changing process parameters or starter cultures used).

-Some of these products, however, are traditional foods now offered with a different marketing strategy.

-The following Table lists some examples of commercially available functional foods and food ingredients that carry bioactive peptides and includes the health claim connected with the respective product.

Examples of commercially available functional foods or food ingredients carrying bioactive peptides.

Product name	Manufacturer	Type of food	Bioactive peptides	Health claim
Calpis AMEEL S (Japan) or Calpico (Europe)	Calpis Co., Japan	Sour milk	VPP, IPP from- and K-CN	Hypotensive
Evolus	Valia, Finland	Fermented milk, calcium-enriched	VPP, IPP from- and K-CN	
BioZate	Davisco, USA	-LG hydrolysate	Whey peptides	
C12 Peption	DMV, Netherlands	Ingredient	Casein-derived dodecapeptide FFVAPFPEVFGK	
Peptide Soup	NIPPON, Japan	Soup	Bonito-derived peptides	
Casen DP Peptic Drnk	Kanebo, Japan	Soft drink	Casein-derived dodecapeptide FFVAPFPEVFGK	
BioPURE-GMP	Davisco, USA	Whey protein hydrolysate	Glycomacropeptide	Anticariogenic, antimicrobial, antithrombotic
CholesteBiock	Kyowa Hakko, Japan	Drink powder	SC!f peptides bound to phospholipids	Hypocholesterolemic
CSPHP				
ProDiet F200	Ingredia, France	Milk drink, confectionery	as$_1$-CN (191–100)[8] YLGYIEQIIR	Reduces stress
Capolac	Arla Foods, Denmark	Ingredient	CPP	HeiJ:E mneral absorption
Tekkotsu Inryou	Suntory, Japan	Soft drink	CPP Kotsu	
Kotsu calcium	Asahi, Japan	Soft drink	CPP	
CE90CPP	DMV, Netherlands	Ingredient	CPP (20%)	
Glutamn peptide	DMV, Netherlands	Dry mik protein hydrolysate	Glutamne-rich peptides	Immunomodulatory
WGESOGPA				
WGESOGPN				
WGESOGPU				

Peptides in molecular biology

-Peptides have recently received importance in molecular biology for several reasons.

-The first is that peptides allow the creation of *peptide antibodies* in animals without the need to purify the protein of interest. This involves synthesizing antigenic peptides of sections of the protein of interest. These will then be used to make antibodies in a rabbit or mouse against the protein.

-Another reason is that peptides have become instrumental in mass spectrometry, allowing the identification of proteins of interest based on peptide masses and sequence. In this case, the peptides are most often generated by in-gel digestion after electrophoric separation of the proteins.

Peptides in molecular biology

-Peptides have recently been used in the study of protein structure and function.

-For example, synthetic peptides can be used as probes to see where protein-peptide interactions occur.

-Inhibitory peptides are also used in clinical research to examine the effects of peptides on the inhibition of cancer proteins and other diseases.