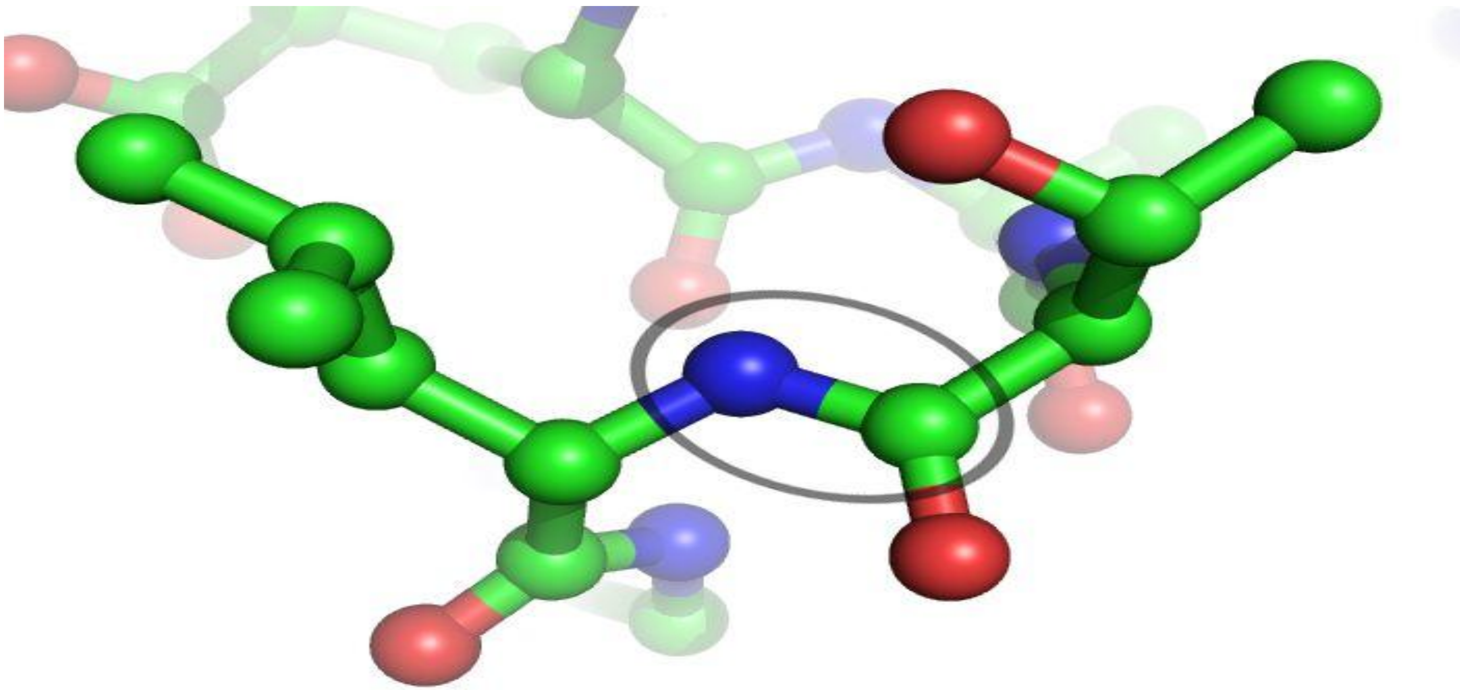
Peptides



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-Introduction to peptides

-Peptide Bond

-Peptide Bond Formation

-Characteristics of Peptide Bonds

-Peptide classes

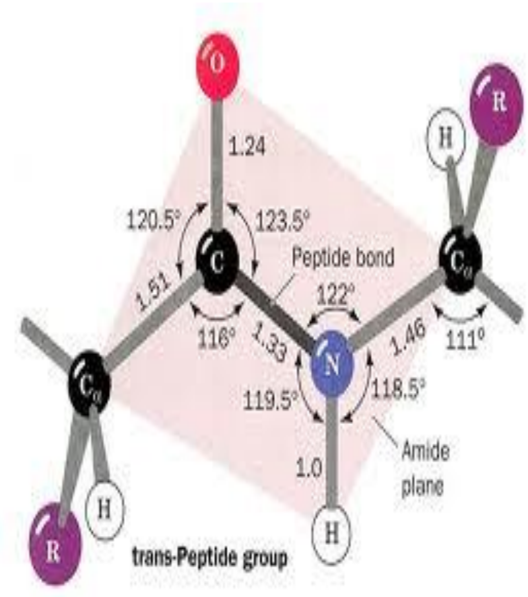
-Physical Properties of Peptides

-Individual Peptides

-Food-derived peptides with biological activity

-Food Applications of bioactive peptides

-Peptides in molecular biology



-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.

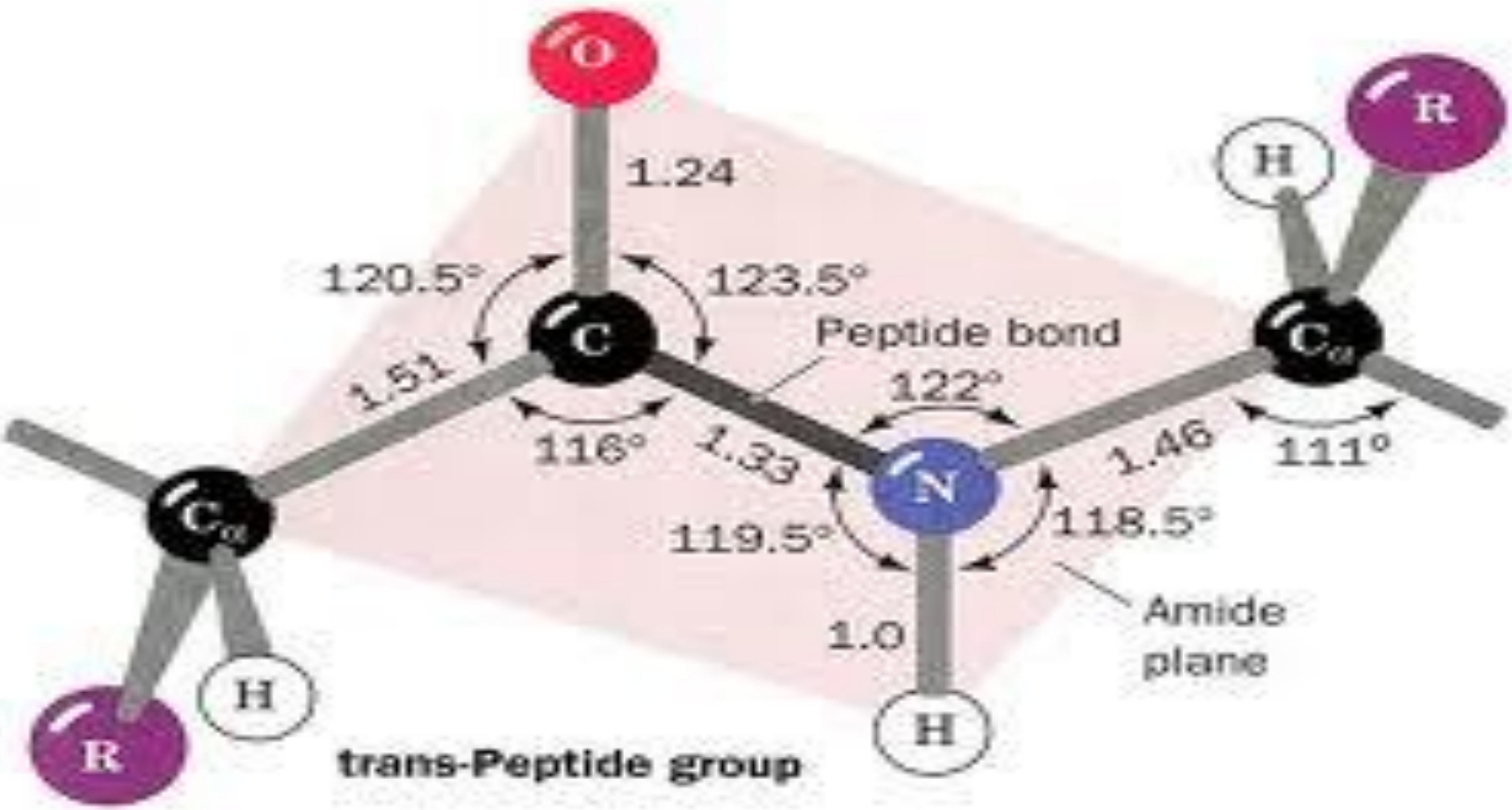
-Amino acids which have been incorporated into a peptide are termed "residues"; every peptide has a N-terminus and C-terminus residue on the ends of the peptide.

-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



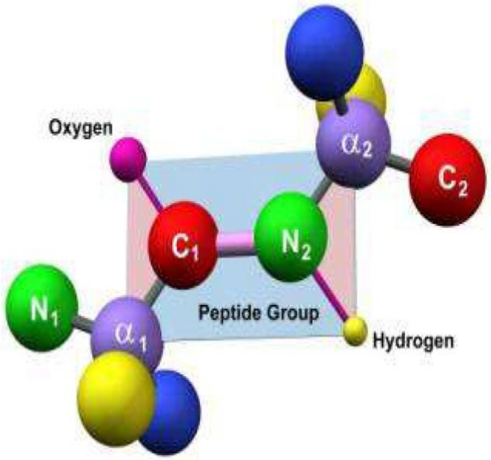
Peptide Bond Formation

-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.

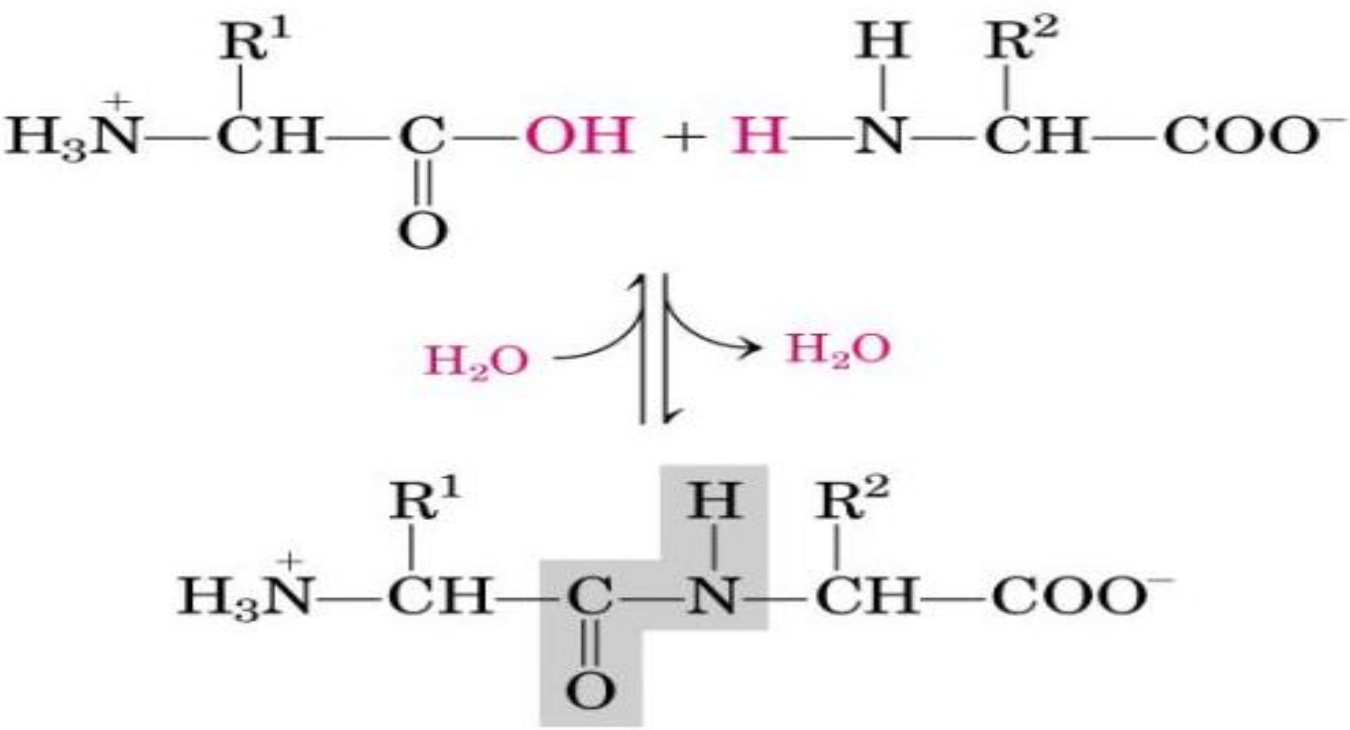
-The product is called a peptide, and named according to the number of amino acids involved: e.g. dipeptide (2), tripeptide (3), decapeptide (10).

-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.

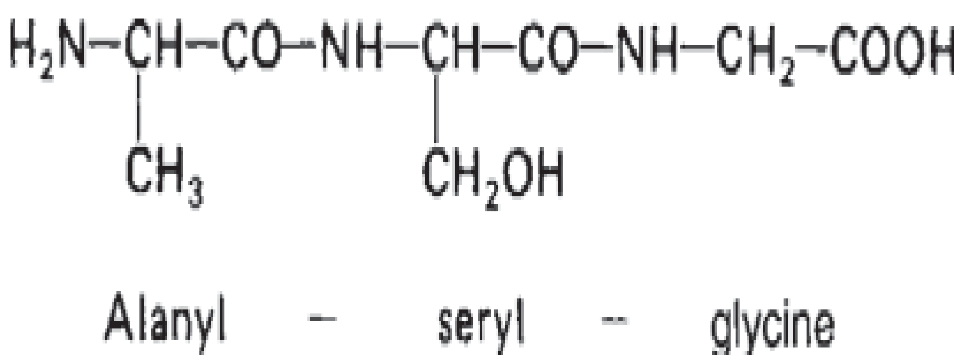
-Functional groups not involved in the peptide synthesis reaction should be blocked. The protecting or blocking groups must be removed after synthesis under conditions which retain the stability of the newly formed peptide bonds.

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

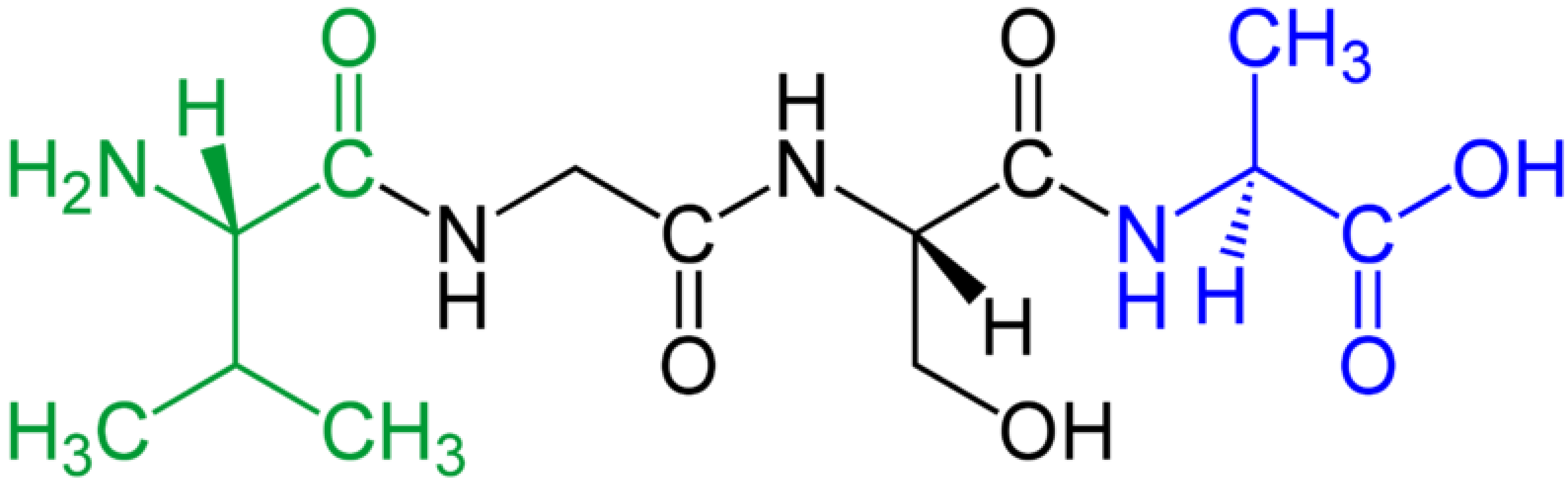
-Higher molecular weight peptides are called polypeptides.

-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

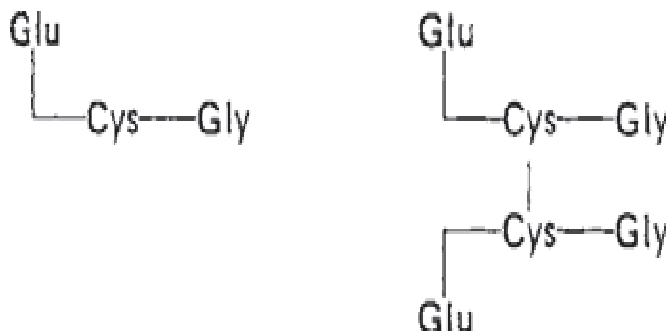


A tetrapeptide (example Val-Gly-Ser-Ala) with green marked amino end (L-Valine) and blue marked carboxyl end (L-Alanine).

-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.

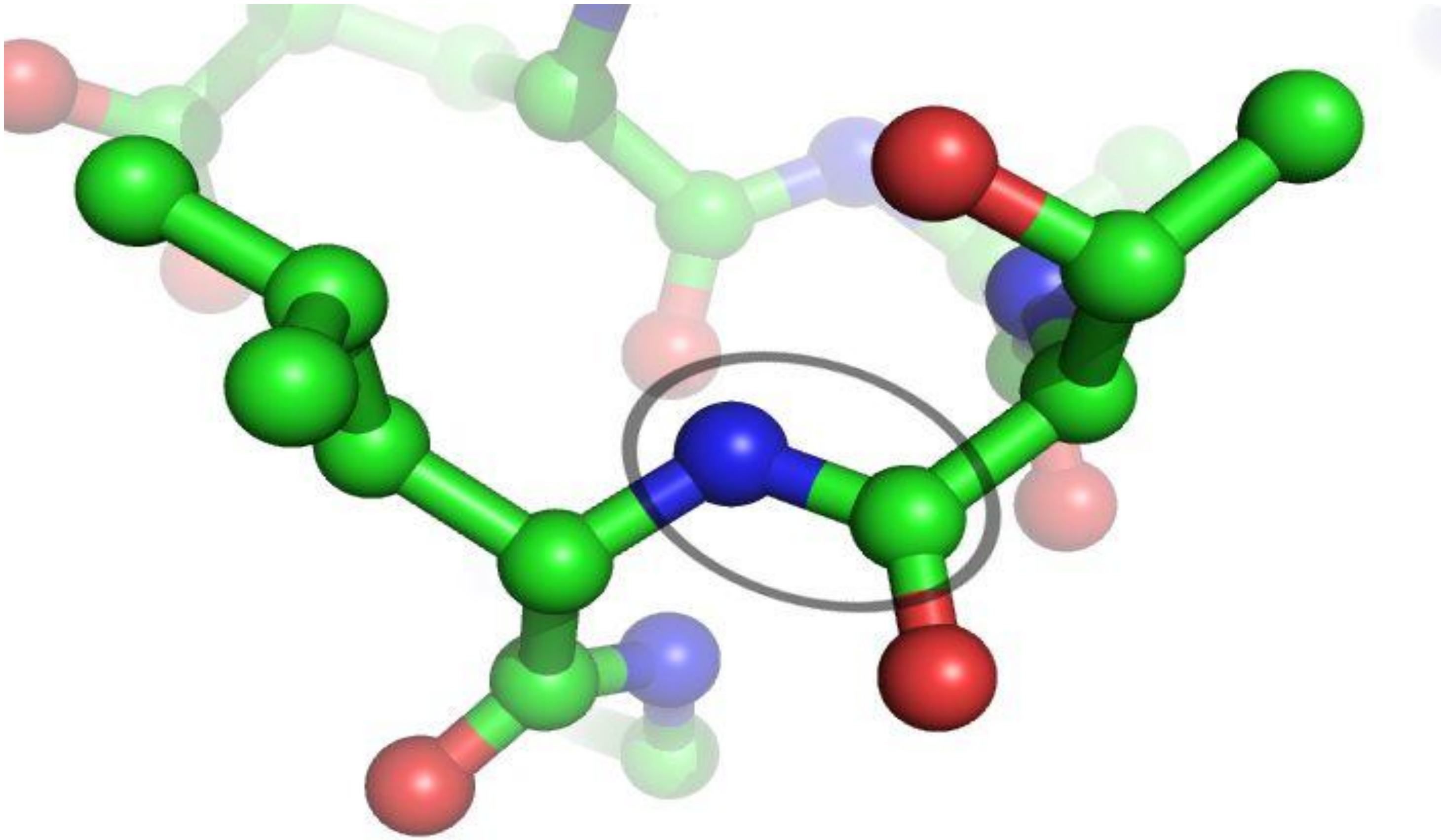
-Peptone is used in nutrient media for growing bacteria and fungi.

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.

2- Sensory Properties

Effect of HCl on the salty taste of Orn-β-Ala

Equivalents HCl	pH	Taste	
		salty[b]	sour[c]
0	8.9	0	
0.79	7.0	0	
0.97	6.0	1	
1.00	5.5	2	
1.10	4.7	3	+/−
1.20	4.3	3.5	+
1.30	4.2	3	++

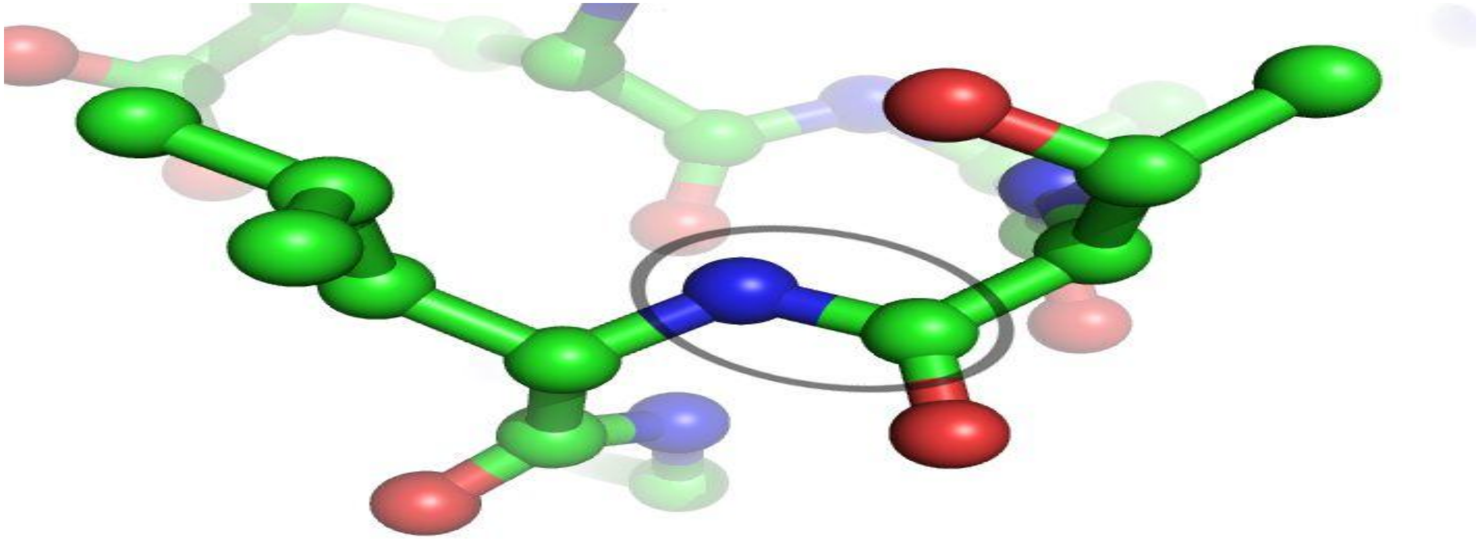
[a] Peptide solution: 30 mmol/L.
[b] The values 1, 3 and 5 correspond in intensity to 0.5%, 0.25% and 0.1% NaCl solutions respectively.
[c] Very weak (+) and slightly sour (++).

-**The intensity of the salty taste of Orn-β-Ala depends on the pH.**

Individual Peptides

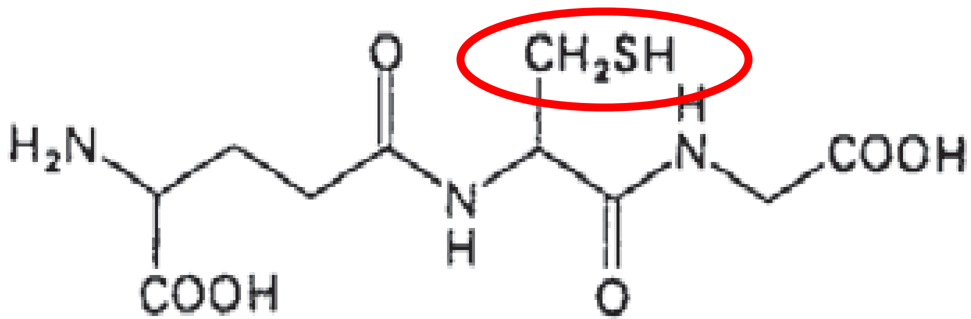
Peptides are widespread in nature.

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



1-Glutathione

-Glutathione (L-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.

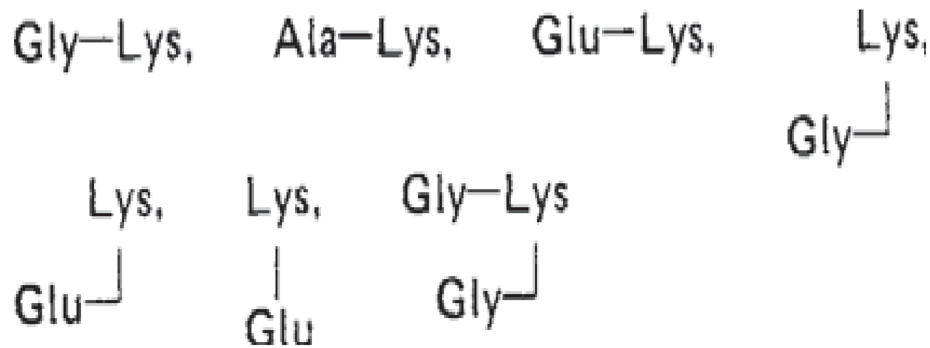
-It is involved in active transport of amino acids and, due to its ready oxidation, is also involved in many redox-type reactions.

-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.

2-Lysine Peptides

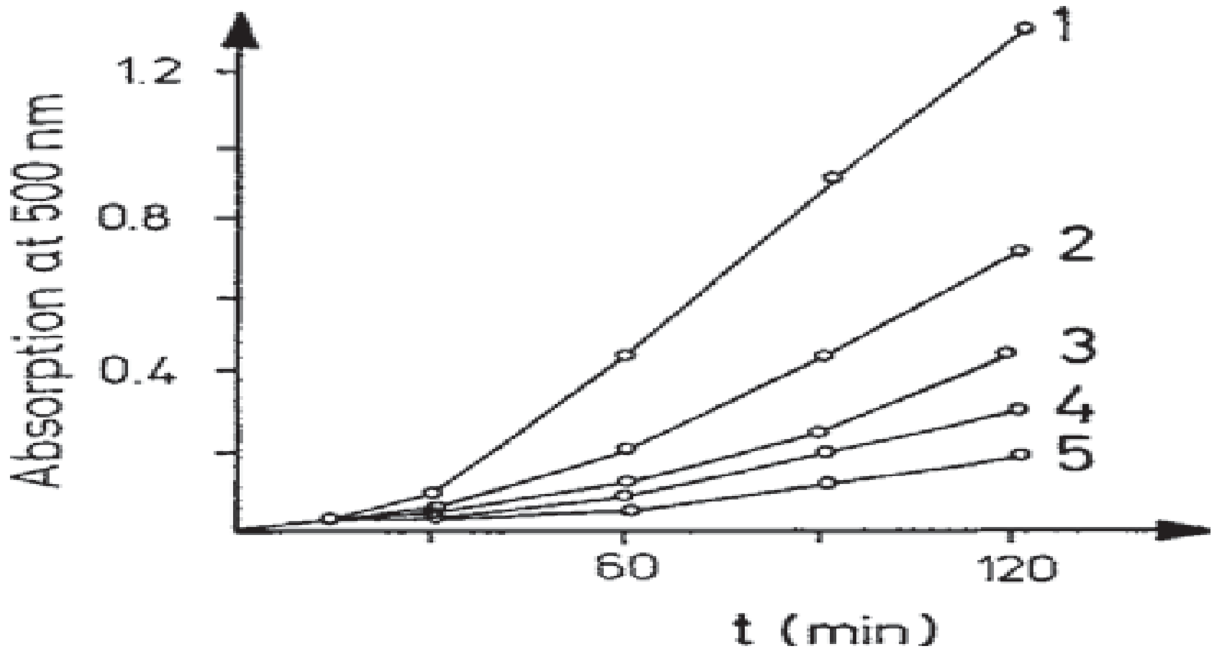
A number of peptides including lysine



have been shown to be as good as lysine in rat growth feeding tests.

-These peptides substantially retard and delay the browning reaction with glucose, hence they are suitable for lysine fortification of sugar-containing foods which must be heat treated.

2-Lysine Peptides



Browning of some lysine derivatives

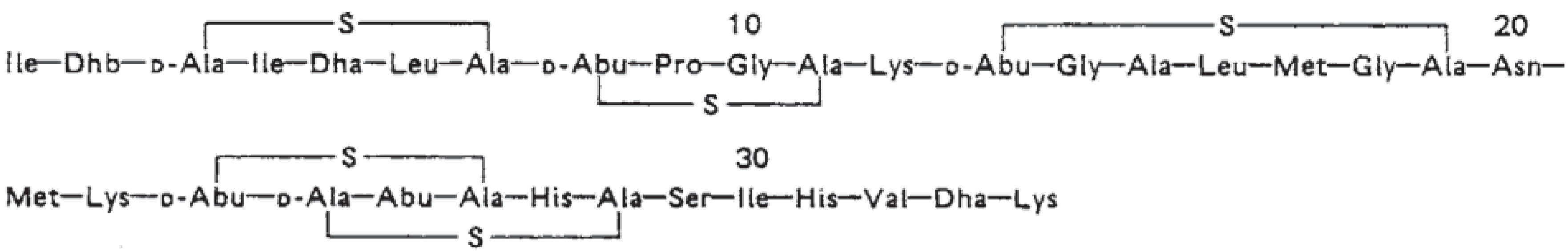
(0.1 M lysine or lysine derivative, 0.1 M glucose in 0.1 M phosphate buffer at pH 6.5 at 100 ◦C in sealed tubes

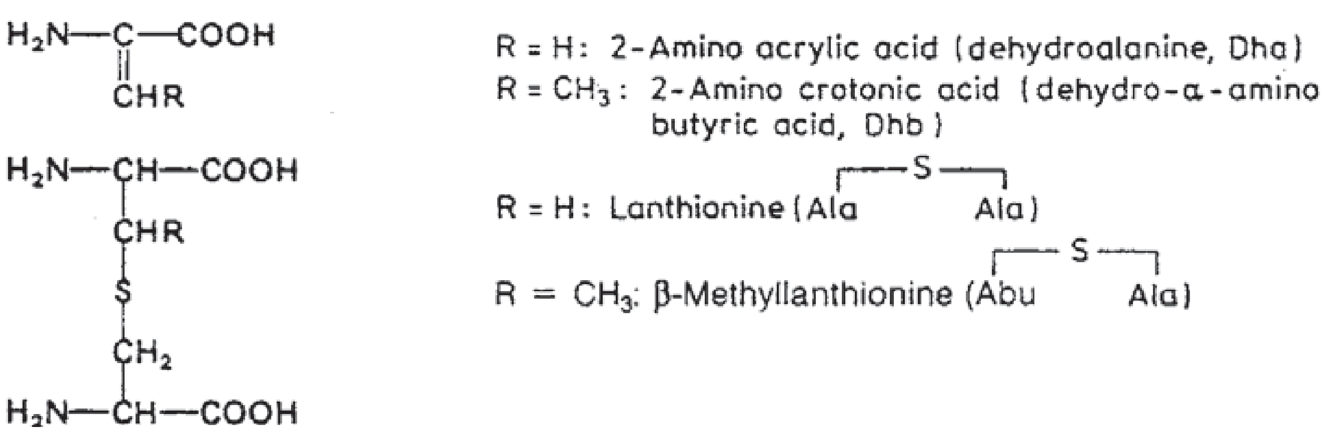
1 Lys, 2 Ala-Lys, 3 Gly–Lys-Gly, 4 Glu–Lys, 5 Lys-Glu)

3- Nisin

-This peptide is formed by several strains of *Streptococcus lactis.*

-It contains a number of unusual amino acids, namely dehydroalanine, dehydro-β-methyl-alanine, lanthionine, β-methyl-lanthionine, and therefore also five thioether bridges.





-Nisin is active against Gram-positive microorganisms (lactic acid bacteria, *Streptococci, Bacilli, Clostridia*).

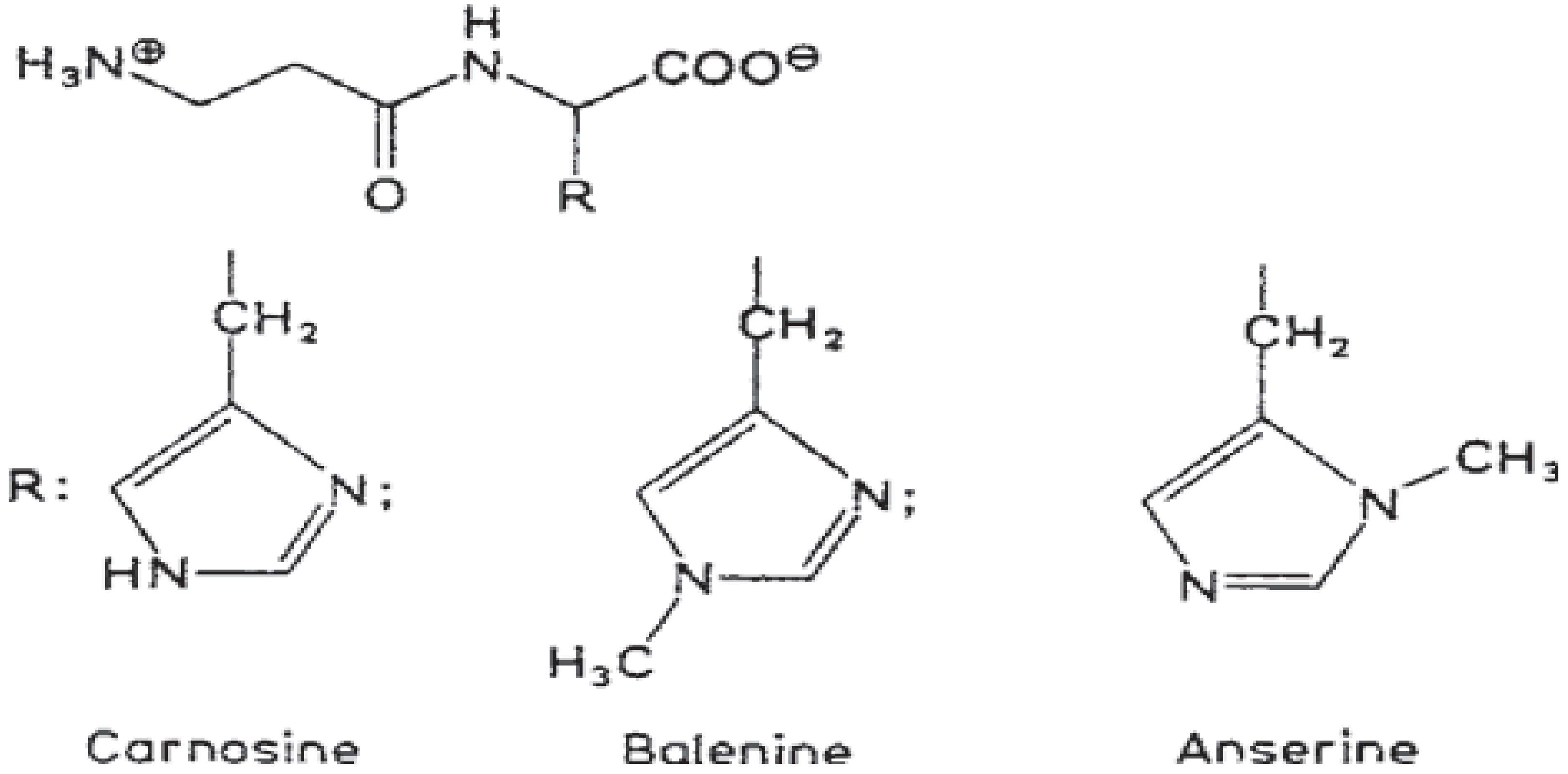
-Nisin begins to act against the cytoplasmic membrane as soon as the spore has germinated. Hence, its action is more pronounced against spores than against vegetative cells.

-Nisin is permitted as a preservative in several countries. It is used to suppress anaerobes in cheese and cheese products, especially in hard cheese and processed cheese to inhibit butyric acid fermentation.

- The use of nisin in the canning of vegetables allows mild sterilization conditions.

4- Carnosine, Anserine and Balenine

-These peptides are noteworthy since they contain a β-amino acid, β-alanine, bound to L-histidine or 1-methyl- or 3-ethyl-L-histidine, and are present in meat extract.



Occurrence of carnosine, anserine and balenine (%) in meat

Meat	Carnosine	Anserine	Balenine	Σ[b]
Beef muscle tissue	0.15–0.35	0.01–0.05		0.2–0.4
Beef meat extract	3.1–5.7	0.4–1.0		4.4–6.2
Chicken meat[c]	0.01–0.1	0.05–0.25		
Chicken meat extract	0.7–1.2	2.5–3.5		
Whale meat				ca. 0.3
Whale meat extract a[d]	3.1–5.9	0.2–0.6	13.5–23.0	16–30
Whale meat extract b[e]	2.5–4.5	1.2–3.0	0–5.2	3.5–12

[a] The results are expressed as % of the moist tissue weight, or of commercially available extracts containing 20% moisture.
[b] β-Alanine peptide sum.
[c] Lean and deboned chiken meat.
[d] Commercial extract mixture of various whales.
[e] Commercial extract mixture, with sperm whale prevailing.

-Carnosine is predominant in beef muscle tissue, while anserine is predominant in chicken meat.

-Balenine is a characteristic constituent of whale muscle.

-These peptides are used analytically to identify the meat extract.

-**Their physiological roles are not clear.**

-They may also be involved in generating exhausted muscle, i.e. in the muscle regaining its excitability and ability to contract.

Food-derived peptides with biological activity

-Many peptides that are released *in vitro* or *in vivo* from animal or plant proteins are bioactive and have health-promoting functions in humans beyond normal and adequate nutrition.

-Different health effects have been attributed to food-derived peptides, including antimicrobial properties, blood pressure-lowering effects, cholesterol-lowering ability, antioxidant activities, enhancement of mineral absorption and/or bioavailability and cyto- or *immuno*-modulatory effects.

-Numerous products are already on the market or under development by food companies that exploit the potential of food-derived bioactive peptides which ascribe scientifically evidenced health claims to consumption of these functional foods.

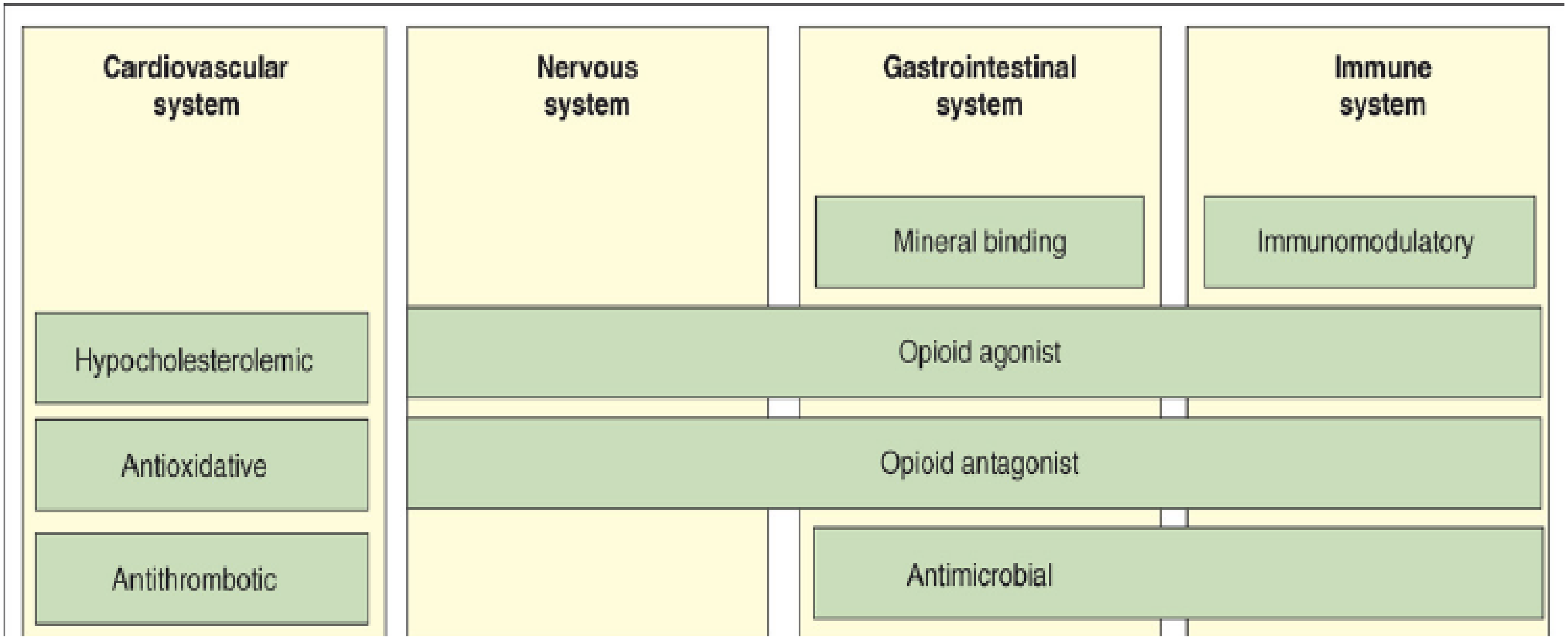
Examples of bioactive peptides from food

Effect	Origin	Encrypting protein(s)	Name/remarks/sequence (in single-letter code)
ACE inhibitory/ hypotensive	Soy	Soy protein	NWGPLV
	Fish	Fish muscle protein	LKP, IKP, LRP (derived from sardine, bonito, tuna, squid)
	Meat	Meat muscle protein	IKW, LKP
	Milk	α-LA, β-LG	Lactokinins (e.g. WLAHK, LRP, LKP)
		α-, β-, κ-CN	Casokinins (e.g. FFVAP, FALPQY, VPP)
	Egg	Ovotransferrin	KVREGTTY
		Ovalbumin	Ovokinin (FRADHPPL)
			Ovokinin (2–7) (KVREGTTY)
	Wheat	Wheat gliadin	IAP
	Broccoli	Plant protein	YPK
Immunomodulatory	Rice	Rice albumin	Oryzatensin (GYPMYPLR)
	Egg	Ovalbumin	Peptides not specified
	Milk	α-, β-, κ-CN, α-LA	Immunopeptides (e.g. α_{S1}-immunocasokinin) (TTMPLW)
	Wheat	Wheat gluten	Immunopeptides
Cytomodulatory	Milk	α-, β-CN	α-Casomorphin (HIQKED(V)), β-casomorphin-7 (YPFPGPI)
Opioid agonist	Wheat	Wheat gluten	Gluten-exorphins A4, A5 (GYYPT), B4, B5, and C (YPISL)
	Milk	α -LA, β-LG	α-Lactorphins, β-lactorphins
		α-, β-CN	Casomorphins
Opioid antagonist	Milk	Lactoferrin	Lactoferroxins
		κ-CN	Casoxins
Antimicrobial	Egg	Ovotransferrin	OTAP-92 (f109–200)[a]
		Lysozyme	Peptides not specified
	Milk	Lactoferrin	Lactoferricin
		α-, β-, κ-CN	Casecidins, isracidin, kappacin
Antithrombotic	Milk	κ-CN (glycomacropeptide)	κ-CN (f106–116)[a], casoplatelin
Mineral binding, anticariogenic	Milk	α-, β-CN	Caseinophosphopeptides
Hypocholesterolemic	Soy	Glycinin	LPYPR
	Milk	β-LG	IIAEK
Antioxidant	Fish	Sardine muscle	MY
	Wheat	Wheat germ protein	Peptides not specified
	Milk	α-LA, β-LG	MHIRL, YVEEL, WYSLAMAASDI

CN, casein; LA, lactalbumin; LG, lactoglobulin.
[a] f, fragment.

Many peptides of plant and animal origin with relevant bioactive potential have been discovered, with by far the most being isolated from milk-based products. Candidate proteins containing these latent biological activities are found in milk, eggs, meat and fish as well as in different plant protein sources such as soy and wheat.

Overview of benefical effects of bioactive peptides from food proteins



-A wide range of activities has been described, including antimicrobial properties, blood pressure-lowering effects, cholesterol-lowering ability, antioxidant activities and enhancement of mineral absorption/bioavailability.

-Moreover, some peptides are multi-functional and can exert more than one of the effects mentioned.

Antimicrobial peptides

-Antimicrobial peptides have been identified from many protein hydrolysates, especially from milk.

-The most well studied are the lactoferricins, which are derived from bovine and human lactoferrin.

-Additionally, a few antibacterial peptides have been identified from α_{S1}-casein and α_{S2}-casein.

-Antimicrobial peptides act against different Gram-positive and Gram-negative bacteria (*Escherichia, Helicobacter, Listeria, Salmonella* and *Staphylococcus*), yeasts and fungi.

-The disruption of normal membrane permeability is at least partly responsible for the antibacterial mechanism of lactoferricins.

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 β- and κ-CN	Hypotensive
Evolus	Valio, Finland	Fermented milk, calcium-enriched	VPP, IPP from β- and κ-CN	
BioZate	Davisco, USA	β-LG hydrolysate	Whey peptides	
C12 Peption	DMV, Netherlands	Ingredient	Casein-derived dodecapeptide FFVAPFPEVFGK	
Peptide Soup	NIPPON, Japan	Soup	Bonito-derived peptides	
Casein DP Peptio Drink	Kanebo, Japan	Soft drink	Casein-derived dodecapeptide FFVAPFPEVFGK	
BioPURE-GMP	Davisco, USA	Whey protein hydrolysate	Glycomacropeptide	Anticariogenic, antimicrobial, antithrombotic
CholesteBlock	Kyowa Hakko, Japan	Drink powder	Soy peptides bound to phospholipids	Hypocholesterolemic
CSPHP				
ProDiet F200	Ingredia, France	Milk drink, confectionery	α_{S1}-CN (f91–100)[a]: YLGYLEQLLR	Reduces stress
Capolac	Arla Foods, Denmark	Ingredient	CPP	Helps mineral absorption
Tekkotsu Inryou	Suntory, Japan	Soft drink	CPP	
Kotsu Kotsu calcium	Asahi, Japan	Soft drink	CPP	
CE90CPP	DMV, Netherlands	Ingredient	CPP (20%)	
Glutamin peptide	DMV, Netherlands	Dry milk protein hydrolysate	Glutamine-rich peptides	Immunomodulatory
WGE80GPA				
WGE80GPN				
WGE80GPU				

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.