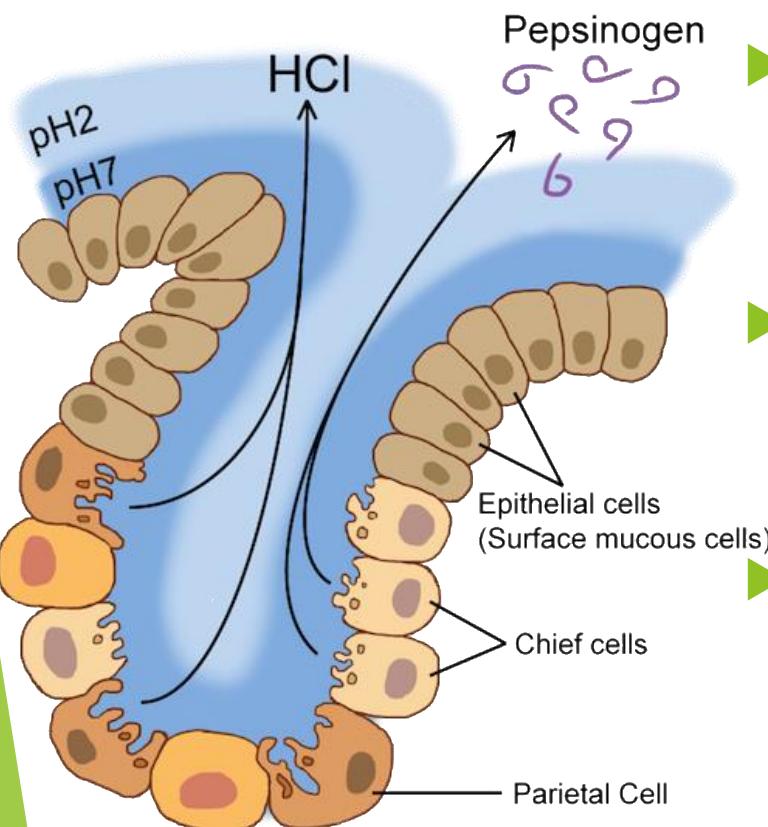


Protein Metabolism

Dietary Protein Degradation



- ▶ Degradation of protein to amino acids occurs in GI tract
- ▶ Protein entry stimulates **gastric mucosa** to secrete a **hormone, gastrin**
- ▶ **Gastrin** stimulates **secretion of HCl** by **parietal cells**; and **pepsinogen** by **chief cells** of gastric glands

Dietary Protein Degradation

- ▶ Acidic gastric juice (pH 1.0 -2.5) has two functions:
 - ✓ **An antiseptic** killing most bacteria & other foreign cells
 - ✓ **A denaturing agent** that unfold globular proteins and render the internal peptide bonds accessible for enzymatic hydrolysis

Dietary Protein Degradation

- **Pepsinogen**, an inactive precursor (zymogen) is converted to active, **pepsin**
- **Pepsin** hydrolyses protein at peptide bonds at amino terminal side of **aromatic side residues**
 - **Phe, Try, Tyr** and **Leu**; cleaving long chain PP chains into a mixture of small peptides

Dietary Protein Degradation

- Acidic stomach content pass into **small intestine**
- **Low pH** triggers secretion of **hormone, secretin** into the blood
- **Secretin** stimulates pancreas to **secrete bicarbonate** into small intestine to neutralize gastric HCl, increasing pH to 7.0

Dietary Protein Degradation

- **Arrival of amino acids** in upper part of intestine (duodenum) cause release into the blood a **hormone, cholecystokinin**
- **Cholecystokinin** stimulates secretion of several **pancreatic enzymes** active at 7.0
- Trypsinogen, chymotrypsinogen and procarboxypeptidase A and B - Zymogens of trypsin, chymotrypsin and carboxypeptidases A and B
- **Zymogens** are synthesized and secreted by **exocrine cells of pancreas**

Dietary Protein Degradation

- **Trypsinogen** is converted to its active form by **enteropeptidase**, a proteolytic enzyme secreted by **intestinal cells**
- **Free trypsin** catalyzes the conversion of additional trypsinogen to trypsin
- **Trypsin** also activates **chymotrypsinogen**, **procarboxypeptidases** and **proelastase**
- Synthesis of enzyme as **inactive precursor** **protect exocrine cells** from **destructive proteolytic attack**

Dietary Protein Degradation

- **Pancreas** further protects itself against self-digestion by making a **special inhibitor protein** called **pancreatic trypsin inhibitor**
- **Inhibitor** effectively prevents premature production of active proteolytic enzymes within the pancreatic cells
- **Trypsin** and **chymotrypsin** hydrolyse the peptides

Specificity of enzymes	Cleavage points
Trypsin	Lys, Arg (C)
Chymotrypsin	Phe, Try, Tyr (C)
Pepsin	Leu, Phe, Try, Tyr (N)
Endoproteinase Lys C	Lys (C)

Dietary Protein Degradation

- Degradation of short peptides in small intestine is then completed by other peptidases
- **Carboxypeptidases A and B** remove successive **carboxy terminal residues** from peptides
- **Aminopeptidases** hydrolyse successive **amino terminal residues** from short peptides
- Resulting mixture of free amino acids is transported into the **epithelial cells lining of small intestine**
- Then, they enter **blood capillaries** in the villi and travel to **LIVER**

Pepsin Cleavage Sites



Trypsin Cleavage Sites



Chymotrypsin Cleavage Sites



Dietary Protein Degradation

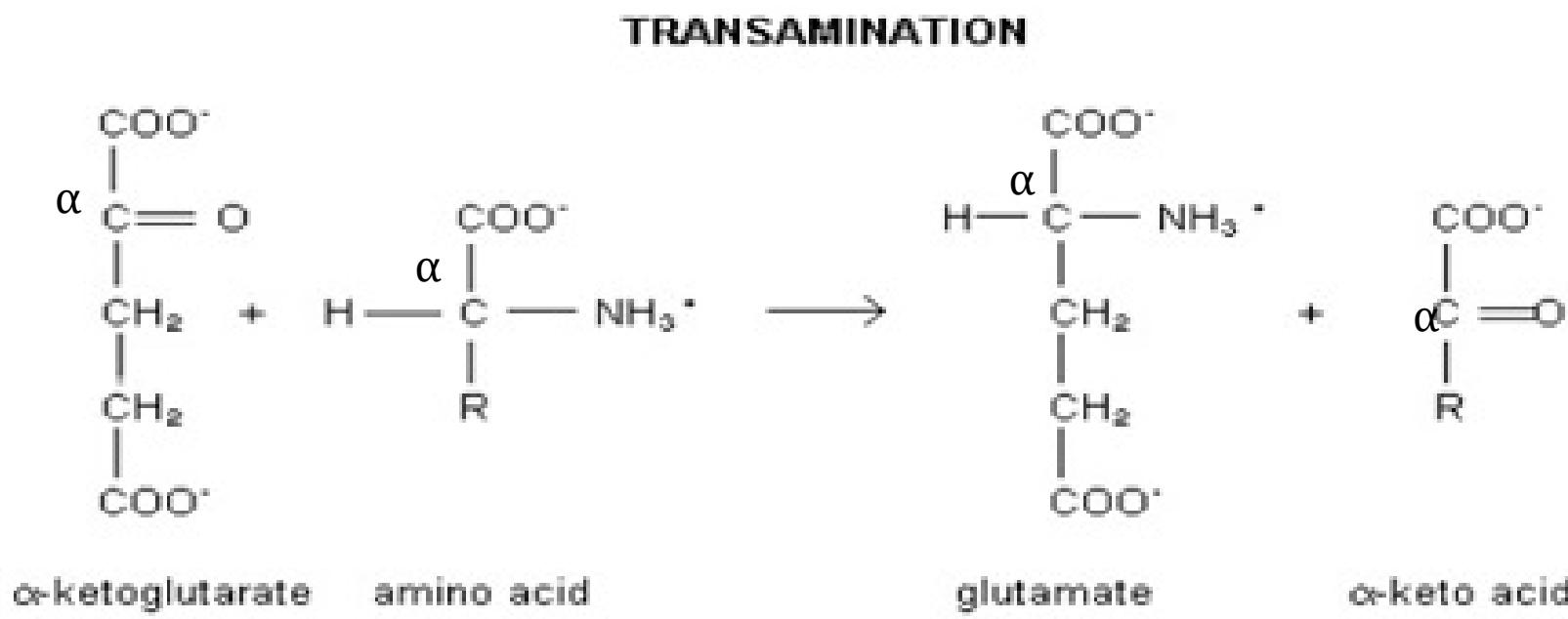
- **Most globular proteins** of animal sources are completely hydrolyzed to amino acids in GI tract
- **Some fibrous proteins** are only partly digested
- Protein of some plant foods is protected against breakdown by indigestible cellulose husks

Amino Acid Catabolism

- ▶ **Carnivores** - 90% energy from AA oxidation
- ▶ **Herbivores** - only small fraction of energy
- ▶ In plants - AA catabolism does not occur
- ▶ In animals - 3 metabolic circumstances
 1. Normal synthesis & degradation of cellular proteins - some AA released from protein breakdown are not needed for protein synthesis
 2. If ingested AA exceeds body's need for protein synthesis, it is catabolized and not stored
 3. Under dietary starvation or diabetes mellitus, cellular proteins are used as fuel

Transamination

- Once AA reach the **liver**, first step is removal of α amino groups by the enzymes called **aminotransferases (or transaminases)**
- α amino groups is transferred to α carbon atom of α ketoglutarate - which forms **α ketoacid** analog of AA and **glutamate**

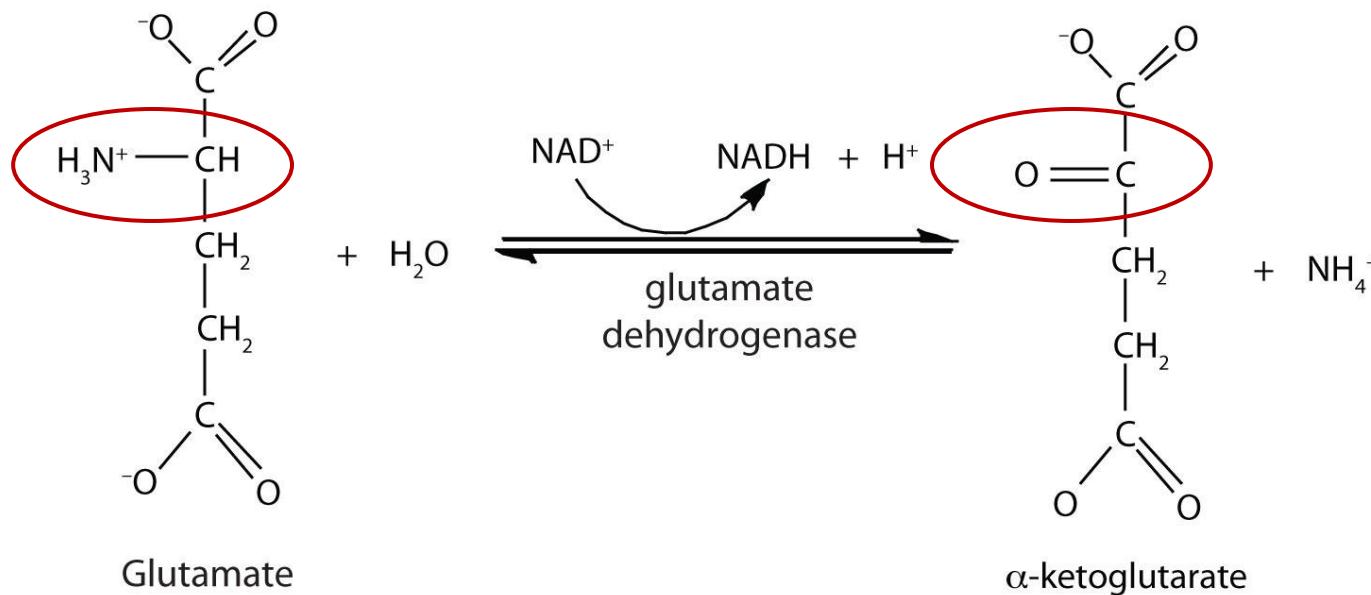


Transamination

- ▶ Role of **transamination reaction** is to **collect the amino groups** from different AA in the form of **L- Glutamate**
- ▶ Glutamate function as **an amino group donor** in Biosynthetic pathway or Excretion pathway
- ▶ Cells contain different types of aminotransferases - alanine AT, aspartate AT, etc.
- ▶ All aminotransferases have prosthetic group - **Pyridoxal phosphate (PLP)** - co-enzyme form of pyridoxine (Vit B6) - **intermediate carrier of amino group**

Oxidative deamination

- ▶ In hepatocytes, **glutamate** is transported from cytosol into mitochondria to undergo **oxidative deamination** - catalyzed by **L-glutamate dehydrogenase**
 - ▶ L-glutamate dehydrogenase use **NAD⁺** or **NADP⁺** as electron acceptor

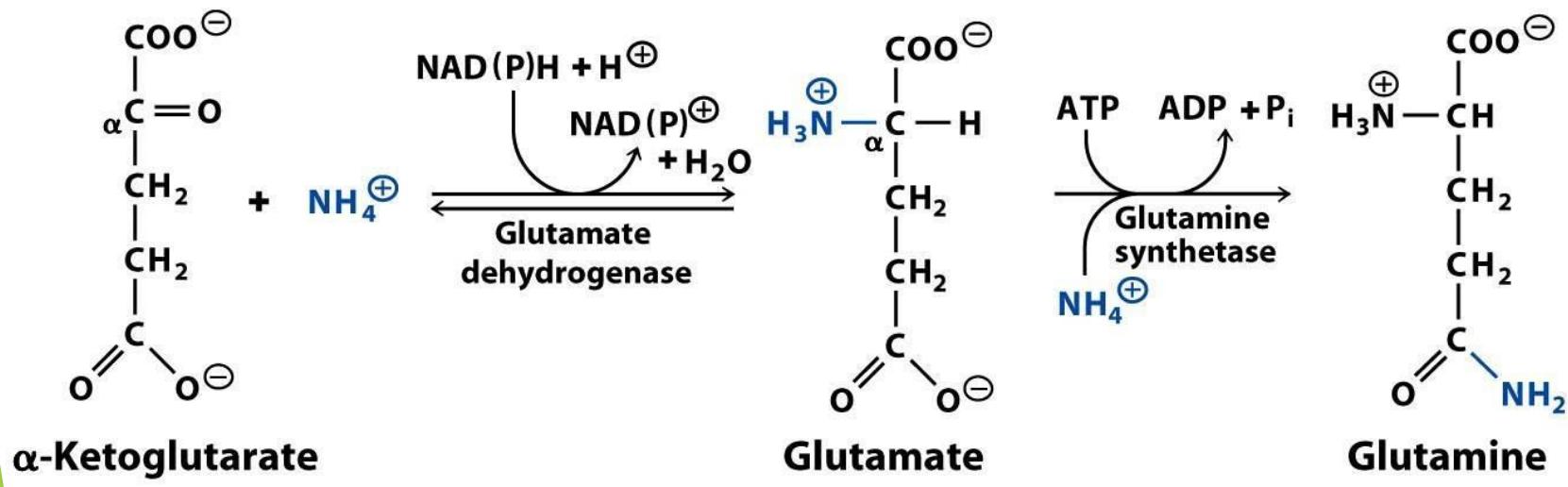


Transdeamination (TDA)

- ▶ Combined action of an aminotransferase and glutamate dehydrogenase is known as transdeamination.
- ▶ α ketoglutarate formed is used in citric acid cycle or for glucose synthesis
- ▶ Glutamate dehydrogenase is the intersection of carbon and nitrogen metabolism

Glutamine transports ammonia

- ▶ Ammonia is quite toxic to animal tissues and its level is regulated in blood
- ▶ Free NH₃ is converted to non-toxic form before export into blood - liver& kidney
- ▶ For this, **free NH₃** combine with **glutamate** to yield **glutamine** by the action of **glutamine synthetase**



Glutamine transports ammonia

- ▶ Excess glutamine is transported through blood to intestine, liver, kidney for processing
- ▶ Amide N₂ is released as NH₄⁺ in mitochondria, where **enzyme, glutaminase** converts **glutamine** to **glutamate**
- ▶ NH₄⁺ in intestine and kidney is transported through blood to liver
- ▶ In liver, ammonia is disposed by **urea synthesis**

Ammonia excretion - Urea Cycle

- ▶ Glutamine synthesis is important pathway for removal of ammonia
- ▶ Glutamine acts as an osmotically active solute (osmolyte) in brain astrocytes
- ▶ Glutamine triggers uptake of water into astrocytes to maintain osmotic balance
- ▶ Glutamine and its derivative α - aminobutyric acid (GABA) are important neurotransmitters

Ammonia excretion - Urea Cycle

- ▶ Amino groups are channeled into a single excretory end product; and not re-used for synthesis of new AA
- ▶ Teleosts excrete amino groups as ammonia (**Ammonotelic**)
- ▶ Terrestrial animals excrete as urea (**Ureotelic**)
- ▶ Birds excrete as uric acid (**Uricotelic**)
- ▶ Plants recycle all aminogroups

Ammonia excretion - Urea Cycle

- ▶ In Ureotelic - Ammonia deposited in mitochondria of hepatocytes is converted into urea in Urea cycle.
- ▶ Urea cycle pathway described 1932 by Hans Krebs and Kurt Henselect
- ▶ Urea produced in liver, passes into blood and to kidneys to be excreted as urine

Urea Cycle

- ▶ Five enzymatic steps (occurs in two compartments)
- ▶ First begins in mitochondria and ends in cytosol
- ▶ First amino group to enter urea cycle is derived from ammonia in matrix - NH_4^+
- ▶ Liver also received NH_4^+ via. portal vein from intestine, ie. due to bacterial oxidation of AA
- ▶ NH_4^+ together with CO_2 (as HCO_3^-) forms carbamyl phosphate in matrix - catalysed by carbamyl phosphate synthetase

Urea Cycle

- ▶ **Carbamyl phosphate** donates its **carbomyl group** to **ornithine** to form **citrulline**, with the release of Pi - catalyzed by **ornithine transcarbamoylase**
- ▶ **Ornithine** plays the role as that of oxaloacetate in citric acid cycle
- ▶ **Citrulline** passes into **cytosol**

Urea Cycle

- ▶ Second amino group now enters from **aspartate** by a condensation reaction between **amino group of aspartate** and **urido group** of citrulline, forming **arginosuccinate** - catalyzed by **arginosuccinate synthetase** that requires ATP
- ▶ **Arginosuccinate** is cleared by **arginosuccinase** to from **free arginine** and **fumurate** (reverse step)
- ▶ **Arginase** cleaves **arginine** to yield **urea** and **ornithine**
- ▶ **Ornithine** is transported into mitochondria to initiate another round

Protein

- ▶ Protein is the second major component in fish
- ▶ Protein in fish muscle is between 15 - 20%, but values <15% or as high as 28% are occasionally found within some species.
- ▶ Extent of variations in protein is comparatively low.
- ▶ Feeding habits, spawning cycle etc. affect the level of protein.

Protein

► Proteins in fish muscle are divided into three groups:

► **Sarcoplasmic proteins:**

Myoalbumin, globulin & enzymes.

Enzymes participating in the cell metabolism- anaerobic energy conversion from glycogen to ATP.

Constitutes 25-30% of the protein

Soluble in salt solutions of low ionic strength ($<0.15 \text{ M}$)

Generally high in pelagic fish - oil sardines, mackerel and low in demersal fish

Protein

- ▶ If the organelles within the muscle cells are broken, this protein fraction will contain the metabolic enzymes localized inside the endoplasmatic reticulum, mitochondria and lysosomes.
- ▶ Composition of the sarcoplasmic protein fraction changes when the organelles are broken.
- ▶ This fraction is used for differentiating fresh from frozen fish, under the assumption that the organelles were intact until freezing.

Protein

- ▶ However, it was later found that some of the enzymes are liberated from the organelles even during iced storage of fish.
- ▶ The proteins in the sarcoplasmic fraction are excellently suited to distinguishing between different fish species, as all the different species have their characteristic band pattern when separated by the isoelectric focusing method.

Protein

► Structural proteins (Myofibrillar proteins):

Actin, myosin, tropomyosin and actomyosin - give muscle its power of contraction.

Constitute 70-80% of total protein (40% in mammals)

Soluble in neutral salt solutions of fairly high ionic strength (0.5 M).

Functional properties - gelling - Surimi & surimi products

Protein

- ▶ **Structural proteins** make up contractile apparatus.
- ▶ Amino-acid composition of fish is same as mammals
- ▶ Physical properties are slightly different - Isoelectric point (pl) is around pH 4.5 - 5.5.
- ▶ Conformational structure change with physical environment.
- ▶ Solubility change after freeze-drying.
- ▶ Treatment with high salt or heat lead to denaturation,

Protein

- ▶ If proteins are denatured under controlled conditions it is utilized for production of **surimi-based products**.
- ▶ After salt and stabilizers are added to a washed, minced muscle protein, and after a controlled heating and cooling procedure, the proteins form a very strong gel.

Protein

► **Connective tissue proteins (Stroma proteins):**

Collagen and elastin - give characteristic texture.

Constitute approx. 3% in teleostei and 10% in elasmobranchii (17% in mammals)

Insoluble in neutral salt solution or in dilute acids or alkalies.

Protein

- ▶ Chemical and physical properties of **collagen proteins** are different in tissues such as skin, swim bladder and myocommata in muscle.
- ▶ Collagen in fish is much more thermolabile and contains fewer but more labile cross-links than collagen from warm-blooded vertebrates.
- ▶ Hydroxyproline content is in general lower in fish than in mammals, a total variation (4.7 and 10% of the collagen)

Protein

- ▶ Different fish species contain varying collagen.
- ▶ The distribution of collagen may reflect the swimming behaviour of the species.
- ▶ The varying amounts and varying types of collagen in different fishes may also have an influence on the textural properties of fish muscle.

Protein in Nutrition

- ▶ All proteins are chains of amino acids linked together.
- ▶ There are 20 amino acids, and some of them are essential in the human diet for the maintenance of good health.
- ▶ Fish protein provides a good combination of amino acids which is highly suited to man's nutritional requirements and compares favorably with that provided by meat, milk and eggs.
- ▶ Fish proteins contain all the essential amino-acids and, like milk, eggs and mammalian meat proteins, have a very high biological value

Amino acids in foods

Amino-acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cystine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

- ▶ Cereal grains are usually low in **lysine** and/or the sulphur-containing amino-acids (**methionine** and **cysteine**)
- ▶ Whereas fish protein is an excellent source of these aminoacids.
- ▶ In diets based mainly on cereals, a supplement of fish can, therefore, raise the biological value significantly.

- ▶ Specific protein fractions are recovered from by-products - **Basic protein or protamines**
- ▶ Found in the milt of the male fish
- ▶ MW < 10 000 kDa and pI >10.
- ▶ Has an extreme amino-acid composition of as much as 65% arginine.
- ▶ Not present in all fish species - best sources are **salmonids and herring**.

- ▶ Protamines adhere to most other proteins less basic and enhance the functional properties of other food proteins.
- ▶ There is a problem of removing all lipids present in the milt during the protein preparation, which often results in an off-flavour development in foods.
- ▶ Another interesting feature is their ability to prevent growth of microorganisms, which appears to be the most promising use of these basic proteins in the future.