Proteins/Amino Acids

Protein metabolism

By

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Preliminary Concepts

- Protein is the principal constituent of organs and soft structures of the animal body
- A continuous supply is needed from feed sources throughout life for growth/repair
- Food protein → body protein
- Food protein: plant or animal
- Unique proteins found in each animal
- No two are alike in physiological behavior
Roles of Protein

1. bulk composition of the body (structural aspects of the cell)
2. oxidative metabolism (used as energy source in energy-deficient diets)
3. enzymes (globular proteins that regulate and influence metabolism)
4. plasma proteins (circulating, mobile proteins such as immune bodies)
5. hormones (regulatory role)
Proteins within Cell Wall
Special Functions

- peptides (formation of proteins)
- purines/pyrimidines (control of protein synthesis)
- histamines (active compounds, allergies)
- conjugated proteins (assist in the excretion of other compounds)
- pigments (e.g., melanin, derived from amino acids)
Elementary Composition of Proteins

- **Chemical composition**: primarily carbon, hydrogen, oxygen
- **additional difference**: contain a fairly constant amount of nitrogen found in amino groups (17%)
- **many also contain sulfur, phosphorus and iron**
- **structure is typically complex, having high molecular weight**
Protein Classification

- **simple proteins**: essentially pure proteins, when **hydrolyzed**, produce individual amino acids (e.g., egg albumin)
- **conjugated**: protein unit linked to another non-protein unit (e.g., **casein**, the protein component of milk with phosphorus esterified to it via the AA serine (ser))
- **derived**: modified proteins such as peptides, modified by heat, acidification, etc.
Conjugated Proteins

- **nucleoproteins**: protein + nucleic acid (e.g., seed germs)
- **glycoproteins**: protein + COH group (e.g., mucus)
- **phosphoproteins**: protein + P-containing compound (e.g., casein)
- **hemoglobins**: protein + hematin or similar substance
- **lecithoproteins**: protein + lecithin (e.g., fibrinogen)
Structure of Protein Molecule

• As mentioned, proteins are sequences of amino acids hooked together by the amino group of one to the carboxyl group of another
• this bond is known as the \textit{peptide linkage}
• AA found in protein are known as \textit{residues}
• protein chains of AA have typically 100-200 residues
• many proteins have more than one chain
The Peptide Linkage

- These C–N bonds cannot rotate.
- Planar peptide bonds and thus cannot rotate freely. They further illustrate the linkage.
Protein Structure

- **primary**: the sequence of AA’s forming the protein
- **secondary**: forces generated by the close proximity of one AA residue to another (e.g., $\alpha$ helix design or $\beta$ pleated sheet)(i.e., certain amino acids can form bonds with others, if close enough, cysteine)
- **tertiary**: bending of one AA chain due to attraction of individual AA’s distant from each other
- **quaternary**: packing of chains together
Protein Structure
Amino Acids (AA)

- As mentioned, proteins are polymerized residues of amino acids.
- The number and proportion of AA vary from protein to protein.
- When proteins are denatured, the AA remain.
- To study protein, you must study AA.
- At least 30 different AA, some essential others non-essential.
Characterizing AA

- Most AA are derived from lower or short-chain fatty acids (FA; such as acetic, propionic or butyric acid)
- naturally-occurring have L-configuration
- synthetic have large proportion of D configs
- soluble in water, amphoteric
- show various types: aliphatic, aromatic, heterocyclic, etc.
“D” vs. “L” Configuration

D-Alanine

L-Alanine

Perspective formulas

Projection formulas
Aliphatic Amino Acids

Glycine (Gly)  Alanine (Ala)  Valine (Val)  Leucine (Leu)  Isoleucine (Ile)

Figure 2-8
Amino acids having aliphatic side chains.
Aromatic Amino Acids

Figure 2-11
Phenylalanine, tyrosine, and tryptophan have aromatic side chains.
Chemical Determination of Protein

- The direct determination of protein in tissue is impractical due to quantity/variation.
- Nitrogen, however, occurs at fairly constant levels:
- \[ [N] \times 6.25 = \text{protein level} \]
- Some proteins have well-known nitrogen levels (e.g., milk @ 15.7% N)
- Determined by Kjehldal N methodology.
Protein/AA Quality

- Amino acids are basically divided into two nutritional categories:
  - essential: those the animal cannot synthesize in sufficient quantity to support maximum growth, typically dietary in nature
  - nonessential: synthesized by animal body, typically non-dietary in nature
- determined first by Rose (1930) working on factorial deletion with rats
Essential AA

- lysine (LYS)
- arginine (ARG)
- methionine (MET)
- histidine (HIS)
- isoleucine (ILE)
- leucine (LEU)
- threonine (THR)
- tryptophan (TRY)
- phenylalanine (PHE)
- valine (VAL)

Exceptions

ser/gly essential for chicks
pigs don’t need ARG, HIS, LEU for maintenance
no big problem for ruminants, why?
All essentials are in “L” form only humans really need HIS
Protein/AE Quantitative Requirements

- A protein requirement is really an EAA requirement (why?)
- Unfortunately, not all sources of protein are “balanced”, not all are digestible
## EAA Requirements of Chickens and Swine

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Channel</th>
<th>Tilapia nilotica</th>
<th>Swine</th>
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<tbody>
<tr>
<td>ARG</td>
<td>4.3</td>
<td>4.2</td>
<td>1.2</td>
</tr>
<tr>
<td>HIS</td>
<td>1.5</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>ILE</td>
<td>2.6</td>
<td>3.1</td>
<td>3.4</td>
</tr>
<tr>
<td>LEU</td>
<td>3.5</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>LYS</td>
<td>5.1</td>
<td>5.1</td>
<td>4.4</td>
</tr>
<tr>
<td>MET + CYS</td>
<td>2.3</td>
<td>3.2</td>
<td>2.3</td>
</tr>
<tr>
<td>PHE + TYR</td>
<td>5.0</td>
<td>5.7</td>
<td>4.4</td>
</tr>
<tr>
<td>THR</td>
<td>2.0</td>
<td>3.6</td>
<td>2.8</td>
</tr>
<tr>
<td>TRY</td>
<td>0.5</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>VAL</td>
<td>3.0</td>
<td>2.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Requirements for EAA

- Requirement for one EAA can be partially mitigated by a NEAA
- **example**: CYS sparing of MET
- CYS replaces about 60% of MET
- often reported as MET-CYS requirement
- **example**: TYR sparing of PHE (about 50%)
Protein Digestion
What is Digestion?

Digestion is the process by which large, complex nutrient molecules are broken down into simpler molecules capable of being used by the organism for food.

Digestion therefore involves the breakdown of complex food molecules into monomers that can be absorbed and utilized by cells. In most animals, digestion is extracellular, external to the body, occurring in a tubular gut with regions specialized for different digestive functions.
Digestion

- **Digestion**: the preparation of food by the animal for absorption
- involves the following processes:
  - 1) mechanical reduction of particle size;
  - 2) enzyme solubilization of organics;
  - 3) pH solubilization of inorganics;
  - 4) emulsification of fats
Different Digestive Systems

- The three different types of digestive systems are:
  - Monogastric
  - Modified Monogastric
  - Ruminant
Monogastric

- Carnivores and omnivores have monogastric digestive systems.
- Monogastric systems have a simple stomach structure and only one compartment.
- Some animals with monogastric digestive systems are pigs and rabbits. Humans also have monogastric digestive systems.
Monogastric Digestive System

- Esophagus
- Esophageal sphincter
- Longitudinal muscle layer
- Circular muscle layer
- Oblique muscle layer
- Gastric folds
- Duodenum
- Pyloric sphincter
Digestive Tract of the Monogastric Mammal
Parts and functions of the monogastric mammal digestive system continued...

• Small Intestine
  - enzymatic digestion and absorption
  - Functions of the small intestine: digestion of proteins, carbohydrates, and fats; absorption of the end products of digestion
    1. duodenum - most digestion occurs here
    2. jejunum - some digestion and some absorption occur
    3. ileum - mostly absorption
  - Bile - made in liver, stored in gall bladder, active in the small intestine, emulsifies fat to aid in digestion
Parts and functions of the monogastric mammal digestive system continued...

- Enzymes in the small intestine

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>trypsin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chymotrypsin</td>
<td>digest proteins</td>
<td>secreted from pancreas</td>
</tr>
<tr>
<td>carboxypeptidases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pancreatic amylase</td>
<td>digests carbohydrates</td>
<td>secreted from pancreas</td>
</tr>
<tr>
<td>lipases</td>
<td>digests lipids</td>
<td></td>
</tr>
<tr>
<td>disaccharidases</td>
<td>digests carbohydrates</td>
<td>secreted from small intestine</td>
</tr>
<tr>
<td>dipeptidases</td>
<td>digest peptides</td>
<td></td>
</tr>
</tbody>
</table>
Hydrolytic enzymes break down proteins, carbohydrates, and fats into their monomeric units. To prevent the organism itself from being digested, these are released as inactive zymogens, only activated when secreted into the gut.

Enzymatic digestion begins in the mouth, where amylase is secreted with saliva. Protein digestion begins in the stomach with pepsin and HCl secreted by the stomach mucosa. The mucosa also secretes mucus, to protect gut tissues.
(a) Stomach

- Esophageal sphincter
- Gastric glands
- Gastric mucosa
- Mucus-secreting cells

- Pyloric sphincter
- Folds

Pepsinogen → Pepsin

HCl

Enzyme-secreting cell

Acid-secreting cell

Gastric pit

(b) Zymogen activation

Inactive zymogen: pepsinogen

Active enzyme: pepsin

Low pH

Masking sequence

Active site

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>SOURCE</th>
<th>ACTION</th>
<th>SITE OF ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary amylase</td>
<td>Salivary glands</td>
<td>Starch $\rightarrow$ Maltose</td>
<td>Mouth</td>
</tr>
<tr>
<td>Pepsin</td>
<td>Stomach</td>
<td>Proteins $\rightarrow$ Peptides; autocatalysis</td>
<td>Stomach</td>
</tr>
<tr>
<td>Pancreatic amylase</td>
<td>Pancreas</td>
<td>Starch $\rightarrow$ Maltose</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Lipase</td>
<td>Pancreas</td>
<td>Fats $\rightarrow$ Fatty acids and glycerol</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Nuclease</td>
<td>Pancreas</td>
<td>Nucleic acids $\rightarrow$ Nucleotides</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Pancreas</td>
<td>Proteins $\rightarrow$ Peptides; activation of zymogens</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Pancreas</td>
<td>Proteins $\rightarrow$ Peptides</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Pancreas</td>
<td>Peptides $\rightarrow$ Peptides and amino acids</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Small intestine</td>
<td>Peptides $\rightarrow$ Peptides and amino acids</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Dipeptidase</td>
<td>Small intestine</td>
<td>Dipeptides $\rightarrow$ Amino acids</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Enterokinase</td>
<td>Small intestine</td>
<td>Trypsinogen $\rightarrow$ Trypsin</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Nuclease</td>
<td>Small intestine</td>
<td>Nucleic acids $\rightarrow$ Nucleotides</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Maltase</td>
<td>Small intestine</td>
<td>Maltose $\rightarrow$ Glucose</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Lactase</td>
<td>Small intestine</td>
<td>Lactose $\rightarrow$ Galactose and glucose</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Sucrase</td>
<td>Small intestine</td>
<td>Sucrose $\rightarrow$ Fructose and glucose</td>
<td>Small intestine</td>
</tr>
</tbody>
</table>
Final enzymatic cleavage of peptides and disaccharides occurs on the cell surfaces of the intestinal mucosa. Amino acids, monosaccharides, and many inorganic ions are absorbed by the microvilli of the mucosal cells. Often specific carrier proteins in the membranes of these cells transport nutrients into the cells. Sodium cotransport is a common mechanism for actively absorbing nutrient molecules and ions.
Control and Regulation of Digestion

Digestion processes are coordinated and controlled by neural and hormonal mechanisms. Salivation and swallowing are autonomic reflexes. Stomach and small intestine actions are largely controlled by the hormones gastrin, secretin, and cholecystokinin. See Figure
Food in stomach

Release of gastrin from stomach mucosal cells

Lowers pH

Stimulates secretion of HCl and pepsin, and increases motility of stomach

Increases delivery of acid chyme to small intestine

Undigested fats and proteins

Acid in chyme

Release of cholecystokinin by intestinal mucosa

Release of secretin by intestinal mucosa

Release of bile from gallbladder

Release of digestive enzymes from pancreas

Release of bicarbonate solution from pancreas

Neutralizes acid

Digestion of food
Parts and functions of the monogastric mammal digestive system continued...

- **Cecum** - essentially non-functioning in many monogastrics. Rabbits and horses have an enlarged cecum that acts like a rumen and is involved with microbial digestion (fermentation)

- **Large Intestine**
  - bacterial activity
  - water absorption
  - waste storage
Processes of the Digestive system
Dynamics of Protein And Amino Acid Metabolism

Dietary Proteins \[\rightarrow\] Digestion to Amino Acids

Transport in Blood to Cells

Protein Synthesis

Amino Acids

Metabolites

Functional Proteins

Protein Degradation In Proteasomes Following Tagging With Ubiquitin
Use of Amino Acids

- Blood
  - Tissue protein synthesis
  - Synthesis of metabolites
    - Enzymes, hormones
  - Deamination or transamination

- Enterocyte
  - Uses amino acids for own needs
Enterocyte Use of Amino Acids

- Energy
  - Primarily glutamine
- Synthesize compounds
  - Apoproteins for lipoprotein formation
  - Digestive enzymes
  - Hormones
  - Other nitrogen-containing compounds
Amino Acid Utilization

Amino Acids and Peptides in Small Intestine

Enterocyte

Free Amino Acids

Liver

Energy and Synthesis of Compounds
Fate of Absorbed Amino Acids

- Three categories
  - Tissue protein synthesis
  - Synthesis of enzymes, hormones, and other metabolites
  - Deamination or transamination
Protein Transport in the Blood

- Amino acids diffuse across the basolateral membrane
  - Enterocytes → portal blood → liver → tissues
  - Transported mostly as free amino acids

- Liver
  - Potential breakdown of many amino acids
    - As needed
  - Synthesis of non-essential amino acids
Protein Metabolism in Cells

- Transport of amino acids from blood into cells
- tRNA immediately ‘picks up’ amino acid
Protein Metabolism in Cells

- No ‘storage’ of amino acids in cells
  - Synthesis of functional proteins
- Proteins degraded to free amino acids
  - Amino acid concentration in blood is fairly constant
Constant Turnover of Proteins

- Degradation and re-synthesis of protein
  - Some amino acids are degraded and must be replaced through the diet
    - ‘Obligatory losses’
- Growth requires that synthesis > degradation
Protein Synthesis

- On-going, semicontinuous activity in all cells but rate varies greatly between tissues
- Rate is regulated by hormones and supply of amino acids and energy
- Energetically expensive
  - requires about 5 ATP per one peptide bond
- Accounts for about 20% of whole-body energy expenditure
## Rate of Protein Synthesis

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pig</th>
<th>Steer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Gut</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>Muscle</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Muscle is far less “metabolically active” so requires fewer cells to be replaced daily.
Hormonal Regulation

- Insulin
  - Glucose availability to cells increases
  - Protein synthesis increases

- Glucagon
  - Protein synthesis decreases
  - Protein degradation increases
Protein Synthesis

- Protein synthesis of any particular protein cannot proceed without an adequate supply of all amino acids that will contribute to the primary structure of that specific protein
Protein Metabolism in Cells

- All 20 amino acids must be present
- If not, must be synthesized in liver
  - Synthesis of non-essential amino acids
    - Transamination: Transfer of NH$_2$-group

Glutamine + ketoacid $\rightarrow$ Glutamate + amino acid
Biosynthesis of Nonessential Amino Acids

- Transamination reactions
  - Allow extensive interconversion between nonessential amino acids
  - Requires vitamin B₆ as a coenzyme
Transamination

• Transfer of amino group from an amino acid to an $\alpha$-keto acid
• Used to synthesize amino acids as needed
  • Some essential amino acids
    • Not lysine or threonine
  • Must have appropriate $\alpha$-keto acid in diet
• Requires vitamin $B_6$ in coenzyme form
  • Pyridoxal phosphate (PLP)
• Catalyzed by amino transferases
Transamination

Amino Acid

\[ \alpha\text{-Keto Acid} \]

\[ \text{Amino transferase} \]

PLP

\[ \alpha\text{-Keto Acid} \]

\[ \alpha\text{-Amino Acid} \]
Transamination

- Alanine
  - PLP
  - Alanine amino transferase (ALT)
  - $\alpha$-amino acid (typically glutamate)

- $\alpha$-ketoadcid (typically $\alpha$-ketoglutarate)
- Pyruvate

- Aspartate
  - PLP
  - Aspartate amino transferase (AST)
  - Oxaloacetate
  - $\alpha$-amino acid (typically glutamate)
Transaminations

Glutamate + Pyruvate

Glutamate-Pyruvate Aminotransferase (Alanine Transferase ALT) → α-Ketoglutarate + Alanine

Glutamate + Oxaloacetate

Glutamate-Oxaloacetate Aminotransferase (Aspartate Transferase AST) → α-Ketoglutarate + Aspartate

Blood levels of these aminotransferases, also called transaminases, are important indicators of liver disease.

DR IDOWU OMO-ANN303-UNAAB
Amino Acids Formed From α-Ketoglutarate

4 Steps

Transamination or Glutamate dehydrogenase

5 Steps

Glutamine synthase

4 Steps

Proline

5 Steps

Ornithine

Urea Cycle

Arginine

Guanidino group

- O₂CCH₂CH₂CCO₂⁻

α-Keto-glutarate

- O₂CCH₂CH₂CHCO₂⁻

Glutamate

O

H₂NCH₂CH₂CH₂CHCO₂⁻

Glutamine

H₂N=CH₂

H₂NCH₂CH₂CH₂CHCO₂⁻

Guanidino group

H₂₂N+NH₂

NH₃⁺

O₂CCH₂CH₂CCO₂⁻

NH₃⁺

O₂CCH₂CH₂CHCO₂⁻

NH₃⁺

O₂CCH₂CH₂CH₂CHCO₂⁻

NH₃⁺
Amino Acid Interrelationships

- Methionine can be converted to Cys
  - If too little Cys in diet, Met is converted to Cys and Met becomes deficient
    - Up to 50% of Cys ‘requirement’ met through Met
- Phe can be converted to Tyr
  - Requirement is typically stated for Phe + Tyr
Protein Catabolism

- Occurs when
  - Dietary protein exceeds protein requirements of body
    - Normal situation in true carnivores
    - Abnormal in omnivores and herbivores
  - Composition of absorbed amino acids is unbalanced
  - Gluconeogenesis is increased
Protein Catabolism

- Some net catabolism of body proteins occurs at all times
  - Expressed as **urinary nitrogen excretion**
    - Use the carbon backbone for energy, excrete the nitrogen as urea
Urinary Nitrogen Excretion

LIVER

Amino acids → keto acids

NH₃ → CO₂ → Urea

Blood

KIDNEY

Urea/uric

Urine
Deamination

- Removal of amino group from an amino acid with no transfer
- Produces ammonia and α-keto acid
  - Ammonia removed by urea/uric cycle
  - α-keto acid is metabolized via several potential pathways
- Pyridoxal phosphate (PLP) required (B₆)
Deamination

\[ \text{Dehydratase} \]

\[ \alpha\text{-Keto Acid} \rightarrow \alpha\text{-Keto Acid} + \text{NH}_4 \]

\[ \text{PLP} \]

\[ \text{H}_2\text{O} \]

\[ \text{Threonine} \rightarrow \alpha\text{-ketobutyrate} + \text{NH}_4 \]

\[ \text{PLP} \]

\[ \text{H}_2\text{O} \]

\[ \text{Ammonium} \]
Use of Keto Acids for Energy

• Keto acids can
  • Enter the TCA cycle and be broken down to CO₂ and H₂O with release of energy
  • Be used for gluconeogenesis
    • Some, not all amino acids
    • In liver (and kidney)
  • Lipogenesis (fatty acid biosynthesis)
  • Ketogenesis
    • Ketone bodies (acetoacetate, acetyl-CoA)
    • Used as energy source in various tissues
Ketogenic Amino Acids

- Leucine and isoleucine
  - Converted to acetoacetate or acetyl CoA in liver
    - Fuel for other tissues
Use of Amino Acids for Energy

- Not economical
  - Energy feeds are less expensive (per kcal) than protein feeds
Disposal of NH₃

- NH₃ is very toxic and must be detoxified and excreted from the body
  - Fish: NH₃
  - Mammals: Urea
  - Birds: Uric acid
- Synthesis of uric acid
  - Same pathway as for purines
- Synthesis of urea—the urea cycle
  - Detoxifies NH₃ to urea
  - Synthesizes arginine
Urea Cycle

- Arginine
- Ornithine
- Ammonia + CO₂
- Citrulline
- Urea
- Ammonia
Urea Cycle

- **Overall reaction**
  \[ 2 \text{NH}_3 + \text{CO}_2 \rightarrow \rightarrow \text{H}_2\text{N}–\text{C}–\text{NH}_2 + \text{H}_2\text{O} \]

  - Energy required (3 ATP)
  - Urea diffuses from liver cells to body fluids
  - Excreted by the kidneys
Amino acids that can become essential in certain physiologic conditions:

- Example: taurine in cats
- Example: proline in young pigs
- Example: tyrosine becomes essential in people with phenylketonuria (PKU)

Hydroxylation of phenylalanine normally forms tyrosine.

Tyrosine is important in adrenaline, noradrenaline, thyroxine, and melanin synthesis.

## Essential Amino Acids

- Threonine
- Methionine
- Isoleucine
- Leucine
- Valine
- Tryptophan
- Phenylalanine
- Histidine
- Lysine

## Branch-Chained Amino Acids

- Isoleucine
- Leucine
- Valine

## Sulphur Amino Acids

- Cysteine
- Methionine

## Essential Amino Acids

- Threonine
- Methionine
- Isoleucine
- Leucine
- Valine
- Tryptophan
- Phenylalanine
- Histidine
- Lysine

## Acidic Amino Acids

- Tyrosine
- Phenylalanine

## Basic Amino Acids

- Arginine
- Lysine
Amino Acid Toxicity/Antagonism

- Toxicity/antagonisms are result of dietary imbalances in EAA
- when one EAA is fed in excess it can also increase the requirement for another, structurally-similar EAA
- toxicity = overfeeding of one EAA and negative effects not mitigated by increasing other EAA
- antagonism = one EAA regulates uptake of another