Aminoacids
Peptides
Proteins

Books

Harper`s Biochemistry

Stryer – Biochemistry

Lehninger – Biochemistry

Moore JT, Langley RH – Biochemistry for Dummies

Salway JG – Medical Biochemistry at a Glance

Kaneko – Clinical Biochemistry

In addition

James Herriot

Jurgen Thorwald

Teaching aims

- getting acquinted with biochemical characteristics and metabolic meaning of aminoacids, peptides and proteins

- getting acquinted with biologically important representatives of aminoacids, peptides and proteins

Learning effects

- ability to join together chemical properties of aminoacids, peptides and proteins with their function in living body and the participation in biochemical pathways

- understanding of the meaning of aminoacids, peptides and proteins for appropriate structure and function of cells

Plasma

Liquid which is obtained from full blood collected with anticoagulant after the centrifugation of morphologic elements.

Plasma depending of used anticoagulant can be citrate, heparin or EDTA related. Moreover, it can be platellet-rich or platellet-free – it can be distinguished depending on the speed of blood centrifugation.

The concentration of total protein in **plasma**

66 – 87 g/dm^3

The concentration of total protein in **serum**

65 – 82 g/dm^3

The role of plasma proteins

- the distrubution of liquids between blood and intercellular space
- transport of hormons, metabolites, metals, drugs etc
- enzymes, regulators
- proteins of immunological system and cloting system
- components of buffers
- hormons and receptors
- components of connective tissue (adhesive)
- nutritional components

Plasma proteins

Albumins are storage material of body which can be used during starvation and the loss of proteins during the course of different diseases. They contain small content of tryptophan.

Albumins maintain constant volume of blood and oncotic pressure. They transport fatty acids, bilirubin, cholesterol and selected ions.

Plasma proteins

Globulins are characterised by higher molecular weight as albumins.

Following fractions are known:

α_1 1-4%

α_2 4-13%

β 7-13%

γ 8-19%

Plasma proteins

- **acute phase proteins** – markers of inflammation. They possess inhibitory properties for proteases (the protection of organism from lysosomal enzymes) or are carriers of different substances (haptoglobin). The representatives are: **C-reactive protein (CRP)**, α_1 antitrypsin, α_1 acid glikoprotein (transports progesteron), haptoglobin (binds and transports extracellular hemoglobin), ceruloplasmin (cooper binding).

Plasma proteins

- proteins of complement system
- transferrin
- fibrinogen
- hormon binding proteins
- **enzymes**
- **hormons**

Plasma proteins

- **immunoglobulins** – contain 2 light and 2 heavy chains bound by disulfide bonds

Classes: **IgG** – they are the component of humoral acquired answer (75%)

IgA – immunological protection of mucosal membranes, are present in saliva, tears, milk (15%)

IgM – primary immunological answer (5-10%)

IgD – biological function not fully recognised

IgE – relevant for alergic diseases

Serum

Is obtained after the centrifugation of blood collected for so called „clot”. Blood collected to dry glass clots and is centrifuged to separate serum from clot. Serum differs from plasma by the lack of fibrynogen.

Hypoproteinemia

The decrease in protein concentration is the result of the loss of proteins, the inhibition of their synthesis or the dilution of blood. Main reason for hipoproteinemia is the decrease in albumin concentration in blood or more seldom the decrease in immunoglobulin. Critical concentration of total protein is – 45g/dm^3 (albumins below 20 g/dm^3) – in this situation edema, transsudation and hypovolemia appear due to the decrease in oncotic pressure and the escape of water from vessels. Often hypoproteinemia is accompaned by dysproteinemia – the alteration in the ratio between concentrations of particular proteins eg. albumin to globulin.

Hyperproteinemia

Is the result of increased production of one or few classes of immunoglobulins. Together with hyperalbuminemia may appear during dehydratation.

Definition

Aminoacids

Organic compounds, derivatives of carobxylic acids containing amino group

Divisions

- **In accordance to configuration:**

1. Protein
2. Rarely present in proteins
3. Non-protein

Aminoacids that are present in proteins

20 aminoacids that are present in proteins and peptides:

- configuration α i L,
- Biosynthesis of proteins (codons)
- Glucose can be obtained from glucogenic aminoacids

Non-protein aminoacids

β Alanine – component of dipeptides carnosin and anserin as well as pantoteic acid which builts coenzyme A

Ornithine, cytruline – intermediates of urea cycle

γ aminobutyric acid – important for nervous system

Rarely present in proteins

- 5-hydroxylysine
- 4-hydroxyproline
- allysine

They are the result of posttranslational modifications:

- Adding of **OH** groups to selected prolins and lysins in collagen and gelatin

- Adding of **methyl** groups to selected lysins and histidins in myosin of muscles

- Adding of **carboxyl** groups to selected glutamins in cloth proteins, blood and bone proteins

- Adding of **phosphate** groups to selected serins, treonins and tyrosins

„New" aminoacids

Selenocysteine – a component of some enzyme proteins. It has a structure resembling cysteine, but instead of sulfur it has selenium built in. It does not have its own codon

Pyrolysine – derivative of lysine

Formylomethionine – derivative of methionine

Divisions

- **In accordance to the possibility to synthesise**

1. Exogenous: branched – isoleucine, leucine, valine
   aromatic – phenylalanine, tryptophan
   containing S – methionine
   basic – arginine, lysine, histidine
   containing OH group – treonine
2. Endogenous: remaining

Consequences: diet; transamination

Divisions

- **In acordance to Karlson**

1. **With apolar side chain**

   alanine, valine, leucine, isoleucine, proline, phenyloalanine, tryptophan, methionine

2. **With polar side chain but without charge**

   glycine, serine, treonine, cysteine, tyrosine, asparagine, glutamine

3. **Monoaminodicarboxylic** (negatively charged)

   asparaginic acid, glutaminic acid

4. **Diaminomonocarboxylic** (positively charged)

   lysine, arginine, histidine

Divisions

- **In accordance to participation in metabolic pathways**

1. **glucogenic:**

   pyruvate pathway (and acetylCoA): glycine, serine, cystine, cysteine, alanine

   glutaminic acid pathway (and α-ketoglutarate): arginine, proline, histidine, glutamine

   succinate pathway: methionine, isoleucine, valine

   oxalacetate pathway: asparagine, asparaginic acid

2. **ketogenic:**

   acetoacetyl-CoA pathway: leucine, lysine

3. **mixed**: isoleucine, phenyloalanine, tyrosine, tryptophane, threonine

Divisions

- **in accordance to the participation in secondary structures of proteins**

1. **Stabilizing of α helix**: alanine, leucine, phenyloalanine, tyrosine, tryptophan, cysteine, methionine, histidine, asparagine, glutamine, valine

2. **Destabilizing of α helix**: serine, isoleucine, threonine, glutaminic acid, asparaginic acid, lysine, arginine, glycine

3. **Interrupting of α helix**: proline, hydroxyproline

Reactions of amino group

- with nitrous acid – nitrogen is produced and the exchange of amino group into hydroxyl group appears (the determination of liberated nitrogen is the basis for quantitative estimation of aminoacids in accordance to the method of van Slyke)
- N-acylation with halides or acid anhydrides - N-acyloaminacids are formatted, it can be used for the protection of amino groups during the synthesis of peptides
- N-alkylation, methylation – betains of appropriate aminoacids are formatted, the donor is adenozyl-methionin
- Ninhydrine reaction – serves for quantitative determination of aminoacids

Reactions of amino group

- Reaction of Sanger with fluorodinitrobenzene – serves for labelling and quantitative determination of amino groups in aminoacids and peptides
- Reaction of Edman with fenyloizotiocyanate – fenylotiokarbamoyl derivatives are formatted, which after treatment with acid undergo cyclisation with the formation of fenylotiohydantoins. Reaction serves for the identification of NH_2-terminal aminoacids in polypeptide chains and the determination of the sequence of aminoacids
- Reaction with dansyl chloride

Reactions of amino group

- deamination –ketoacids are formated
- transamination

Reactions of carboxyl group

- Reduction – aminoalkohols are formated
- Esterification – serves for the protection of carboxyl groups in the synthesis of peptides
- Decarboxylation – amines are formated (biogenic), the reaction requires pirydoxal phosphate

Biogenic amines

Histidine – **histamine** – tissue hormon which regulates blood pressure, responsible for alergic reactions

Asparaginic acid - **β alanine** – element of CoA

Glutaminic acid – **γ aminobutyric acid (GABA)**

Serine – **kolamine** – element of conjugated lipids

Threonine - **propanolamine** – element of vitamin B12

Cysteine – **cysteamine** – element of CoA

Tyrosine – **tyramine** – tissue hormon

- **dopamine** – substrate for adrenalin synthesis

Tryptophan – **tryptamine** – tissue hormon

- **serotonine** – tissue hormon

Reactions of functional groups

- Thiol group of cysteine

- Participates in redox reactions
- Participates in the formation of disulfide bonds

– with ions of heavy metals – mercaptic derivatives are formated (inactivation of active centers of enzymes)

- Oxidation – cystine is formated

Reactions of functional groups

- hydroxyl group of serine and threonine
  - Stabilizes protein structures by the formation of hydrogen bonds

Reakcje grup R

- imidazol group (cyclic) of histidine
  - Serves as ligand for metal ions

Hemoglobin contains 4 iron atoms which are bound to proteins via histidine

Physico-chemical properties

Acid-base properties depend on environmental pH

- **in acidic environment** aminoacid accepts proton, behaves like cation and in accordance to Bronsted is proton-donor

$NH_3^+ - CHR - COOH$

- **in basic environment** aminoacid returns proton, behaves like anion and is proton-acceptor

$NH_2 - CHR - COO^-$

- **in isoelectric point**

$NH_3^+ - CHR - COO^-$

Physico-chemical properties

Isoelectric point – pH where aminoacid contains balance between positive and negative charges and is neutrally charged - jon obojnaczy. In this pH value aminoacid is characterised by the lowest solubility and is not moving in electric field.

Consequences – activity of enzymes

- buffers
- isolation

Physical properties of aminoacids

Smell – glutaminic acid – przyprawa

- products of reaction between prolin and glucose – fresh bred

Taste – glutaminic acid – „umami” (5th taste)

Toxicity – pelagra – excess of Leu, insufficiency of Trp

- neurotoxicity – excess of Tyr

The determination of aminoacids

- ninhydrin method – blue product is formated (except from prolin and hydroxyprolin - yellow)
- cysteine method – black sediment of PbS is formated
- Millon`s method – red product is formated
- Method of Adamkiewicz-Hopkins – purple ring on the border of two phases is formated
- xantoprotein method – yellow nitro derivatives of aromatic aminoacids are formated
- formol titration in accordance to Sorensen

The separation of aminoacids

- electrophoresis
- chromatography

Laboratory methods for the synthesis of aminoacids

- Acidic hydrolysis
- Basic hydrolysis
- Enzymatic hydrolysis
- Microbiologic methods
- Synthetic methods
- Prebiotic methods

The transportation of aminoacids via membranes

- system A – transports majority of neutral aminoacids
  - ma cechy wtórnego transportu aktywnego
  - zależy od wewnątrzkomórkowego stężenia aminokwasów
  - podlega regulacji hormonalnej
- układ ASC – transportuje alaninę, serynę i cysteinę
- układ Gly- transportuje glicynę
- układ N – transportuje histydynę, glutaminę i asparaginę
- układ L – transportuje leucynę, izoleucynę, walinę i fenyloalaninę
  - działa na zasadzie dyfuzji ułatwionej

Peptids

Definition

Chain molecules containing from 2 up to 100 aminoacids, which are bound by amide bond named peptide bond. These molecules can cross dialysis membranes (molecular weight up to 10 000 D) and are not susceptible to denaturation due to the lack of secondary structure.

Divisions

- from 2 up to 10 aminoacids – OLIGOPEPTIDS
- more than 10 aminoacids – POLYPEPTIDS

- homeomeric – contain ONLY aminoacids
- heteromeric (peptolides) – contain additional structural elements

- homodetic – contain ONLY peptide bonds
- heterodetic – other bonds such as ester, disulfide, thioester may occur

The meaning of peptides

- biologically active hormons
- antybiotics
- toxins
- in food industry

Peptide bond

Substituted amide bond that binds amino group of one aminoacid with carboxyl group of next aminoacid (water is liberated). It has the character of a covalent bond and shows the following features:

- **semiunsaturated** – single bond of C-N posseses in 40% character of double bond while double bond between C=O posseses in similar range the character of single bond
- **planar** – atoms of C and N, as well as C adjacent to them are in the same plane
- **cross substituted** – C atoms are always in trans position to each other and to peptide bond
- **polar**

The synthesis of polypeptide chain

Aims:

1. The confirmation of proposed primary structures by chemical synthesis
2. The examination of the relationship between structure and biologicalactivity via synthetic analogs
3. The chemical changes of biologically active peptides in order to modify pharmacological effects
4. Economic requirements
5. The synthesis of model peptides

The synthesis of dipeptide

The stages for the determination of aminoacid sequence

1. The identification of terminal NH_2 and COOH aminoacid
2. The hydrolysis of polypeptide chain with trypsin
3. Electrophoretic or chromatographic separation of obtained fragments as well as the indetification of terminal NH_2 i COOH aminoacids
4. Gradual degradation of chain in accordance to Edman
5. The hydrolysis of chain with the use of different peptidase

Method of Sanger with 2,4-dinitrofluorobenzen

After hydrolysis newly formated DNP-aminoacids are extracted and determined chromatographically

Method of Edmana with phenyl-isotiocyanate

‘labelled” aminoacid and the chain shorter for one aminoacid are obtained

Biologically important oligopeptids

- dipeptids - carnosine and anserine, aspartam (more sweet than sacharose)
- tripeptids - glutathione
- tetrapeptids - endomorfin
- pentapeptids - enkefalins
- heksapeptids – angiotensin IV
- heptapeptids – angiotensin III
- oktapeptids - angiotensin II
- nonapeptids – bradykinin, vazopresin, oxytocin
- dekapeptids – kinin, angiotensin I

Biologically important polypeptids

- 16-27 – endorfins α i ɣ
- 27 - secretin
- 29 – Glukagon
- 32 - Calcitonin
- 39 - ACTH
- 51 – Insulin (86 – proinsulin)
- 84 - Parathormon

Glycine (1820r.)

- characteristic for connective tissue
- is able to neutralise toxic substances via connection with their carboxyl groups for example:

  benzoic acid + glycine $\rightarrow$ hippuric acid
- participates in the synthesis of purine bases
- does not contain assymetric carbon atom
- binds to bile acids

Alanine (1888 r.)

- is present nearly in each protein
- after deamination pyruvate is formated – the substrate for gluconeogenesis

Serine (1865)

- after decarboxylation colamine is formated – the compound of conjugated lipids
- easily undergoes esterification with phosphate acid

Threonine (1935)

- easily undergoes esterification with phosphate acid

Phenyloalanine (1879) and Tyrosine (1846)

- precursor of thyroid gland hormons, adrenaline and melanine
- metabolic blocks in biochemical pathways:

- Alkaptonuria – the lack of brak homogentisin oxygenase
- Fenyloketonuria – the lack of phenyloalanin hydrolase
- Albinizm – the lack of o-difenol oxygenase

Tryptophan (1901)

- precursor in the synthesis of vitamin B3 – nicotinic acid
- after decarboxylation formates serotonine
- thanks to the presence of aromatic rings may absorb ultraviolet light at 280 nm – it provides with the possibility of spectrophotometric determinations
- formates yellow nitro derivatives with nitric acid (quantitative determinations)
- metabolic blocks in pathways:

- Disease of Hartnup –

-the lack of tryptophan

oxygenase

Histidine (1896)

- after decarboxylation histamine is formated

Cysteine (1884)

- the component of glutathione
- derivative of cysteine – taurine – creates complexes with bile acids
- derivative – cystine (1810)
- oxidation leads to the formation of cystein acid
- heating in basic solutions causes the liberation of sulfur, ammonia and pyruvate

Methionine (1922)

- donor of single carbon fragments do reactions of synthesis (as S-adenosyl-metionine)
- cysteine reaction is negative

Valine (1901), Leucine (1819), Isoleucine (1904)

- exogenous – due to branched chain

Lysine (1889), Arginine (1895)

Proline (1901)

- characteristic compound of collagen – protein of connective tissue
- derivative of proline is hydroxyproline

Asparaginic acid (1868) and Asparagine

- ability to bind ammonia

Glutaminic acid (1866) and Glutamine

- ability to transport ammonia ions