Plasma Proteins
General properties and selected examples

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Harpers Biochemistry Chapter 49

Plasma is Obtained from Centrifuged Blood
Plasma vs. serum

**Blood with anticoagulant**

- Spin blood
- Liquid, separate into 2 parts
- Cells

**Serum**

**Clotted blood**

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**Separation of Components**

- **Plasma** = Less Dense
- **Platelets / WBC’s**
- **Hematocrit**
  - “Packed Cells”
  - More Dense

**Diagram**

- Plasma (55%): Yellow
- White blood cells and platelets (<1%): Blue
- Red blood cells (45%): Red
## Components of Plasma

**Blood plasma Consists of:**
- Water 90%
- Plasma Proteins 6-8 %
- Electrolytes (Na⁺ & Cl⁻) 1%

**Other components:**
- Nutrients (e.g. Glucose and amino acids)
- Hormones (e.g. Cortisol, thyroxine)
- Wastes (e.g. Urea)
- Blood gases (e.g. CO₂, O₂)

## Plasma Proteins

**Plasma Proteins:** A complex mixture that includes hundreds of proteins
- albumin
- globulins
- fibrinogen
### Plasma Proteins

- Maintaining colloid osmotic balance (albumin)
- Buffering pH changes
- Transport of materials through blood (such as water-insoluble hormones)
- Antibodies (e.g. gamma globulins, immunoglobulins)
- Clotting factors (e.g. fibrinogen)
- Antiproteases

### Separation of Plasma Proteins

- **Solubility**
  - Salting out

- **Electrophoresis**

- **Isoelectric Focusing**

- **Use of antibodies**
### Fractions of Plasma Proteins

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Amount (%)</th>
<th>$c$ (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Albumins</strong>: albumin pre-albumin ( transthyretin)</td>
<td>52 – 58</td>
<td>34 – 50</td>
</tr>
<tr>
<td>$\alpha_1$-globulins: thyroxin-binding globulin, transcortin, $\alpha_1$-acid glycoprotein, $\alpha_1$-antitrypsin, $\alpha_1$-lipoprotein (HDL), $\alpha_1$-fetoprotein</td>
<td>2.4 – 4.4</td>
<td>2-4</td>
</tr>
<tr>
<td>$\alpha_2$-globulins: haptoglobin, macroglobulin, ceruloplasmin</td>
<td>6– 10</td>
<td>5 – 9</td>
</tr>
<tr>
<td>$\beta$-globulins: transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system</td>
<td>8.5 – 14.5</td>
<td>6 – 11</td>
</tr>
<tr>
<td>$\gamma$-globulins: IgG, IgM, IgA, IgD, IgE</td>
<td>10 – 21</td>
<td>8 – 15</td>
</tr>
</tbody>
</table>

### Polymorphism of Plasma Proteins

- The presence of two or more genetic variants of the same protein.
- The variants are encoded by a single locus.
- The variant appears in at least 1% of the population.
- The variants differ slightly in their amino acid sequence.
- Migrate differently under electrophoresis.
Acute Phase Proteins

- Levels of certain proteins increase during acute inflammatory states
- Level also increase
  - chronic inflammatory states
  - Cancer
- Elevation vary from 50% to 1000 fold
- These proteins play a role in body’s response to inflammation
- Examples: C-reactive protein, $\alpha_1$-antitrypsin, haptoglobin

Half-life of Plasma Proteins

- Each protein has a characteristic half life
- Determination of half-life
- Isolate protein
  - Label with $^{131}$I
  - Determine its radioactivity
    - inject into a normal subject
    - Withdraw blood sample at various time intervals
  The time for radioactivity to decline from its peak value to one half of its peak value
Most plasma proteins are glycoproteins
Albumin

- 60% of the total plasma proteins.
- Level varies with age ≈ 3.4-4.7 g/100ml
- 12 grams are produced daily in the liver
  25% of the total protein synthesis by liver,
  ≈ one half of the secreted proteins.
- 69 kDa (One polypeptide chain, 585 A. Acids)
- Half-life ~ 20 days

Synthesis of Albumin

Preproalbumin
  ↓
  signal peptide

Proalbumin
  ↓
  hexapeptide

Albumin
Albumin Structure

- Ellipsoidal shape
- Partial digestion with protease → three domains
- Highly polar with three hydrophobic clefts
- Anionic at pH 7.4 with 20 negative charges

Albumin is responsible for ≈ 80 % of the Colloidal pressure
Albumin binds to a variety of substances

- Free fatty acids
- Bilirubin
- Some steroid hormones
- Some drugs like:
  - Salicylic Acid (Aspirin)
  - Sulfonamide
- Ca$^{2+}$
- Cu$^{+}$

Binding of drugs to albumin can result in drug-drug interaction

A and B are two drugs that bind to albumin
Decreased concentration of albumin

- Inadequate source of amino acids.
  Malnutrition and muscle wasting.
- Liver disease
  Total protein can be normal (↑ globulin, ↓ albumin)
- Gastrointestinal loss
  Leakage of fluid from inflamed or diseased mucosa
- Renal disease
  Penetration of albumin through glomerulus
Analbuinemia

- Absence of Albumin from the plasma
- Rare condition
- Defect in the processing of mRNA of albumin
- Autosomal recessive inheritance
- Moderate edema
  - Compensation by ↑ synthesis of other plasma proteins

Prealbumin

- Migrates ahead of albumin
- Combines with
  - $T_4$ (Thyroxine) and $T_3$
  - Retinol binding protein
- Short half life (2 Days)
  - Sensitive marker of poor protein nutrition
- Decreased in
  - Hepatic damage
  - Tissue necrosis
**α₁-Antiproteinase**

- Also known as α₁-antitrypsin
- Inhibits trypsin, elastase, and other proteases
- ~52 kDa Glycoprotein
  - (394 A.Acidswith 3 oligosaccharide chains)
- > 90% of the α₁-fraction of human plasma
  - Principal serine protease inhibitor

**α₁-Antiproteinase (polymorphism)**

- Many polymorphic forms
- The major genotype is MM
- Alleles $P_i^M$, $P_i^S$, $P_i^Z$, $P_i^F$
- Deficiency can develop a lung disease (emphysema)
  - Individuals with ZZ genotype and PiSZ heterozygotes
  - Individuals with MS or MZ genotype usually not affected
Tissue damage in α₁-antitrypsin deficiency + pulmonary inflammation

Leukocytes ➔ Elastase

α₁-antitrypsin ➔ Inactive Elastase

Lung tissue not affected

Smoking in top of α₁-antitrypsin deficiency

• Specific methionine (met 358) is involved in binding α₁-antitrypsin to proteinase

• Smoking oxidizes this methionine

• Oxidized can not inhibit proteinases

• Individuals with PiZ phenotype at higher risk.
α₁-antitrypsin deficiency liver disease

- Polymerization of ZZ molecules in the endoplasmic reticulum of hepatocytes
- Aggregation is due to interaction between a loop in one molecule and a prominent β-pleated sheet in another molecule.
- α₁-antitrypsin is not released from cells
  ➔ Liver cirrhosis in 10% of patients

Loop-sheet polymerization of the ZZ molecules

Prominent β-pleated sheet in the Z molecules

Molecule of the M phenotype
**Haptoglobin (Hp)**

- $\alpha_2$ glycoprotein (90kDa)
- Synthesized mainly by hepatocytes
- Two kinds of polypeptide chains
  - Two $\alpha$ chains
  - One $\beta$ chain
- Three polymorphic forms
  - Hp 1-1, Hp 2-1, Hp 2-2
  - Polymorphism is associated with many inflammatory diseases.
- Acute phase protein
  - ↑ Level in a variety of inflammatory states, also in burn, nephrotic syndrome

**Haptoglobin Function**

- Binding of free hemoglobin
- 10% of the Hb degraded each day is released into the plasma

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Hb (65 kDa)

Kidney
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Haptoglobin level

- Measured as Hb binding capacity
  - Normally 40-180 mg of Hb binding capacity

- ↓↓ Level in hemolytic anemia

- Hb-Hp complex has shorter half-life than that of Hp
  \[ t_{1/2} \text{ of } Hb  \approx 90 \text{ minutes} \]
  \[ t_{1/2} \text{ of } Hp  \approx 5 \text{ days} \]
  Hb-Hp complex is cleared 80 times faster than Hp

α₁-fetoprotein (AFP)

- Was first discovered in the serum of the fetus.
- Very low level in adult.
- Detectable in the maternal blood in pregnancy
- Elevated level in some congenital defects
- Low level ⇔ increased risk of Down’s syndrome
- High level in many cases of liver cancer
**α₂-Macroglobulin**

- Large protein 720 kDa
- Tetramer of 4 identical chains
- 8-10 % of the total plasma proteins
- Synthesized by
  - Monocytes
  - Hepatocytes
  - Astrocytes
- Level varies with age, gender
- Increased level in nephrotic syndrome

**α₂-Macroglobulin**

- Pan proteinase inhibitor
  - Trypsin, pepsin, plasmin, thrombin
  - inhibitor of
    - Coagulation
    - Fibrinolysis
  - Forms complex with the proteinase followed by clearance

- Binding to many cytokines (signal molecules) and directing them to their target cells
Ceruloplasmin

- α2-globulin (160 kDa)
- Binds copper
  - Tight binding
  - 90% of the plasma copper (6 atoms/molecule)
  - Albumin binds the remaining 10%
- Has oxidase activity (ferroxidase)
- Low level in Wilson disease.

Copper: an essential trace element

- Daily intake 2-4 mg
- Body contains ~ 100 mg
- Cofactor for a number of enzymes
  - Cytochrome oxidase, amine oxidase
  - Can alternate between Cu^{2+} and Cu^{+}
- Excess Copper can be harmful
- Metallothioneins, a group of small proteins regulate tissue level of copper
C-Reacrive Protein

• Undetectable in healthy individuals
• In patients with diverse inflammatory diseases
  – Acute rheumatic fever, bacterial infection, gout ..
  – Tissue damage
• Reacts with C substance (polysaccharide in Pnumococci and molecular groups on a wide variety of bacteria
Blood Overview

plasma

~55% of blood

\[ \text{H}_2\text{O} \quad 92\% \]

plasma proteins \( 7\% \)

ions

nutrients

wastes

hormones

Blood plasma

proteins:

albumins

60% of plasma proteins

made in liver

transport:

- fatty acids
- hormones
- other stuff
Blood plasma proteins:

**globulins**

35% of plasma proteins

two types

**immunoglobulins**

aka., antibodies (Ab)

**transport globulins**

hormone-binding (thyroid H)

metalloproteins (iron)

apolipoproteins (lipids)

steroid-binding (testosterone)

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**Causes of decreased plasma albumin:**

I. Decreased synthesis
   A. malnutrition
   B. malabsorption
   C. advanced chronic liver disease

II. Abnormal distribution or dilution
   A. overhydration
   B. increased capillary permeability like in septicemia

III. Abnormal excretion or degradation
   A. nephrotic syndrome
   B. burns
   C. hemorrhage
   D. certain catabolic states
   E. loss of protein from the digestive tract

IV. Rare congenital defects
   A. hypoalbuminemia
   B. analbuminemia