TRANSAMINASE ENZYME ACTIVITIES

The α-amino group of an amino acid is transferred to an α-ketoacid by transaminase enzymes. The prosthetic group of transaminase enzymes is pyridoxal-5'-phosphate, a derivative of B₆ vitamin. The most important transaminase enzymes in diagnostics are the aspartate-aminotransferase and alanine-aminotransferase.

The aspartate-aminotransferase, ASAT (EC 2.6.1.1)

The aspartate-aminotransferase (former glutamate-oxaloacetate-transaminase GOT, GOT activity of serum is called sGOT) localising to all organs, - its concentration is extremely high in the liver and heart - catalyses the following reaction (Fig. 1.):

\[
\begin{align*}
\text{COO}^- & \quad + \quad \text{CH}_2\text{CH} - \quad \text{CH} - \quad \text{NH}_3^+ \\
\text{CH} & \quad + \quad \text{CH}_2\text{CO} - \quad \text{COO}^- \\
\text{CH} & \quad + \quad \text{CH}_2\text{CH} - \quad \text{NH}_3^+ \\
\text{COO}^- & \quad + \quad \text{CH}_2\text{CO} - \quad \text{COO}^-
\end{align*}
\]

Figure 1: The ASAT (GOT) enzyme activity

The alanine-aminotransferase, ALAT (EC 2.6.1.2)

The alanine-aminotransferase (former glutamate-pyruvate-transaminase GPT, GPT activity of serum is called sGPT) enzyme activity is localized to the liver, kidney, heart, and skeletal muscle cells. Its activity is high in the liver cells in particular. The ALAT enzyme catalyses the following reaction (Fig. 2.):
The ALAT activity is detectable in both mitochondria and about 50-85% of total enzyme activity of cells in the cytosol.

**Determination of the aspartate-aminotransferase, ASAT [GOT] activity**

**The principle of the determination**

\[ \text{L-Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ASAT}} \text{Oxaloacetate} + \text{L-Glutamate} \]

\[ \text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+ \]

\(\text{MDH} = \text{malate-dehydrogenase}\)

**Reagents**

**Reagent 'ASAT':**

- 80.00 mmole/l TRIS buffer pH=7.8
- 200.00 mmole/l L-aspartate
- 12.00 mmole/l \(\alpha\)-ketoglutarate
- 0.60 kU/l Malate dehydrogenase (MDH)
- 0.18 mmole/l NADH

**Test samples:**

serum of a 'normal' and an 'ill' patient
Procedure

Please, determine the ASAT activity of the 'ill' patient only after the complete determination - incubation, photometry - of the 'normal' patient.

Set up the reaction in photometer cuvettes

<table>
<thead>
<tr>
<th>Reagents</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagens 'ASAT'</td>
<td>1,0</td>
<td>1,0</td>
</tr>
<tr>
<td>Serum of 'normal' patient</td>
<td>*0,1</td>
<td>0,0</td>
</tr>
<tr>
<td>Serum of 'ill' patient</td>
<td>0,0</td>
<td>*0,1</td>
</tr>
</tbody>
</table>

*Start of reaction

Read the optical densities of the samples each minute for a 5 min. period at 340 nm. Distilled water serves as blank. Calculate the $\Delta A/\text{min}$ for each tube.

Calculation of the ASAT (SGOT) enzyme activity

An enzyme unit is defined as the amount of enzyme catalyzing the transamination of 1 µMol aspartate in 1 min.

$$\text{IU/l} = \frac{\Delta A/\text{min} \times 1000 \times V}{\varepsilon \times d \times v}$$

Where

- $V$ volume of reaction mixture [ml]
- $\varepsilon$ NADH$_{340\text{ nm}}$: 6.22 $[\text{cm}^2 \times \mu\text{mol}^{-1}]$
- $d$ the length of the optical way in [cm]
- $v$ volume of serum added to the test [ml]

Linearity: If the $\Delta A/\text{min}$ values of samples are higher than 0.150 (340 nm), dilute samples fivefold or tenfold with a 0.9 % solution of NaCl. Determine the enzyme activity with the diluted sample and multiply the results by 5 or 10.
The alanine amino transferase, ALAT [GPT]

The principle of determination

\[
\begin{align*}
\text{L - Alanine} + \alpha - \text{ketoglutarate} & \xrightarrow{\text{ALAT}} \text{Pyruvate} + \text{L - Glutamate} \\
\text{Pyruvate} + \text{NADH} + H^+ & \xrightarrow{\text{LDH}} \text{L - Lactate} + \text{NAD}^+
\end{align*}
\]

LDH = lactate-dehydrogenase

Reagents

**Reagent 'ALAT'**
- 100.00 mmole/l TRIS buffer pH=7.5
- 500.00 mmole/l L-alanine
- 15.00 mmole/l \(\alpha\)-ketoglutarate
- 1.20 kU/l Lactate dehydrogenase (LDH)
- 0.18 mmole/l NADH

**Test samples**
- serum of a 'normal' and an 'ill' patient

Procedure

Determine the ASAT activity of the 'ill' patient only after the complete determination - incubation, photometry - of 'normal' patient.

**Set up the reaction in photometer cuvettes**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 'AL'</td>
<td>1,0</td>
<td>1,0</td>
</tr>
<tr>
<td>Serum of 'normal' patient</td>
<td>*0,1</td>
<td>0,0</td>
</tr>
<tr>
<td>Serum of 'ill' patient</td>
<td>0,0</td>
<td>*0,1</td>
</tr>
</tbody>
</table>

*Start of reaction

Read optical activities for 5 minutes at 340 nm. Distilled water serves as blank. Calculate the \(\Delta A/\text{min}\) for each tubes.
Calculation of the ALAT (SGPT) enzym activity

An enzyme unit is defined as the amount of enzyme catalyzing the transamination of 1 µMole alanine in 1 min.

\[
IU/l = \frac{\Delta A/min \times 1000 \times V}{\varepsilon \times d \times v}
\]

Where
- \( V \) volume of reaction mixture [ml]
- \( \varepsilon \) NADH \( 340 \text{ nm} \): \( 6.22 \text{ [cm}^2 \times \mu\text{mol}^{-1}\text{]} \)
- \( d \) the length of the optical way in [cm]
- \( v \) volume of serum added to the test [ml]

Linearity: If the \( \Delta A/min \) values of samples are higher than 0.150 (340 nm), dilute samples fivefold or tenfold with a 0.9 % solution of NaCl. Determine the enzyme activity with the diluted sample and multiply the results by 5 or 10.

Biomedical aspects of transaminase activity

The transaminase enzymes show a low level in the plasma under normal circumstances. With the accelerated death of cells - virus infection, necrosis - soluble enzymes enter the bloodstream. Parallel determination of both transaminase activities is of great importance in the diagnosis and evaluation of liver and heart diseases.

Myocardial infarction significantly enhances the activity of ASAT (SGOT) while the activity of ALAT (SGPT) is elevated moderately. On the other hand, hepatocellular tissue destruction enhances both activity, but the level of ALAT (SGPT) is higher, than that of ASAT (SGOT). Acute hepatitis is likely when the quotient of activities of ASAT/ALAT is under the value of 1.3; on the other hand, acute myocardial infarction is followed by a higher value of ASAT/ALAT quotient.

The ASAT activity is enhanced

1. In myocardial infarction.
2. In acute rheumatoid carditis.
3. In the first 10 days of heart surgical interventions.
4. After catheter treatment of the heart.
5. After heart massage.
6. During the first 48 hours of acute pancreatitis.
7. Acute destruction of liver cells

<table>
<thead>
<tr>
<th></th>
<th>U/I 25 °C</th>
<th>U/I 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult: men</td>
<td>≤ 19</td>
<td>≤ 35</td>
</tr>
<tr>
<td>women</td>
<td>≤ 15</td>
<td>≤ 40</td>
</tr>
</tbody>
</table>

Table 1. The normal values of ASAT [GOT] activities.

The SGPT activity is enhanced

1. In acute hepatitis.
2. In liver cirrhosis
3. Mononucleosis

Normal ALAT (SGPT) activities are summarized in the table 2.

<table>
<thead>
<tr>
<th></th>
<th>U/I 25 °C</th>
<th>U/I 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult: men</td>
<td>≤ 23</td>
<td>≤ 35</td>
</tr>
<tr>
<td>women</td>
<td>≤ 19</td>
<td>≤ 40</td>
</tr>
</tbody>
</table>

Table 2: The normal values of ALAT [GPT] activities
Questions

a.) Explain the function of transaminases.

b.) A man (40 years/185 cm/95 kg) was admitted to the local hospital (0 day), complaining of pains in his chest. The activity of ASAT was determined every day. Explain the following data of the patient:

<table>
<thead>
<tr>
<th>Hours</th>
<th>0</th>
<th>12</th>
<th>18</th>
<th>24</th>
<th>30</th>
<th>72</th>
<th>192</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/l</td>
<td>15</td>
<td>45</td>
<td>90</td>
<td>105</td>
<td>108</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

Comment on the results and suggest a possible diagnosis for the patient.

c.) Propose determination of enzyme activities different from ASAT to confirm of your diagnosis.

d.) What disorders result in a raised level of activity of ALAT?

e.) Determine the ASAT and ALAT activities of 'normal' and 'ill' plasma samples supplied by the service of practical rooms. Indicate briefly how you could measure their activities.