3 Energy-rich compounds

3.1 Theoretical part

Cell as a complicated system requires the large amount of free energy for all important activities: the performance of mechanical work in the muscle contraction, and other cellular movements, the active transport of molecules and ions, and the synthesis of macromolecules and other biomolecules. Free energy used in these processes maintains an organism in a state that is far from the equilibrium.

All these processes mentioned above are energetically very demanding and cells use energy which is special for all living systems – chemical energy.

This form of energy is puts in some chemical compounds and is liberated in hydrolysis of some of group which is bounded to this compound by the high energy bonds (\(\sim\)).

There is nothing special about the bonds themselves. There are high-energy bonds in the sense that much free energy is released when they are hydrolysed for the reason given above.

If high energy bonds are hydrolysed, products of reaction go to the energetically lower state what is expressed as marked change of Gibbs energy of system \((\Delta G)\).

Most of chemical bonds in compounds of organism as ester, peptide and glycoside bonds are not high energy and in hydrolysis of these bonds liberated energy is about 10–15 kJ/mol of compound. If high energy compound (bond) is hydrolysed much more free energy is liberated (30–60 kJ/mol of high energy compound).

Energy-rich molecules are formed by the oxidation of substrates which cell obtains from the environment.

The creation of energy-rich compounds in cells is carried out by three main ways:

1. During oxidation of substrates (for instance glucose) are formed intermediates with high-energy phosphate group.

2. ATP – the most important high-energy phosphate compound and its phosphoanhydride bonds are referred to as high-energy bonds and is created in the process of oxidative phosphorylation in mitochondria.

3. Some energy-rich compounds are produced so that phosphate is transferred from ATP to another molecule in the reaction which is catalysed by kinase and high-energy bond is preserved.

3.1.1 Kinds of high-energy bonds

Energy-rich compounds in cells comprise five kinds of high-energy bonds: phosphoanhydride, acyl phosphate, enolphosphate, guanidine phosphate and thioester bonds (Fig. 3.1).

**Phosphoanhydride bond** is formed between two molecules of phosphoric acid (\(\text{H}_3\text{PO}_4\)). In hydrolysis of 1 mol of this bond is liberated approximately 30.5 kJ/mol bond. These bonds we can find in nucleotides. Typical representative of high-energy compound with phosphoanhydride bond (diphosphate bond) is ATP (adenosine triphosphate). In this compound are two high-energy diphosphate bonds (phosphoanhydride bonds). The third phosphate bond between phosphate and ribose is not energy-rich, it is phosphate ester bond.

Similar diphosphate bonds are in all di- and triphosphates of purine and pyrimidine nucleosides.

Energy of diphosphate bonds has a great importance in the metabolism of a cell. ATP serves as the principal immediate donor of free energy in biological systems in most endergonic reactions of the cell, in the active transport of molecules across membranes, muscle contraction, transmission of nerve impulse, and the other processes which require energy.

Despite that fact that ATP is the principal donor of energy (source of energy), for some metabolic pathways can be used energy of diphosphate bonds of another nucleosides as GTP (guanosine triphosphate) which is donor of energy in the proteosynthesis and also in the gluconeogenesis. UTP (uridine triphosphate) is important nucleotide in the metabolism of saccharides and CTP (cytidine triphosphate) in the metabolism of lipids.
Enolphosphate bond is formed when phosphate group is attached to the hydroxyl group which is bonded to carbon with double bond.

This bond is energetically the richest bond in hydrolysis of which is liberated 61 kJ/mol bond. Such a bond is in phosphoenolpyruvate which is formed in the breakdown of glucose in the glycolysis.

This energy-rich bond can be transferred by means of kinase to ADP to form ATP (this process is called phosphorylation of the substrate).

Acylphosphate bond is formed by the reaction of carboxylic acid with phosphate group. In the hydrolysis is liberated approximately 49 kJ/mol of energy. This type of bond is in 1,3-bisphosphoglycerate and is formed in the glycolysis and can be also transferred from this compound to ADP to form ATP.

This type of high-energy bonds is formed also in the activation of fatty acids and amino acids when these react with ATP. Product is acyladenylate resp. aminoacyl adenylate.

Guanidine phosphate bond is formed if phosphate group is attached to guanidine group. Energy of the hydrolysis is 43 kJ/mol. The most important compound with this bond is phosphocreatine. Phosphocreatine is first of all in muscle cells where is a reserve of energy for this tissue.

In some animals as a storage form of energy is arginine phosphate where phosphate group is bonded to guanidine group of arginine.

Thioester bond – is not typical high-energy bond because here is not energy-rich phosphate, but here is acyl rest of carboxylic acid attached to sulphur from –SH group. Energy liberated in the hydrolysis of this bond is around 41 kJ/mol.

In cells this type of bond is formed when the rest of carboxylic acid is attached to coenzyme A. This acid is by this way activated and can enter the different chemical reactions without the supplement of energy.

Especially important is acetyl-CoA which is one of the principal energetical substrates of the cell.
3.1.2 Energy-rich compounds in muscle cells

The muscle contraction is mechanical work and muscle cell is actually „machine“ where chemical energy of high-energy compounds is changing to mechanical work.

As the principal source of energy in the muscle contraction serves ATP. In resting muscle the muscle cell creates definite level of ATP and ratio of ATP:ADP is about 10:1. During the muscle work the great amount of ATP is used. The muscle cell has the enzymatic equipment for producing ATP in the glycolysis and also in the oxidative phosphorylation.

In active muscle level of ATP decreases. The reduced energy charge of active muscle stimulates the glycogen breakdown, glycolysis, citric acid cycle, and the oxidative phosphorylation. These processes are the relative contributions to the generation of ATP.

In the state when the muscle is active (vigorously working) appears the anaerobic conditions and so the generation of ATP by the oxidation of substrates is considerably limited.

So in the resting state muscle cell uses phosphocreatine which contains phosphoguanido group to store high-potential phosphoryl group in the muscle. The concentration of phosphocreatine in the muscle in the resting state is five more times higher than the level of ATP. Working muscle is able to use energy of phosphoguanido group of phosphocreatine for the regeneration of ATP.

Phosphocreatine is formed by the reaction:

\[
\text{ATP + creatine} \rightleftharpoons \text{phosphocreatine + ADP}\]

And in the muscle contraction the reaction is reverse:

\[
\text{phosphocreatine + ADP} \rightleftharpoons \text{ATP + creatine}\]

The reaction is catalysed by kinase. High activities of this enzyme are first of all in muscle cells and also in myocard.

3.2 The aim of practical exercise

The aim of laboratory practice is to determine activity of creatine kinase in the muscle, the myocard and the liver of a rabbit. We compare activity in homogenates of these tissues. First of all in the muscle, but also in the myocard this enzyme plays important role in the energetics of cell and its activity in these tissues is high. On the contrary the liver cell does not use phosphocreatine as reserve of energy and so activity of creatine kinase in the liver tissue is low.

The activity of creatine is also determined in the clinical laboratory. In injury of myocard this enzyme is liberated into blood and so the determination of activity of creatine kinase is one of the basic parameters in diagnosis of the infarct of myocard.

3.3 Practical part

3.3.1 The determination of phosphocreatine kinase in tissues of muscle, myocard liver of rabbit

Principle

Creatine kinase forms ATP from phosphocreatine and ADP:

\[
\text{HN=C} \overset{\text{NH}}{\sim} \overset{\text{P}}{\text{N-CH}_2\text{COOH}} \quad + \quad \text{ADP} \quad \xrightarrow{\text{CK}} \quad \text{HN=C} \overset{\text{NH}_2}{\sim} \overset{\text{P}}{\text{N-CH}_2\text{COOH}} \quad + \quad \text{ATP}
\]
The activity of enzyme is determined from the amount of generated product of the enzymatic reaction. The product creates with diacetyl in α-naphthol coloured complex. Intensity of the colour is proportional to the amount of creatine formed in the reaction.

Reagents
1. 2.10^-3 mol.l^-1 phosphocreatine in Tris-HCl buffer solution, pH 7,0 and 5.10^-3 mol.l^-1 MgCl₂ (activator of the reaction)
2. 10^-3 mol.l^-1 ADP
3. 25 % TCA
4. 2 % α-naphthol in solution of 1,5 mol.l^-1 NaOH and 1,5 mol.l^-1 Na₂CO₃
5. 0,05 % diacetyl
6. Extract of muscle of a rabbit (2 mg/100 ml of extract)
7. Extract of myocard (100 mg/100 ml extract)
8. Extract of liver of a rabbit (2 g/100 ml extract)

Procedure

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Samples (ml)</th>
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<tbody>
<tr>
<td></td>
<td>Muscle</td>
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<tr>
<td>Phosphocreatine in buffer solution, pH 7</td>
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<tr>
<td>Homogenate of muscle</td>
<td>0,5</td>
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<tr>
<td>Homogenate of myocard</td>
<td>-</td>
</tr>
<tr>
<td>Homogenate of liver</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
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<tr>
<td>ADP</td>
<td>0,5</td>
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Incubation 15 minutes

<table>
<thead>
<tr>
<th>Samples</th>
<th>Muscle</th>
<th>Myocard</th>
<th>Liver</th>
<th>Reference solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 % TCA</td>
<td>0,5</td>
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<tr>
<td>α-naphthol</td>
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<td>0,5</td>
<td>0,5</td>
<td>0,5</td>
</tr>
<tr>
<td>Diacetyl</td>
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</tr>
</tbody>
</table>

Measure absorbance against the reference solution at 525 nm

Table 3.1

3.4 The evaluation of exercise

3.4.1 Physiological values

Physiological values of creatine kinase in various tissues are very different. For instance in the liver activity of creatine kinase is only 12 nkat/g fresh tissue, in the myocard is activity about 6 μkat/g tissue and in the muscle 35 μkat/g tissue. If we compare activity in the liver and in the muscle, here is activity 3 000 times higher.

In the blood serum activity of creatine kinase is 0.8 nkat/ml serum (50 U/l serum).
3.4.2 The evaluation

From obtained absorbance values of various tissues we read the amount of created creatine in the reaction from the analytical curve.

Then from these values we calculate the amount per 1 g of fresh tissue. Activity of creatine kinase we express in nkat or μkat per gram of fresh tissue.

Illustrate the activity of enzyme in column graphs and determine how many times is the activity of creatine kinase higher in the muscle tissue than in the liver and the myocard.

<table>
<thead>
<tr>
<th>Solution 1</th>
<th>Solution 2</th>
<th>Solution 3</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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<td>0.5</td>
</tr>
<tr>
<td>2.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

3.3 Practical part

3.3.1 The determination of phosphocreatine

Liver of rabbit