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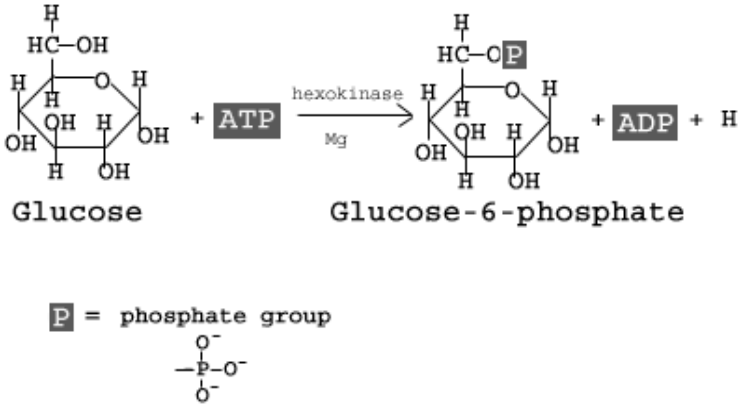
Carbohydrate metabolism. Glycolysis. Lactic acid determination

Glycolysis is the metabolic process that serves as the foundation for both aerobic and anaerobic cellular respiration. In glycolysis, **glucose is converted into pyruvate**. Glucose is found in the blood and is usually a result of the breakdown of carbohydrates into sugars. It enters cells through specific transporter proteins that move it from outside the cell into the cell's cytosol. All of the glycolytic enzymes are found in the cytosol.

The overall reaction of glycolysis which occurs in the cytoplasm is represented simply as:

$C_6H_{12}O_6$ + 2 NAD^+ + 2 ADP + 2 P ——> 2 pyruvic acid, ($CH_3(C=O)COOH$ + 2 ATP + 2 NADH + 2 H^+

Step 1: Hexokinase

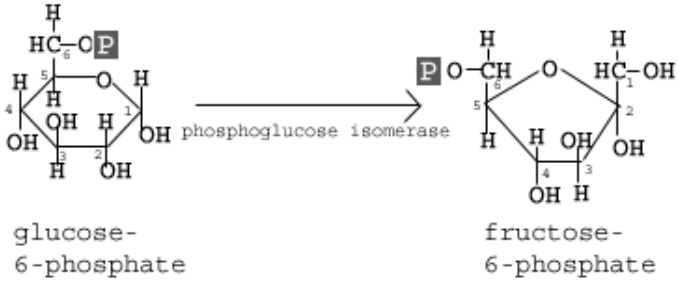


The first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate. The enzyme that catalyzes this reaction is hexokinase.
Details:

The glucose ring is phosphorylated. Phosphorylation is the process of adding a phosphate group to a molecule derived from ATP. As a result, at this point in glycolysis, 1 molecule of ATP has been consumed.

The reaction occurs with the help of the enzyme **hexokinase**. The result of this phosphorylation is **glucose-6-phosphate** (G6P).

Step 2: Phosphoglucose Isomerase

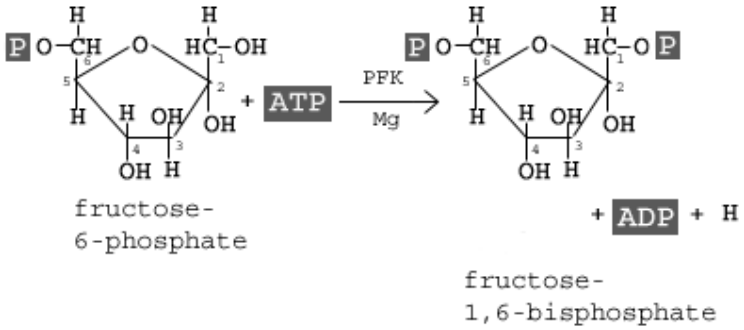


The second reaction of glycolysis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase.

Details:

The second step of glycolysis involves the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P). This reaction occurs with the help of the enzyme phosphoglucose isomerase.

Step 3: Phosphofructokinase



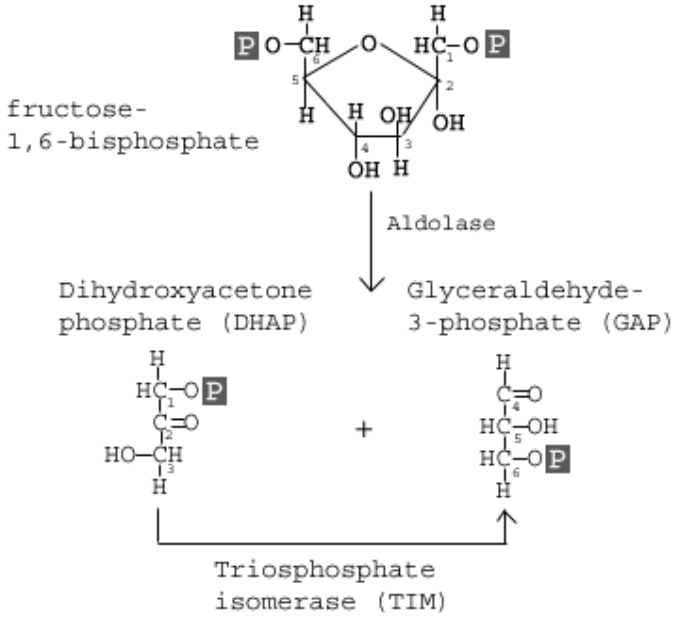
Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-bisphosphate.

Details:

In the third step of glycolysis, fructose-6-phosphate is converted to fructose- 1,6-*bis*phosphate (FBP). Similar to the reaction that occurs in step 1 of glycolysis, a second molecule of ATP provides the phosphate group that is added on to the F6P molecule.

The enzyme that catalyzes this reaction is phosphofructokinase (PFK).

Step 4: Aldolase

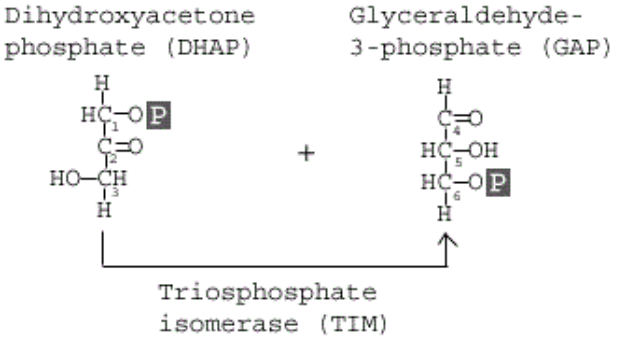


The enzyme aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).

Details:

This step utilizes the enzyme aldolase, which catalyzes the cleavage of FBP to yield two 3-carbon molecules. One of these molecules is called glyceraldehyde-3-phosphate (GAP) and the other is called dihydroxyacetone phosphate (DHAP).

Step 5: Triphosphate isomerase

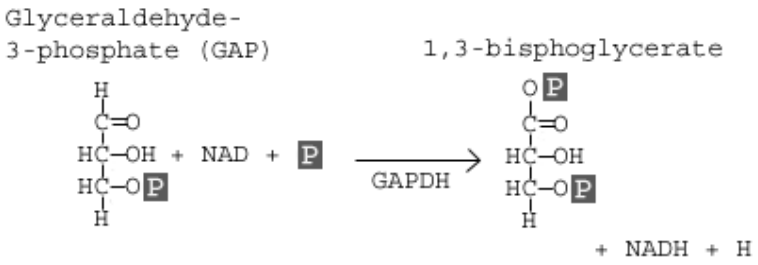


The enzyme triophosphate isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed/used in next step of glycolysis.

Details:

GAP is the only molecule that continues in the glycolytic pathway. As a result, all of the DHAP molecules produced are further converted into GAP so it can continue in glycolysis.

Step 6: Glyceraldehyde-3-phosphate Dehydrogenase

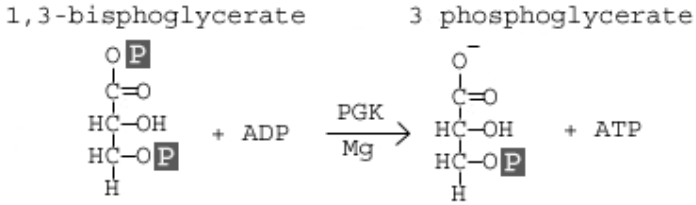


Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-bisphosphoglycerate.

Details:

In this step, two main events take place: 1) glyceraldehyde-3-phosphate is oxidized by the coenzyme nicotinamide adenine dinucleotide (NAD); 2) the molecule is phosphorylated by the addition of a free phosphate group. The enzyme that catalyzes this reaction is glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Step 7: Phosphoglycerate Kinase

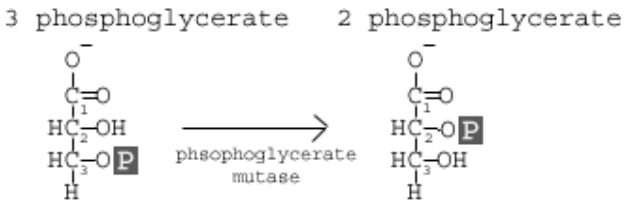


Phosphoglycerate kinase transfers a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.

Details:

In this step, 1,3-bisphoglycerate is converted to 3-phosphoglycerate by the enzyme phosphoglycerate kinase (PGK). This reaction involves the loss of a phosphate group from the starting material.

Step 8: Phosphoglycerate Mutase

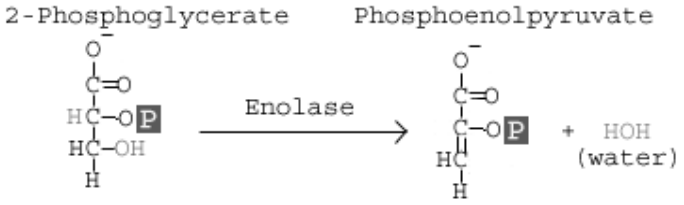


The enzyme phosphoglycero-mutase relocates the P from 3- phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.

Details:

This step involves a simple rearrangement of the position of the phosphate group on the 3 phosphoglycerate molecule, making it 2 phosphoglycerate. A *mutase* is an enzyme that catalyzes the transfer of a functional group from one position on a molecule to another.

Step 9: Enolase

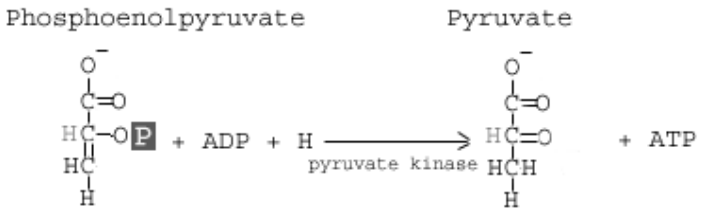


The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP).

Details:

This step involves the conversion of 2 phosphoglycerate to phosphoenolpyruvate (PEP). The reaction is catalyzed by the enzyme enolase. Enolase works by removing a water group, or *dehydrating* the 2 phosphoglycerate.

Step 10: Pyruvate Kinase



The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in step 10.

Details:

The final step of glycolysis converts phosphoenolpyruvate into pyruvate with the help of the enzyme pyruvate kinase. As the enzyme's name suggests, this reaction involves the transfer of a phosphate group. The phosphate group attached to the 2′ carbon of the PEP is transferred to a molecule of ADP, yielding ATP.

Role of Glycolysis

Glycolysis is the most fundamental system for sugar metabolism in the body. It contributes to the production of the energy currency ATP, as well as NADH, which is used to create ATP in the electron transfer system.

The pyruvate obtained by glycolysis is used in the TCA cycle only after pyruvate has bound with coenzyme A (CoA) to obtain acetyl-CoA. Glycolysis has two roles. The first role is the generation of ATP. In addition to ATP production in glycolysis, metabolism in the TCA cycle and oxidative phosphorylation of acetyl-CoA supply much more ATP. In fact, only two moles of ATP

per mole of glucose are produced under anaerobic conditions, whereas about 38 moles of ATP can be produced under aerobic conditions. The second role is the formation of intermediate metabolites, used as precursors for many biosynthetic pathways. For example, acetyl-CoA is a precursor for fatty acid synthesis. Acetyl-CoA is synthesized from proteins, sugars and lipids, as well as from pyruvate by joining the acetyl residue and CoA together. Acetyl-CoA produced in this way goes into the TCA cycle, which consists of nine steps in the matrix of mitochondria. The enzyme complex of the mitochondrial inner membrane performs several reaction steps. The end-product of glycolysis, pyruvate, is changed into acetyl-CoA by a combination of decarboxylation and CoA. Acetyl-CoA is also obtained from β-oxidation of fatty acids and metabolism of amino acids. Under anaerobic conditions, pyruvate is converted into lactate by lactate dehydrogenase. In patients with acidosis, such as in hyperventilation or diabetes, lactate is converted to pyruvate. Therefore, the reaction of lactate dehydrogenase is reversible and depends on the levels of oxygen *in vivo*.

Regulation of glycolysis

The regulatory enzymes of glycolysis are: hexokinase, glucokinase, phosphofructokinase and pyruvate kinase.

Anaerobic glycolysis is the transformation of glucose to lactate when limited amounts of oxygen (O_2) are available. Anaerobic glycolysis is only an effective means of energy production during short, intense exercise, providing energy for a period ranging from 10 seconds to 2 minutes.

Normal values: 5 – 20 mg lactic acid /100 ml blood

Physiological variations:
Lactic acid concentration is increased at new born children, after muscular intense activity, strong emotions, in the last semester of pregnancy.

PLEASE REVIEW THE ASTRUP METHOD!!
Experimental part
A. Determination of serum lactate dehydrogenase activity *(E.C. 1.1.1.27)*

Principle
The reaction catalyzed by LDH is:

$$\text{pyruvate} + \text{NADH} + {}^{+}\text{H} \underset{\text{pH = 9,0}}{\overset{\text{pH = 7,5}}{\rightleftharpoons}} \text{lactate} + \text{NAD}^{+}$$

The reaction is preceded at pH 7.5, so the pyruvate is reduced to lactate by NADH. The disappearance of NADH is followed by the decrease in absorbance at 340 nm, which is measured for several minutes either continuously or at frequent intervals. The change in the absorbance per minute (ΔA/min) is related directly to micromoles of NADH oxidized and, in turn, to micromoles of substrate transformed per minutes (International Units).

Reagents
1. Phosphate buffer, pH = 7.40. 13.97 g anhydrous K_2HPO_4 and 2.69 g anhydrous KH_2PO_4, are dissolved and brought to 1000 ml with distilled water.
2. NADH + H^+ in phosphate buffer, contains 2.5 mg/ ml
3. Sodium pyruvate in phosphate buffer, 2.5 mg sodium pyruvate/ml
4. Serum

Procedure

Pipette in the spectrophotometer cuvette:

- 2.7 ml phosphate buffer, pH 7.4
- 0.1 ml blood serum
- 0.1 ml NADH

Mix slowly and maintain the mixture at room temperature for about 20 minutes. Introduce the cuvette into the spectrophotometer and watch the absorbency at 340 nm. If the absorbency does not vary in time, add in the cuvette:

- 0.1 ml pyruvate solution

Mix, reintroduce the cuvette into the spectrophotometer, and read the absorbency at 340 nm (time 0). Read then the absorbance every 30 seconds for 5 minutes.

Plot the absorbency as a function of time. Calculate, using the graph – the linear part - the value ΔE/minute. More correct is to draw the tangent of the curve in the origin point and to use this one to calculate ΔE/minute.

The results are expressed as activity in IU.

Calculation

Calculate the LDH activity using the formula:

$$UI = \frac{\Delta E/min}{6220 \cdot 1} \cdot \frac{3.0}{0.10} \cdot 10^6 = \Delta E/min \text{ x } 4825$$

where 6220 is the molar absorbency of NADH.

At absorbencies higher than 0.1/min at 340nm, the determination is repeated with a diluted serum (1/10 with NaCl 0.9%).

Normal values

LDH : 138 - 276 IU/l

Clinical significance

Moderate increasing: viral hepatitis, diseases of skeletal muscle, acute myocardial infarct, infectious mononucleosis, acute hemolysis.

B. Determination of lactic acid and pyruvic acid in blood

The normal concentration of pyruvic acid in plasma is approx. 0.9 mg%. If anaerobic glycolysis takes place in a tissue, the concentration of lactic acid in plasma will increase, comparing with pyruvic acid concentration and this fact is illustrated by the excess lactate index-ELI (Huckabee).

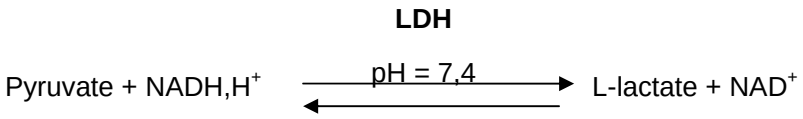
$$ELI = (L_{act} - L_o) - (P_{act} - P_o) \text{ x } L_0 / P_0$$

Where L_{act}, P_{act} = the actual (real) lactate, pyruvate respectively, concentration

L_o, P_o = the normal concentration of lactate (pyruvate)

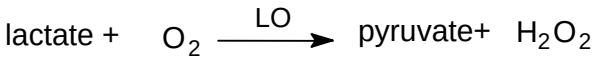
There are different methods for the determination of lactic and pyruvic acid. The quantity of acid is directly proportional with the quantity of the reaction product which can be determined by various techniques: photometry, titrimetry, gas measuring, chromatography.

The enzymatic techniques for lactic acid use lactate dehydrogenase (LDH) or lactate oxidase (LO). For LDH the reaction is:



Measures must be taken for the reaction to lead to lactic acid production (pH=9, excess of NAD^+). The resulted NADH is measured spectrophotometrically or by fluorimetry.

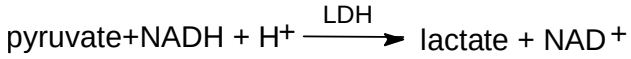
For LO, the reaction is:



The hydrogen peroxyde can be determined spectrophotometrically or using a specific electrode.

Special care must be taken at sample collection: venous stasis elevates lactic acid level and metabolic active erythrocytes produce pyruvic acid. Cooled blood (4 ^{0}C) can be used up to two hours after collection. Iodoacetate presence is recommended when collecting the sample and sodium fluoride can be used as glycolysis inhibitor.

For enzymatic determination of pyruvic acid, LDH is used:



The NADH consumption is monitored at 340 nm, being directly proportional with pyruvate consumption. Care must be taken to ensure a NADH excess and a pH of 7.40.

Pathological variations:

Hyperlactacidemia appears in diseases in which the ratio lactic acid / pyruvic acid is constant: massive perfusion of glucose, massive insulin administration, hepatic glycogenoses with glucose-6-phosphatase deficit, alcalosis by pulmonary hyperventilation.

Also it appears in diseases in which the ratio lactic acid / pyruvic acid is increased more than 20/1: epilepsy, convulsions, infections, cardiac or postsurgery shock, myocardial infarct, drug intoxication with antihistamines, neoplasm.

Hypolactacidemia appears in renal insufficiency.