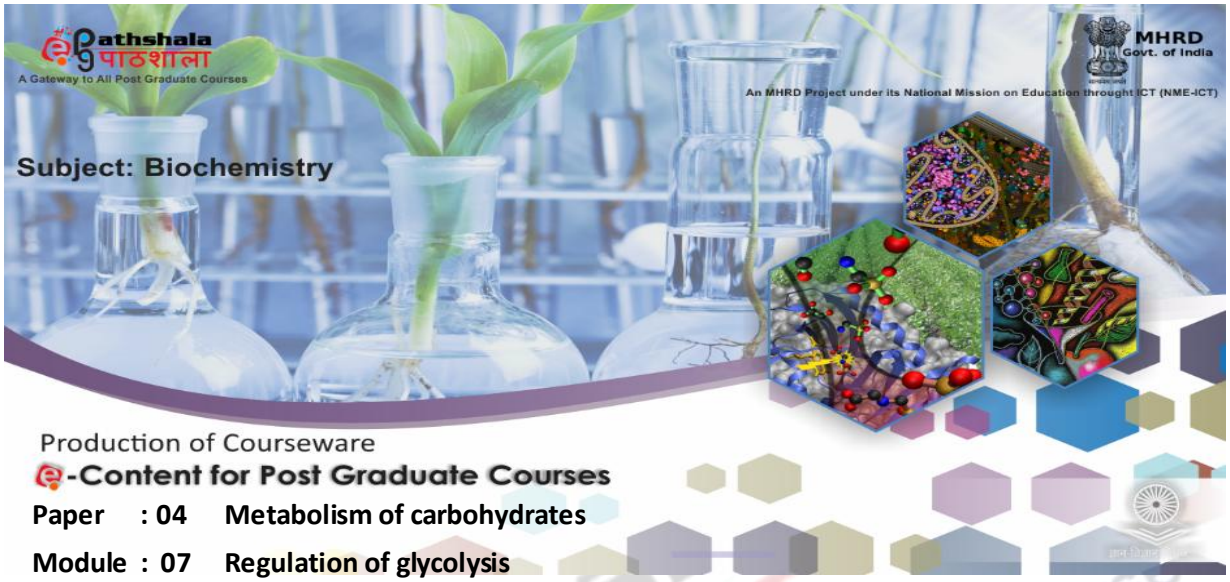

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Paper : 04 Metabolism of carbohydrates

Module : 07 Regulation of glycolysis

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MHRD Govt. of India

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Description of Module	
Subject Name	Biochemistry
Paper Name	04 Metabolism of carbohydrates
Module Name/Title	07 Regulation of glycolysis

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Regulation of glycolysis

Objectives

1. To understand importance of glucose and its metabolism
2. Glycolysis pathway
3. Glycolysis enzymes and checkpoints
4. Energy account of glycolytic reactions

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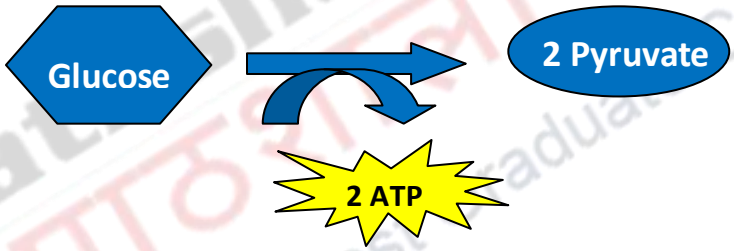
Introduction

- The word is derived from the Greek term glyk-, "sweet," and the word lysis, means "solubilization"
- Glycolysis essentially means glucose + lysis i.e breakdown of glucose
- This is one of the most ancient pathway known to human kind
- There are a series of biochemical reactions through which 1 molecule of glucose is metabolized to yield 2 moles of pyruvate (pyruvic acid) and 2 ATP as net energy yield



- Preliminarily glycolysis is a process that takes place in cytosol
- Both aerobic and anaerobic organisms have this pathway for glucose metabolism
- This process is regarded as anaerobic as oxygen is not required at any stages of glucose metabolism. However even in aerobic organisms glycolysis functions in same manner as presence of oxygen does not affect the reactions.
- The end product of glycolysis is pyruvate which can further be metabolised in two ways **(i) Anaerobic** and **(ii) Aerobic**

- The anaerobic reactions are popularly called **fermentation**e.g. pyruvate to lactate (lactic acid production) or pyruvate to ethanol (alcohol production)

- Majority of organic acid are produced from sugar fermentation in this manner

- While fate of pyruvate underaerobic conditions, generates ample amount of ATP as pyruvate is completely oxidized to CO_2 and H_2O, generating higher amount of ATP

- In the late eighteenth century Louis Pasteur carried out extensive research on yeast and studied fermentative reactions in it. Later in 1897, E. Buchner moved one step ahead and brought about fermentation using cell free extracts and launched the branch of biochemistry wherein various metabolic processes can be studied under *in-vitro* conditions.

- In the latter half of nineteenth century a complete map of glycolysis was elucidated. G. Embden, O. Meyerhof and J. Pernas were the pioneers in the work and hence the pathway is also known as the Embden-Meyerhof-Pernas pathway. Contributions by C. Neuberg, O. Warburg, G, Cori and C. Cori also noteworthy in shaping the present knownledge of glycolysis and its related biosynthetic mechanisms in a cell.Entner-Doudoroff pathway is a name given to glycolysis reactions specifically when occurring in prokaryotes specifically archaea after the contributions of M.Doudoroff and N. Entner.

For Your Information (FYI):

Entner-Doudoroff pathway varies from a typical glycolysis. Heretwo different enzymes viz. 6-phosphogluconate dehydratase and 2-keto-3-deoxyphosphogluconate aldolase are employed for the formation of pyruvate. In fact the energy efficiency of ED pathway is half of EMP pathway as only single ATP is generated by hydrolysis of one molecule of glucose is comparison to 2 ATP by EMP pathway.

Glycolysis pathway

- Glycolysis pathway has a total of 10 reactions at the end of which two moles of pyruvate is formed from single glucose molecule. Below mentioned are the reactions and enzymes involved in it.
- Glucose to Glucose-6-phosphate is a phosphorylation reaction requires energy from ATP. The reaction is irreversible and is catalysed by enzyme hexokinase (glucokinase)

For your information (FYI):

Hexokinase is the enzyme that initiates glycolysis in muscles, brain and other vital tissues while glucokinase is the enzyme that does similar reactions in liver and pancreas.

- Glucose-6-phosphate to Fructose-6-phosphate an isomerisation reversible reaction catalysed by glucose-6-phosphate isomerase enzyme (also called phosphoglucomutase)
- Fructose-6-phosphate to Fructose-1-6-bisphosphate is another irreversible reaction of this pathway and is catalysed by phosphofructokinase enzyme utilizing energy from ATP
- Fructose-1-6-bisphosphate to 2 molecules of glycerladehyde-3-phosphate. Fructose-1,6-bisphosphate actually splits into 2 3-carbon moieties, an aldehyde and a ketone: glyceraldehyde 3-phosphate (GAP) and di-hyroxy-acetone-phosphate (DHAP). The reaction is catalysed by fructose-bis-phosphate aldolase enzyme

For your information (FYI):

In glycolytic pathway dihydroxy-acetone-phosphate (DHAP) and Glyceraldehyde-3-phoshpate (GAP) [also called Phosphoglyceraldehyde (PGAL]) are isomers and are easily interconvertible. A reaction catalysed by triose-phosphate isomerase. Glyceraldehyde-3-phoshpate acts as a substrate for glycolytic reactions and hence the entire keto-product

DHAP is converted to PGAL (GAP). Hence we now have 2 molecules of GAP from each molecule of glucose

- 2 molecules of glycerladehyde-3-phosphate to 1-3-bisphosphoglycerate a dehydrogenation reaction catalysed by glyceraldehydes-3-phosphate dehydrogenase. Here in this reaction, NAD^+ is reduced to NADH + H^+ from NAD. Additionally above oxidation reaction is coupled with a phosphorylation reaction that yields 1-3-bisphosphoglycerate

- 1-3-bisphosphoglycerate to 3-phosphoglycerate is an exergonic reaction catalysed by phosphoglycerate kinase high energy bond in 1-3-bisphosphoglycerate is hydrolyzed to a carboxylic acid and the energy liberated in that is used conveniently for the formation of energy rich phosphate bond of ATP from ADP

- 3-phosphoglycerate to 2-phosphoglycerate reaction where phosphate group shifts from 3rd position to the 2nd position. The reaction is an isomerisation reactions and is catalysed by phosphoglycerate mutase

- 2-phosphoglycerate to phospho-enol-pyruvate a dehydration reaction catalysed by an enolase (phosphor-puryvate hydratase)

- Phospho-enol-pyruvate to pyruvate, an irreversible reaction is catalysed by transferase activity followed by dephosphorylation. The enzyme involved is Pyruvate kinase. The enolphosphate intermediate has a high energy phosphate bond which when hydrolyzed is converted to enolic form of Pyruvate along with formation of ATP. Enol pyruvate quickly changes to keto pyruvate which is far more stable

Glycolysis enzymes and checkpoints (regulation)

- As we saw in above glycolytic pathways, there are certain irreversible enzyme catalyzed reactions they have negative ΔG. These can be potential regulatory sites of the pathway
- The preliminary function of glycolysis is to produce energy (ATP), it must be regulated so that energy is released(ATP is generated) under needful situations
- In glycolysis, the reactions catalyzed by hexokinase (glucokinase), phosphofructokinase, and pyruvate kinase are such irreversible reactions and can behave as checkpoints for regulation of the entire pathway
- These enzymes regulated by
    1. Availability of substrate
    2. Concentration of enzymes responsible for rate-limiting steps
    3. Allosteric regulation of enzymes regulates pathway with in milliseconds
    4. Covalent modification of enzymes (e.g. phosphorylation) regulates pathway within few seconds
    5. Transcriptional control (hours)
- The intracellular concentration as well as transcription of all three enzymes is well regulated by hormonal action.
- Hormone pair of insulin-glucagon secreted by pancreas in response to sudden rise and fall in blood glucose levels. Insulin is also released in response to sudden rise in amino acid levels in the blood. As a universal effect insulin promotes the storage of excessive energy under fed state while glucagon acts antagonist to insulin in every manner.
- Insulin promotes the transcription of glucokinase (hexokinase), phosphofructokinase, and pyruvate kinase, while glucagon demotes the transcription of these 3 enzymes

- The visible effects can be seen when an individual is well-fed or is continually starved.

Checkpoint 1: Regulation of Hexokinase/glucokinase

- Hexokinase enzyme initiates glycolysis in muscles, brain it has high affinity for glucose so even at suboptimal glucose levels in blood the enzyme favors glycolysis
- The end product of this reaction glucose-6-phosphate though acts as a feedback inhibitor the overall reaction helps expenditure of too much of cellullar ATP when glucose is abundant
- Whereas the counterpart of hexokinase enzyme in liver and pancreas is glucokinase, acts at a higher concentration of glucose
- Thus when glucose concentration is at peak after rich carbohydrate intake the enzyme helps liver to remove excess glucose and thereby regulate blood glucose after meals. Moreover the enzyme activity is not inhibited by glucose-6-phosphate

Checkpoint 2: Regulation of Phosphofructokinase

- Phosphofructokinase (PFK) catalyses the phosphorylation and converting fructose-6-phosphate to fructose-1-6-bisphosphate an irreversible as well as rate limiting reaction of glycolytic pathway
- Phosphofructokinase is a tetramer having four identical subunits. ATP is allosteric inhibitor of PFK hence glycolysis is down regulated when intracellular ATP level is high
- The allosteric mechanism includes binding of ATP at a distinct site on PFK different from catalytic site and inducing a conformational change that rotates positions of two amino acids Glu161 and Arg162
- When low affinity for substrate is observed the charges are imbalanced as phosphate on F6P is repeled by Glu161

- While under high affinity for substrate F6P results in stabilization of charges between phosphate at 6th position and arginine at 162nd position
- This conformational change helps maintain intracellular ionic strength and regulate blood pH further reducing glycolysis and preventing accumulation of acids due to rigorous muscular activity under low oxygen environment. It also helps regulate and minimize lactoacidosis
- Other regulators of this enzyme are ATP(feedback inhibition), AMP(reverse inhibition), ADP(allosteric inhibition), citrate(feedback inhibition) and β-D-fructose 2-6-bisphosphate (feed-forward inhibition)

Checkpoint 3: Regulation of pyruvate kinase

- This is the last enzyme catalyzed reaction of glycolysis that is irreversible and can regulate entire pathway
- At low glucose concentration covalent phosphorylation inhibits the pyruvate kinase activity
- But if fructose 1,6 bisphosphate is formed, the reaction is activated instead and as a feed forward activator fructose-1,6-bisphosphate leads the enzyme catalysed reaction in forward direction
- Other regulators are AMP and ADP that positively regulate the reaction while ATP is a negative effector of the reaction

Energy account of glycolytic pathway

- **Energy consumed during glycolysis**

  Step 1 Hexokinase *: 1 ATP*

  Step 3 Phosphofructokinase *: 1 ATP*

- **Energy produced during glycolysis**

  Step 6 Phsophoglycerate kinase *: 2 ATP*

  Step 10 Pyruvate kinase *: 2 ATP*

- Total/ molecule of glucose : 4 ATP
- Net ATP/molecule of glucose : 2 ATP

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