4.1: Glycolysis and the pyruvate dehydrogenase complex (PDC)

Glycolysis can be divided into two parts, the preparative phase, which requires two ATP, and the energy producing phase, which produces NADH and ATP. The net result of glucose oxidation through glycolysis is two ATP, two NADH and two pyruvate. Briefly, the process of glycolysis starts with the phosphorylation of a glucose molecule (six-carbon sugar). The addition of a phosphate group traps the glucose in the cell where it will undergo isomerization to fructose 6-phosphate and further phosphorylation to fructose 1,6-bisphosphate. From here, fructose 1,6-bisphosphate is cleaved by aldolase B into two three-carbon compounds, which will ultimately produce two pyruvate. Under aerobic conditions, the pyruvate will enter the mitochondria and be oxidized to acetyl-CoA, which will enter the TCA cycle. When oxygen is limited or energy demands exceed oxygen delivery for ATP, the cell will rely on anaerobic glycolysis. In this case, lactate dehydrogenase will oxidize the NADH generated from glycolysis by reducing cytosolic pyruvate to lactate. Under these conditions oxygen is not required to reoxidize NADH, and therefore the process is referred to as anaerobic. The energy produced through this process is much less than through aerobic oxidation and therefore less favorable (figure 4.1).
Regulation of glycolysis

Glycolysis in the liver has three primary regulated and irreversible steps (figure 4.1).

**Glucokinase: Glucose to glucose 6-phosphate**

In the liver, glucose is taken up through an insulin-independent process mediated by GLUT2 transporters. Following this, glucose must be phosphorylated to be trapped in the cell. The phosphorylation of glucose to glucose 6-phosphate is catalyzed by glucokinase (figure 4.2) in the liver.
Figure 4.2: Regulatory step committed by hexo or glucokinase. The first regulatory step in glycolysis is the phosphorylation of glucose by hexo or glucokinase. The reverse reaction, which is part of gluconeogenesis, is catalyzed by glucose 6-phosphatase.

In skeletal muscle, and most other peripheral tissues, glucose is phosphorylated by hexokinase.

Glucokinase and hexokinase perform the same reaction but have very different enzyme kinetics. Glucokinase (in the liver) has a higher $K_m$ (lower affinity for glucose) when compared to hexokinase. In the liver, this enzyme will phosphorylate glucose only when glucose concentrations are high such as in the fed state. Glucokinase also has a high $V_{max}$ and is therefore not rapidly saturated. This allows for continuous glucose uptake when glucose levels are high allowing for glucose storage and the rapid removal of glucose from circulation, minimizing the likelihood of hyperglycemia. In contrast, hexokinase has a lower $K_m$ and a high affinity for glucose (figure 4.3). This enzyme becomes rapidly saturated over a very small range of glucose concentrations.

Figure 4.3: Comparison of glucokinase and hexokinase kinetics.
Regulation of glucokinase and hexokinase

Hexokinase is regulated through feedback inhibition where glucose 6-phosphate will compete with glucose for substrate binding. On the other hand, glucokinase is regulated through an alternative mechanism involving the glucokinase regulatory binding protein (GKRP). This protein will bind glucokinase and trap it in the nucleus. When glucose is high, glucokinase is released into the cytosol to phosphorylate glucose. As fructose 6-phosphate levels increase, this will inhibit the glucokinase reaction by enhancing the rebinding of glucokinase to GKRP, trapping it in the nucleus (figure 4.4).

Phosphofructokinase 1 (PFK1): Fructose 6-phosphate to fructose 1,6-bisphosphate (figure 4.5)

Following glucose phosphorylation to glucose 6-phosphate, the glucose 6-phosphate can be used for glycogen synthesis or the pentose phosphate pathway. Substrate that continues through glycolysis is isomerized to fructose 6-phosphate, which is the substrate for the reaction catalyzed by phosphofructokinase 1 (PFK1).
Regulation of phosphofructokinase 1 (PFK1)

Regulation of phosphofructokinase 1 is primarily through allosteric activation by AMP and fructose 2,6-bisphosphate. High AMP levels would indicate a lack of energy within the cell, and this would increase flux through glycolysis by enhancing the activity of PFK1. PFK1 is also inhibited by citrate and ATP; levels of these compounds are indicative of a high energy state, suggesting there are sufficient oxidation productions and glucose is diverted to storage pathways.

Fructose 2,6-bisphosphate is an important regulator of glycolysis, formed by a shunt in the glycolytic pathway. When there is an excess of fructose 6-phosphate in the cell, this substrate is accepted by phosphofructokinase 2 (PFK2) and converted to fructose 2,6-bisphosphate. This compound, fructose 2,6-bisphosphate, functions as an allosteric activator of PFK1. Additionally, PFK2 can be regulated by covalent modification such as phosphorylation. PFK2 is a bifunctional enzyme and only functions as a kinase when insulin is high and it is dephosphorylated. Under fasted conditions, when glucagon is high, this leads to the phosphorylation and inactivation of PFK2; when the enzyme is phosphorylated, it functions as a phosphatase and is referred to as fructose 2,6-bisphosphatase (FBP2) (figure 4.5).

Pyruvate kinase: Phosphoenol pyruvate to pyruvate

Following the synthesis of fructose 1,6-phosphate, aldolase will cleave this substrate into dihydroxyacetone and glyceraldehyde 3-phosphate. These three carbon compounds will be used to synthesize pyruvate in the final regulatory step of the pathway catalyzed by pyruvate kinase (PK).

Regulation of pyruvate kinase (PK)

The final regulatory step of glycolysis is the reaction catalyzed by pyruvate kinase. The enzyme converts phosphoenol pyruvate (PEP) to pyruvate. PK can be regulated by phosphorylation and allosteric means. PK is subject to feed-forward activation by fructose 1,6-bisphosphate, which allosterically activates the enzyme, increasing flux in the downward direction. As energy levels in the cell increase, ATP levels will reduce enzyme activity through allosteric inhibition (figure 4.6). PK can also be regulated through phosphorylation. Similar to PFK2, PK is dephosphorylated and active in the fed state but phosphorylated during the fasted state, which renders the enzyme inactive; the phosphorylation is glucagon mediated.

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Figure 4.6: Regulation of pyruvate kinase phosphorylation and fructose 1,6-bisphosphate.

**Movement of NADH from the cytosol to the mitochondria**

The NADH generated in the cytosol by glycolysis must be oxidized back to NAD$^+$ in order to maintain a pool of NAD$^+$ needed for glucose oxidation. As NADH oxidation takes place in the mitochondria, and the membrane is not permeable to NADH, two shuttles are used to move cytosolic NADH into the mitochondria. These processes are a way to get energy out of cytoplasmic NADH into the mitochondria.

**Glycerol 3-phosphate shuttle**

The glycerol 3-phosphate shuttle is the major shuttle used in most tissues to move NADH from the cytosol to the mitochondria for oxidation. In this pathway, NAD$^+$ is regenerated by glycerol 3-phosphate dehydrogenase, which transfers electrons from NADH to dihydroxyacetonephosphate to generate glycerol 3-phosphate. Glycerol 3-phosphate can diffuse across the mitochondrial membrane where it will donate electrons to membrane bound FAD (bound to succinate dehydrogenase) (figure 4.7).
NAD

**Glycolysis**

In glycolysis, glucose is broken down into two pyruvate molecules. Pyruvate dehydrogenase complex (Pyruvate dehydrogenase complex) is a key transition point between cytosolic and mitochondrial metabolism.

Under aerobic conditions, the pyruvate produced by glycolysis will be oxidized to acetyl-CoA using the pyruvate dehydrogenase complex (PDC).

**Malate-aspartate shuttle**

Many tissues also contain the malate-aspartate shuttle, which can also carry cytosolic NADH into the mitochondria. Cytosolic NADH is used to reduce oxaloacetate (OAA) to malate, which can cross the mitochondrial membrane. Once inside the mitochondria, malate can be oxidized to OAA to produce NADH. OAA can’t pass through the mitochondrial membrane, so it requires transamination to aspartate, which can be shuttled into the cytosol to regenerate the cycle (figure 4.8).

**Pyruvate dehydrogenase complex**

Under aerobic conditions, the pyruvate produced by glycolysis will be oxidized to acetyl-CoA using the pyruvate dehydrogenase complex (PDC). This enzyme is a key transition point between cytosolic and mitochondrial metabolism.
This complex is composed of three subunits, which require the cofactors thiamine pyrophosphate, lipoic acid, and FADH$_2$; NADH is also required for the reaction to move forward. The enzyme is highly regulated by both covalent and allosteric regulation. Deficiencies of the PDC can be recessive or X-linked (depending on the subunit deficient) and present with symptoms of lactic acidosis after consuming a meal high in carbohydrates. This metabolic deficiency can be managed by delivering a ketogenic diet and bypassing glycolysis all together.

**Regulation of the pyruvate dehydrogenase complex (PDC)**

The PDC is regulated by allosteric and covalent regulations. The complex itself can be allosterically activated by pyruvate and NAD$^+$. Elevation of substrate (pyruvate) will enhance flux through this enzyme as will the indication of low energy states as triggered by high NAD$^+$ levels. The PDC is also inhibited by acetyl-CoA and NADH directly. Product inhibition is a very common regulatory mechanism, and high NADH would signal sufficient energy levels, therefore decreasing activity of the PDC.

The PDC is also regulated through covalent modification. Phosphorylation of the complex will decrease activity of the enzyme.

The enzyme responsible for phosphorylation of the PDC is pyruvate dehydrogenase kinase. The kinase is regulated inversely to the PDC (figure 4.9). The kinase is most active when acetyl-CoA and NADH are high. These compounds will stimulate the kinase to phosphorylate and inactivate the PDC. The PDC can be dephosphorylated by a calcium-mediated phosphatase.

![Figure 4.9: Regulation of the pyruvate dehydrogenase complex (PDC).](https://med.libretexts.org/Bookshelves/Basic_Science/Cell_Biology_Genetics_and_Biochemistry_for_Pre-Clinical_Students/04%2F4%2F4.9.png)
Summary of pathway regulation

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<tr>
<td></td>
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<td>Acetyl-CoA, NADH, ATP (-)</td>
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Table 4.1: Summary of pathway regulation.

References and resources

Text


**Figures**

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