12: Pyruvate Oxidation and the TCA Cycle

Oxidation of Pyruvate and the TCA Cycle

Overview of Pyruvate Metabolism and the TCA Cycle

Under appropriate conditions, pyruvate can be further oxidized. One of the most studied oxidation reactions involving pyruvate is a two part reaction involving NAD$^+$ and a molecule called co-enzyme A, often abbreviated simply as "CoA". This reaction oxidizes pyruvate, leads to a loss of one carbon via decarboxylation, and creates a new molecule called acetyl-CoA. The resulting acetyl-CoA can enter several pathways for the biosynthesis of larger molecules or it can be routed to another pathway of central metabolism called the Citric Acid Cycle, sometimes also called the Krebs Cycle, or Tricarboxylic Acid (TCA) Cycle. Here the remaining two carbons in the acetyl group can either be further oxidized or serve again as precursors for the construction of various other molecules. We discuss these scenarios below.

The different fates of pyruvate and other end products of glycolysis

The glycolysis module left off with the end-products of glycolysis: 2 pyruvate molecules, 2 ATPs and 2 NADH molecules. This module and the module on fermentation will explore what the cell may now do with the pyruvate, ATP and NADH that were generated.

The fates of ATP and NADH

In general, ATP can be used for or coupled to a variety of cellular functions including biosynthesis, transport, replication etc. We will see many such examples throughout the course.
What to do with the NADH however, depends on the conditions under which the cell is growing. In some cases, when an external electron acceptor is not available and the electrons carried by NADH are not required for some biosynthetic reduction reaction, the cell will opt to simply recycle the NADH back into NAD\(^+\) by "dumping" the electrons onto an internal metabolite—preferably a useless waste product—and excreting that reduced metabolite. This process is called fermentation (described in more detail in the module on fermentation). Alternatively, NADH can be recycled back into NAD\(^+\) by donating electrons to something known as an electron transport chain (ETC- this is covered in the module on respiration and electron transport, titled "Cashing in on Redox"). However, please note that NADH is not generally "junk" that needs to be recycled. NADH acts as a universal and essential source of reducing power, just as ATP is a source of both energy and of phosphate for a large variety of reactions. It only needs to be processed via fermentation when there is an excess of NADH and/or a lack of NAD\(^+\) in the cell. Fermentation will help to maintain NAD\(^+\)/NADH homeostasis.

**The fate of cellular pyruvate**

- Pyruvate can be used as a terminal electron acceptor (either directly or indirectly) in fermentation reactions; this is discussed in the fermentation module. The reduced form of pyruvate could be secreted from the cell as a waste product.
- Pyruvate could be further oxidized to extract more free energy from this fuel.
- Pyruvate can serve as a valuable intermediate compound linking some of the core carbon processing metabolic pathways.

**The further oxidation of pyruvate**

In respiring bacteria and archaea, under appropriate conditions and as needed, pyruvate is further oxidized. In aerobically respiring eukaryotic cells, the pyruvate molecules produced at the end of glycolysis (which occurs in the cytoplasm) are transported into mitochondria, which are sites of cellular respiration and house oxygen-consuming electron transport chains. Here O\(_2\) will act as a externally supplied "terminal electron acceptor". Organisms from all three domains of life share similar mechanisms to further oxidize the pyruvate to CO\(_2\). First pyruvate is decarboxylated and covalently linked to co-enzyme A via a thioester linkage to form the molecule known as acetyl-CoA. While acetyl-CoA can feed into multiple other biochemical pathways we now consider its role in feeding the circular pathway known as the Tricarboxylic Acid Cycle, also referred to as the TCA cycle, the Citric Acid Cycle or the Krebs Cycle. This process is detailed below.

**Conversion of Pyruvate into Acetyl-CoA**

In a multistep reaction catalyzed by the enzyme pyruvate dehydrogenase, pyruvate is oxidized by NAD\(^+\), decarboxylated, and covalently linked to a molecule of co-enzyme A via a thioester bond. Remember: there are two pyruvate molecules produced at the end of glycolysis for every molecule of glucose metabolized; thus, two of the six original carbons will have been eliminated as CO\(_2\) at the end of this step. The CO\(_2\) will diffuse out of the cell. In addition, one molecule of NAD\(^+\) is reduced to NADH during this process per molecule of pyruvate oxidized.
<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyruvate → Acetyl CoA</td>
<td>A carboxyl group is removed from pyruvate, releasing carbon dioxide.</td>
</tr>
<tr>
<td>2</td>
<td>NAD$^+$ → NADH + CO$_2$</td>
<td>NAD$^+$ is reduced to NADH.</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>An acetyl group is transferred to coenzyme A, resulting in acetyl CoA.</td>
</tr>
</tbody>
</table>
Upon entering the mitochondrial matrix, a multi-enzyme complex converts pyruvate into acetyl CoA. In the process, carbon dioxide is released and one molecule of NADH is formed. Pyruvate is oxidized—something must simultaneously be reduced—what is it?
Ask yourself:
By the end of the TCA cycle, all of glucose's original carbons will have been lost as CO₂. So... what remains in the cell? What did the cell harvest? Why did the cell destroy this sugar molecule?

Suggested discussion

Describe the flow and transfer of energy in this reaction using good vocabulary - (e.g. reduced, oxidized, redox,
The Tricarboxylic Acid (TCA) Cycle

In the presence of a suitable **terminal electron acceptor**, acetyl CoA delivers (exchanges a bond) its acetyl group to a four-carbon molecule, oxaloacetate, to form citrate (designated the first compound in the TCA cycle).

In bacteria and archaea reactions in the TCA cycle happen in the cytosol. In eukaryotes, the TCA cycle takes place in the matrix of mitochondria. Most of the enzymes of the TCA cycle are water soluble (not in the membrane), with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion (in eukaryotes). Unlike glycolysis, the TCA cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one ATP equivalent (GTP), 3 NADH, and an FADH₂. If you enjoy bookkeeping, remember these are the values for each acetyl coA entering the cycle.

In the TCA cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD⁺ molecules are reduced to NADH, one FAD molecule is reduced to FADH₂, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the TCA cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants. (credit: modification of work by "Yikrazuul"/Wikimedia Commons)
Steps in the TCA Cycle

**Step 1:**

The first step of the cycle is a condensation reaction involving the two-carbon acetyl group of acetyl-CoA with one four-carbon molecule of oxaloacetate. The products of this reaction are the six-carbon molecule citrate and free co-enzyme A. This step is considered irreversible because it is so highly exergonic. Moreover, the rate of this reaction is controlled through negative feedback by ATP. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases. If not already, the reason will become evident shortly.

**Step 2:**

In step two, citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

**Step 3:**

In step three, isocitrate is oxidized by NAD$^+$ and decarboxylated. Keep track of the carbons! This carbon now leaves the cell as waste and is no longer available for building new biomolecules. The oxidation of isocitrate therefore produces a five-carbon molecule, α-ketoglutarate, a molecule of CO$_2$ and reduced NADH. This step is also regulated by negative feedback from ATP and NADH, and a positive effect from ADP.

**Step 4:**

Step 4 is catalyzed by the enzyme succinate dehydrogenase. Here, α-ketoglutarate is further oxidized by NAD$^+$. This oxidation again leads to a decarboxylation and thus the loss of another carbon as waste. So far two carbons have come into the cycle from acetyl-CoA and two have left as CO$_2$. At this stage, there is no net gain of carbons assimilated from the glucose molecules that are oxidized to this stage of metabolism. Unlike the previous step however succinate dehydrogenase - like pyruvate dehydrogenase before it - couples the free energy of the exergonic redox and decarboxylation reaction to drive the formation of a thioester bond between the substrate co-enzyme A and succinate (what is left after the decarboxylation). Succinate dehydrogenase is regulated by feedback inhibition by ATP, succinyl-CoA, and NADH.

Suggested discussion

We have seen several steps in this and other pathways that are regulated by allosteric feedback mechanisms (ATP and/or ADP concentrations). Is there something(s) in common about these reactions? Why might these be good steps to regulate?

**Step 5:**

In step five, there is a substrate-level phosphorylation event, where inorganic phosphate (P$_i$) is added to GDP (or ATP) to form GTP (an ATP equivalent for our purposes) or ATP. The energy that drives this substrate level phosphorylation event comes from the hydrolysis of the CoA molecule from succinyl-CoA to form succinate. Why is either GTP or ATP
produced? In animal cells there are two isoenzymes (different forms of an enzyme that carries out the same reaction) for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. As we'll see later, the process of protein synthesis primarily uses GTP as an energy source. Most bacterial systems produce GTP in this reaction.

**Step 6:**

Step six is another redox reactions in which succinate is oxidized by FAD$^+$ into fumarate. Two hydrogen atoms are transferred to FAD$^+$, producing FADH$_2$. The difference in reduction potential between the fumarate/succinate and NAD$^+$/NADH half reactions is insufficient to make NAD$^+$ a suitable reagent for oxidizing succinate with NAD$^+$ under cellular conditions. However, the difference in reduction potential with the FAD$^+$/FADH$_2$ half reaction is adequate to oxidize succinate and reduce FAD$^+$. Unlike the NADH formed during the TCA cycle, FADH remains attached to the enzyme and transfers electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion or plasma membrane (depending on whether the organism in question is eukaryotic or not).

**Step 7:**

Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate using NAD$^+$. Another molecule of NADH is produced in the process.

**Summary**

Hopefully you are still awake at this point. Note that this process completely oxidizes 1 molecule of pyruvate, a 3 carbon organic acid, to 3 molecules of CO$_2$. During this process, 4 molecules of NADH, 1 molecule of FADH$_2$, and 1 molecule of GTP (or ATP) are produced. For respiring organisms this is a significant source of energy, since each molecule of NADH and FAD$_2$ can feed directly into the electron transport chain, and as we will soon see, the subsequent redox reactions will indirectly energetically drive the synthesis of additional ATP. This suggests that the TCA cycle is primarily an energy generating mechanism evolved to extract or convert as much potential energy form the original energy source to a form cells can use, ATP (or the equivalent) or an energized membrane. However, - and let us not forget - the other important outcome of evolving this pathway is the ability to produce several precursor or substrate molecules necessary for various catabolic reactions (this pathway provides some of the early building blocks to make bigger molecules). As we will discuss below, there is a strong link between carbon metabolism and energy metabolism.

**Link to Learning**

Click through each step of the citric acid cycle [here](https://bio.libretexts.org/Courses/University_of_California_Davis/BIS_2A%3A_Introductory_Biology_(Britt)/Readings/12%3A_...).
Connections to Carbon Flow

One hypothesis that we have started exploring in this reading and in class is the idea that "central metabolism" evolved as a means of generating carbon precursors for catabolic reactions. Our hypothesis also states that as cells evolved, these reactions became linked into pathways: glycolysis and the TCA cycle, as a means to maximize their effectiveness for the cell. A side benefit to this evolving metabolic pathway was the generation of NADH from the complete oxidation of glucose - we saw the beginning of this idea when we discussed fermentation. We have already discussed how glycolysis not only provides ATP from substrate level phosphorylation, but also yields a net of 2 NADH molecules and 6 essential precursors: glucose-6-P, fructose-6-P, trios-P, 3-phosphoglycerate, phosphoenolphyruvate, and of course pyruvate. However, we have not yet described why the synthesis of NADH from NAD+ is so helpful for the cell - we'll get to that in "Cashing in on Redox".

During the process of pyruvate oxidation via the TCA cycle 4 additional "essential precursors" are formed: acetyl~CoA, alpha-ketoglutarate, oxaloacetate, and succinyl~CoA. Three molecules of CO₂ are lost and this represents a net loss of mass for the cell. These precursors, however, are substrates for a variety of catabolic reactions including the production of amino acids, fatty acids, and various co-factors, such as heme. This means that the rate of reaction through the TCA cycle will be sensitive to the concentrations of each metabolic intermediate. A metabolic intermediate is a compound that is produced by one reaction (a product) and then acts as a substrate for the next reaction. This also means that metabolic intermediates, in particular the 4 essential precursors, can be removed at any time for catabolic reactions, if there is a demand.

Not all cells have a functional TCA cycle

Since all cells require the ability of make these precursor molecules, one might expect that all organisms would have a fully functional TCA cycle. In fact, the cells of many organisms DO NOT have a the enzymes to form a complete cycle - all cells, however, DO have the capability of making the 4 TCA cycle precursors noted in the previous paragraph. How can the cells make precursors and not have a full cycle? Remember that most of these reactions are freely reversible, so, if NAD⁺ is required for the oxidation of pyruvate or acetyl~CoA, then the reverse reactions would require NADH. This "backwards" process is often referred to as the reductive TCA cycle. The reductive TCA cycle is used, by some organisms, to construct glucose and other carbon containing molecules from CO₂! To drive these reactions in reverse (with respect to the direction discussed above) requires energy and a source of reducing electrons, in this case carried by both ATP and NADH. If you can create ATP and NADH via the TCA cycle, it stands to reason that driving it in reverse will require ATP and NADH as "inputs".

Additional Links

Here are some additional links to videos and pages that you may find useful.

Chemwiki Links

- Chemwiki TCA cycle

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