Looking more closely at metabolism…Enzymes and Cofactors

Model 1. When yeast make ethanol and CO_2 from glucose, they break down glucose over *many* steps. Each of these steps is a chemical reaction (bonds are broken and formed), and each chemical reaction is catalyzed by a different *enzyme*.

An *enzyme* is a type of protein. An enzyme is composed of *amino acids* linked together by *peptide bonds*. Enzymes are proteins that can *catalyze* a chemical reaction – that makes an enzyme a *catalyst*. When the enzyme catalyzes the chemical reaction it accelerates the rate (or speed) of the reaction without being a reactant or a product. The enzyme (or catalyst) is unchanged by the reaction; it only helps the reaction go faster. Often the un-catalyzed reaction is SO slow that it doesn't seem to react at all, but in the presence of the enzyme catalyst the reaction is much faster – typically 1 million to 1 trillion times faster – fast enough to sustain life.

Key Concept
Enzymes are proteins that catalyze (accelerate the rate of) chemical reactions

Table 15.1 The enzyme catalyzed steps of glycolysis

		Substrate(s) consumed	**Product(s) produced**	**Enzyme catalyst**
These steps = 1X per glucose	1	Glucose + ATP	Glucose-6-phosphate + ADP	Hexokinase
	2	Glucose-6-phosphate	Fructose-6-phosphate	Phosphoglucoisomerase
	3	Fructose-6-phosphate + ATP	Fructose-1,6-bisphosphate + ADP	Phosphofructokinase
	4	Fructose-1,6-bisphosphate	Dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	Aldolase
	5	Dihydroxyacetone phosphate	glyceraldehyde-3-phosphate	Triose phosphate isomerase
These steps = 2X	6	Glyceraldehyde-3-phosphate + Phosphate + NAD^+	1,3-bisphosphoglycerate + NAD-H	Glyceraldehyde-3-phosphate dehydrogenase
	7	1,3-bisphosphoglycerate + ADP	3-phosphoglycerate + ATP	Phosphoglycerate kinase
	8	3-phosphoglycerate	2-phosphoglycerate	Phosphoglycerate mutase
	9	2-phosphoglycerate	phosphoenolpyruvate + H_2O	enolase
	10	Phosphoenolpyruvate+ ADP	Pyruvate + ATP	Pyruvate kinase

The name of an enzyme tells you about what it does. An enzyme name is typically formed by adding the suffix –*ase* to the name of the *substrate* (that is, the molecule that

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the enzyme performs a reaction on) and the type of reaction the enzyme catalyzes. Reactions catalyzed by enzymes are classified based on the kind of chemistry going on.

The process of *glycolysis* is a sequence of 10 enzyme catalyzed reactions that *lyse* or "split" a glucose molecule into two pyruvate molecules. In glycolysis. a single glucose molecule ($C_6H_{12}O_6$) becomes two pyruvate molecules (C_3H_3O) and two water molecules (H_2O) while producing two molecules of the energy molecule ATP[1]. Steps 6-10 are repeated for each of the two glyceraldehydes-3-phosphate molecules produced in steps 4 and 5.

Questions.

1. Explain the following expression: "*All enzymes are proteins, but not all proteins are enzymes*".

Some proteins have other purposes (ie. structure) and are not enzymes.

2. The steps of glycolysis net 2 ATP molecules – the high-energy molecule that organisms need to conduct the chemistry of life. And yet from Table 15.1, it appears that two ATP are consumed and two ATP are produced – which appears like a net of zero. How is it that glycolysis yields 2 molecules of ATP for every molecule of glucose?

Glucose splits in to 2 pyruvate.
↓
2 molecules of ATP formed per pyruvate molecule

[1] See activity 14 for a lesson on ATP

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3. There are 4 steps in Table 15.1 in which ATP is involved. What do you notice about the *names* of the enzymes catalyzing these steps?

End in "ase"

4. At the start of glycolysis, there is one glucose molecule ($C_6H_{12}O_6$). At the end of glycolysis those atoms have be rearranged to form 2 pyruvate ($C_3H_3O_2$) and 2 water (H_2O) molecules. An additional two hydrogens end up attached to NAD^+ as NAD—H.

   Explain how the process of glycolysis is consistent with the *Law of Conservation of Mass* which says, *During a chemical reaction, no atoms can be destroyed formed or changed*.

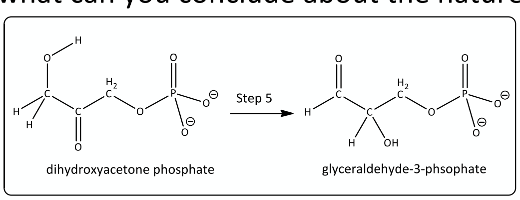
$C_6H_{12}O_6 \longrightarrow 2 \times C_3H_3O_2 + 2 \times H_2O + 2H$

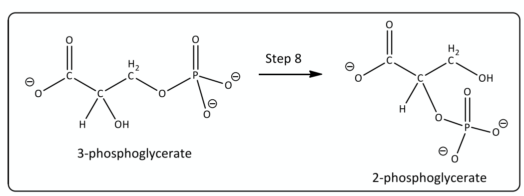
glucose

$C_6H_6O_4 + H_4O_2 + 2H$

same $\longrightarrow C_6H_{12}O_6$

5. The reactions catalyzed by *triose phosphate isomerase* and *phosphoglycerate mutase* are shown below. Notice each has only one substrate and one product. Both of these steps are examples of *isomerization* reactions. Based on these examples, what can you conclude about the nature of an *isomerization* reaction.





   Is an isomerization reaction consistent with the *Law of Conservation of Mass*? Why or why not?

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Model 2. At the conclusion of glycolysis, there is a net production of the energy molecule ATP, but a net *consumption* of the molecule NAD^+. In step 6 (Table 15.1), one molecule of NAD^+ was converted to NAD—H. Every organism that breaks down glucose via glycolysis – that includes humans and *S. cerevisiae* (baker's/brewer's yeast) – must have a way to regenerate the NAD^+ from the NAD—H or *metabolism* – the chemical reactions that create life – will cease.

In organisms that breathe oxygen – like you and I – the regeneration of NAD^+ occurs through conversion of pyruvate to acetyl-CoA. That acetyl-CoA molecule is further metabolized to two molecules of carbon dioxide (CO_2) via the *Krebs Cycle*[2] - producing additional ATP and NAD^+/NAD-H molecules for use elsewhere in metabolism.

But in baking and brewing, the yeast don't have much oxygen, and under these conditions they regenerate the NAD^+ using the reactions of ethanol fermentation.

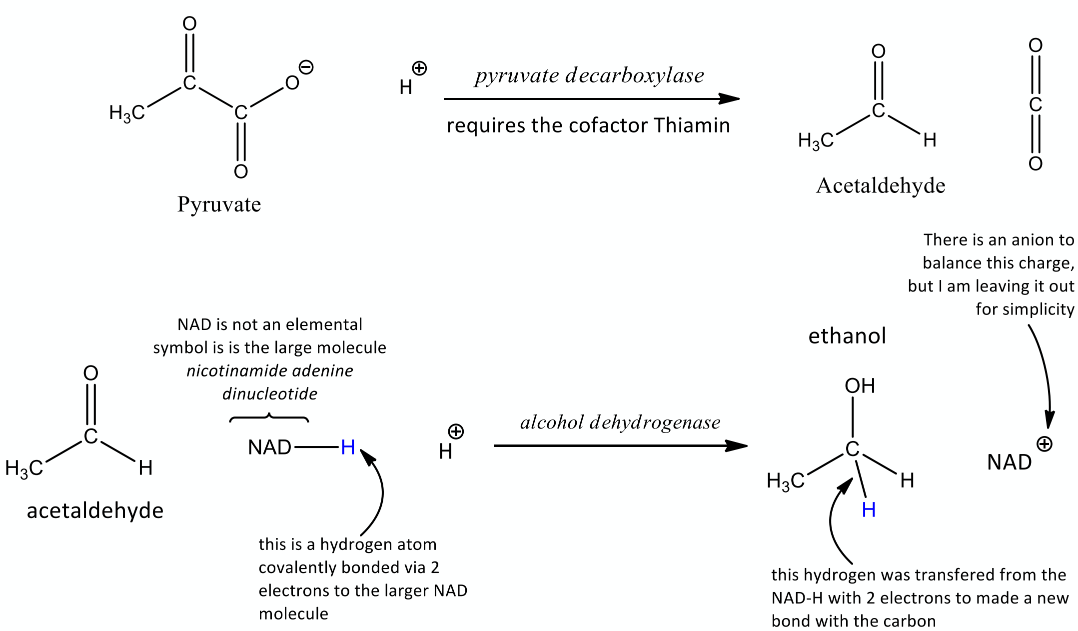

Figure 15.1. In yeast, the final two steps of the metabolism of Glucose to ethanol and CO_2 are catalyzed by the *enzymes* pyruvate decarboxylase and alcohol dehydrogenase. The alcohol dehydrogenase step regenerates the NAD^+ needed to balance glycolysis.

[2] The Krebs cycle is also known at the Citric Acid Cycle or the Tricarboxylic Acid (TCA) cycle.

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When the enzyme catalyzes the chemical reaction it accelerates the rate (or speed) of the reaction without being a reactant or a product. The enzyme (or catalyst) is unchanged by the reaction; it only helps the reaction go faster. But sometimes, enzymes need a little help to catalyze difficult chemical reactions. In the reactions of Figure 15.1 both thiamine and NAD/NAD-H are *cofactors.* Cofactors can be organic (that is, containing carbon atoms) or inorganic (that is, containing no carbon atoms) molecules that are required by an enzyme to catalyze its reaction. Compared to the enzyme itself, which is a large macromolecular protein, cofactors are relatively small.

Key Concept
Cofactors are small molecules that are necessary for some enzyme catalyzed reactions.

Table 15.2. Cofactors for enzyme catalyzed reactions that are also Vitamins.

Cofactor	Vitamin name	Disease caused by deficiency
NAD – *nicotinamide adenine dinucleotide*	Niacin	Pellagra
FAD – *flavin adenine dinucleotide,* FMN – *flavin mononucleotide*	Riboflavin	Growth retardation
Thiamin	Vitamin B1	Beriberi
Coenzyme A	Vitamin B3	Deficiency is very rare
Biotin	Biotin	Dermatitis
Pyridoxal phosphate	Vitamin B6	Various symptoms
Tetrahydofolate	Folate/Folic Acid	Anemias
Adenosylcobalmin	Vitamin B12	Pernicious anemia
L-Ascorbic Acid	Vitamin C	scurvy

A short list of cofactors is shown below in Table 15.2. Interestingly, cofactors also appear frequently on nutrition labels! (Figure 15.2) Why? Because cofactors like thiamin and NAD^+ are more commonly known to us as *vitamins*. We know *vitamins* as elements of nutrition essential for human health, but vitamins are molecules too – and most vitamins are *cofactors* for different types of enzyme catalyzed reactions.

PERCENTAGE OF U.S. RECOMMENDED DAILY ALLOWANCES (U.S. RDA)

	CEREAL	WITH SKIM MILK
PROTEIN	4	15
VITAMIN A	25	30
VITAMIN C	**	2
THIAMIN	25	30
RIBOFLAVIN	25	35
NIACIN	25	25
CALCIUM	**	15
IRON	100	100
VITAMIN D	10	25
VITAMIN B_6	25	25
FOLIC ACID	25	25
VITAMIN B_{12}	25	35
PHOSPHORUS	15	25
MAGNESIUM	15	20
ZINC	25	30
COPPER	8	10

Figure 15.2. Excerpt from a nutrition label for breakfast cereal[3]

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That means that yeast need vitamins too! All organisms require cofactors or *vitamins* for some of their enzyme catalyzed reactions.

Questions:

6. Which enzyme catalyzes the reaction in yeast that is responsible for the rising of bread dough? Explain.

pyruvate decarboxylase
↳ reaction makes CO_2

7. By definition, a catalyst (like an enzyme) can be used over and over again to *catalyze* the same reaction. For example, one molecule of catalyst could catalyze the same chemical reaction hundreds, even thousands of times. How is this consistent with the fact that the catalyst is neither a starting material (i.e. the thing on the left side of the reaction arrow) nor product? Rather the catalyst appears "over the arrow". Use the word *bonds* in your answer.

Starting materials are consumed
Catalyst break bonds in starting material + make the bonds to form products

* 8. Using the definition of catalyst, explain why NAD^+, the cofactor in Figure 15.1 is *not* a catalyst. Use the word *bonds* in your answer.

The NAD^+ molecules are consumed when they form bonds with H.

* You can skip this question

9. Hypothesize about the role of riboflavin, vitamin B6, folate….etc from Table 15.2 above. Why might it be important for humans to eat these molecules. Explain your reasoning using the word *enzyme*.

These molecules are needed for the enzyme to fuction

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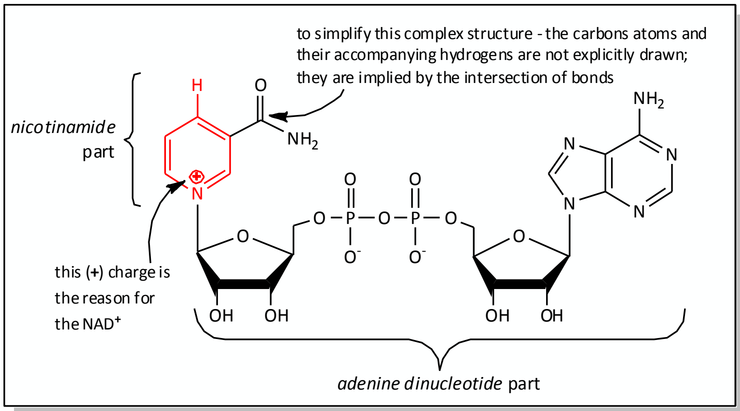
Model 3. Let's take a closer look at a very important cofactor, *nicotinamide adenine dinucleotide* and see how this molecule participates in the reaction to generate ethanol.

NAD⁺ (*nicotinamide adenine dinucleotide)*



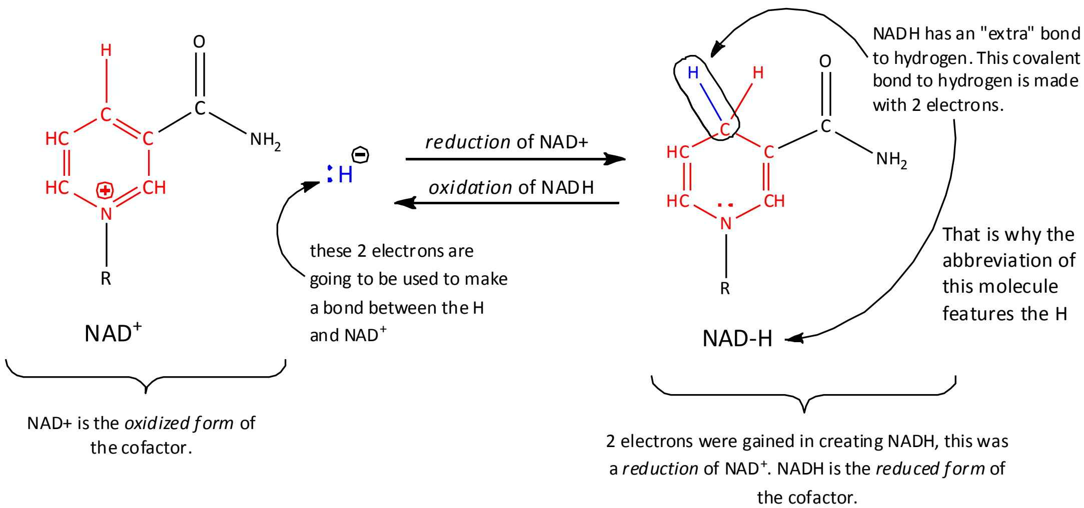


Figure 15.3. The reduced and oxidized forms of the cofactor Nicotinamide Adenine Dinucleotide (NAD^+)

NAD^+ is a mediator of *oxidation-reduction* reactions – called *redox* reactions for short. A *reduction* is the gain of electrons, while an *oxidation* is the loss of electrons.

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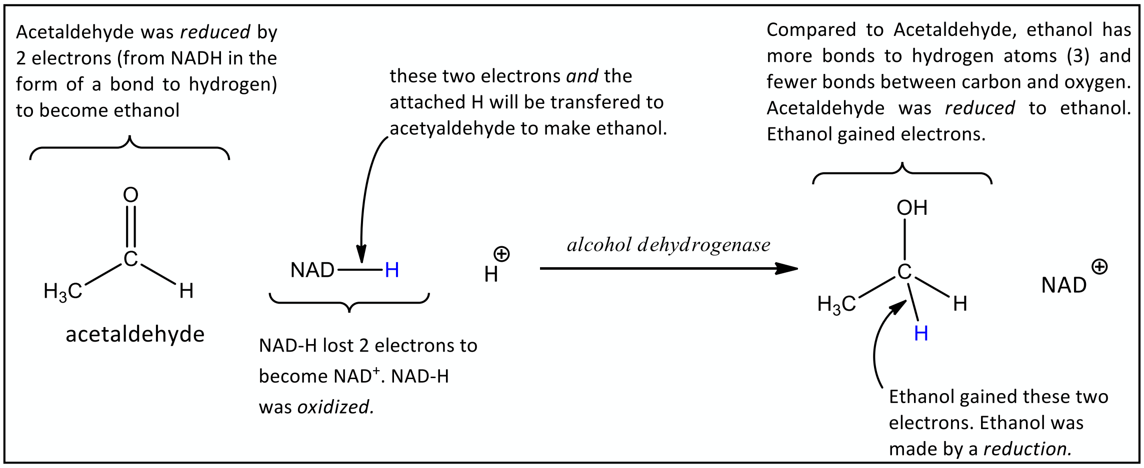

Figure 15.4. The reduction of acetaldehyde to ethanol via alcohol dehydrogenase.

Questions:

10. Define *oxidation* and *reduction* using the word *electrons.*

Oxidation - loss of electrons

reduction - gain of electrons

11. In *redox* reactions, when something is oxidized, another molecule must be reduced. You can't have one without the other.

a) Why are *oxidation* and *reduction* – by definition – linked?

When one molecule loses electrons, another molecule must pick them up or gain them

b) The alcohol dehydrogenase reaction of Model 3 is a *redox* reaction. What is getting *oxidized* and what is being *reduced*?

NAD^+ → being reduced

$H^{\ominus}$ → getting oxidized

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12. NAD+/NADH appears throughout metabolism, and it is often nicknamed the "electron carrier". Why is this a fitting description of NAD+/NADH?

$NAD^+ \rightarrow NADH$ reduction

oxidation

Readily cycles back and forth.

13. The two forms of *nicotinamide adenine dinucleotide* are also referred to as the *reduced* and *oxidized* forms of the cofactor. Which form of *nicotinamide adenine dinucleotide* is the *reduced* form (that is – *has more electrons*), and which is the *oxidized* form (that is – *has fewer electrons*)?

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