DR. BOGDAN FEDELES: Hello, and welcome to 5.07 Biochemistry online. I'm Dr. Bogdan Fedeles. Let's metabolize some problems. Today we're discussing problem 2 of problem set 6. Here we're going to explore in more detail the mechanism of phosphoglycerate mutase, which is the eighth enzyme in glycolysis. It's the enzyme that catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate.

Generally speaking, mutases are enzymes that catalyze the shift of a functional group between two similar positions of a molecule. In the case of phosphoglycerate mutase, this enzyme catalyzes the transfer of the phosphate group from the 3 position of glycerate to the 2 position of glycerate. In 5.07, you will encounter several mutases. Similar to phosphoglycerate mutase, there is a bisphosphoglycerate mutase, which converts 1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate.

Now, this reaction is very important when it happens in the red blood cells. Another mutase you will encounter is in the glycogen breakdown pathway. It's called phosphoglucomutase and converts glucose 1-phosphate to glucose 6-phosphate. Now finally, the most intriguing of them all is the methylmalonyl-coa mutase, which is a fascinating enzyme that converts methylmalonyl-coA to succinyl-coA.

In this reaction, it rearranges this carbon skeleton of the molecule, and it requires adenosylcobalamin, which is a co-factor derived from vitamin B12. Back to phosphoglycerate mutase, this is a fascinating enzyme because it uses a phosphorylated histidine in the active site. And this is actually an example of a phosphorous-nitrogen bond, one of the very few available in biochemistry.

Here is a schematic of the mechanism of phosphoglycerate mutase. Now, the reaction starts where the enzyme is already phosphorylated. We'll call it a phospho enzyme. And the histidine in the active site contains the phosphate group. Then the enzyme binds the substrate 3-phosphoglycerate. And then it's going to transfer this phosphate group onto the 2 position, the

2-hydroxyl of the 3-phosphoglycerate to generate the 2,3-bisphosphoglycerate.

Then the phosphate at the 3 position is transferred to the histidine to generate the product of the reaction 2-phosphoglycerate and regenerate the phosphoenzyme. Note that the phosphate group is in fact not transferred. This phosphate group here is not the same that ends up on the 2 position. But rather, this phosphate group gets transferred to the enzyme. And the phosphate group from the enzyme ends up on the second position of glycerate.

As we just mentioned, the active form of the enzyme has already the phosphate bound to histidine. Now the question is, how did this phosphate get there in the first place? Presumably, the enzyme is first synthesized in a form that we call apo form, which does not have the phosphate. And the phosphate is then added as a post-translational modification.

Now, our problem suggests that one source for this phosphate is phosphoenolpyruvate. And there's some experimental evidence that phosphoenolpyruvate can transfer their phosphate and phosphorylate the histidine in this enzyme. Now, we are asked to comment on how reasonable this proposal is. We're going to evaluate the proposed transformation between phosphoenolpyruvate and phosphoglycerate mutase from two points of view.

First of all, is this transformation thermodynamically accessible? And second, is this structurally feasible? We know that phosphoenolpyruvate contains a high-energy phosphate bond that can release a lot of energy upon hydrolysis. Now, if we look in our book, this is the Voet & Voet, Third Edition. If we look here, phosphoenolpyruvate, it says, releases about 62 kilojoules per mole upon hydrolysis. Now this is significantly more than what energy is released by the hydrolysis of ATP going to ADP, which is only about 31 kilojoules per mole.

Now, this should not be surprising to you because PEP, phosphoenolpyruvate, is used in the last step of glycolysis, the pyruvate kinase, to phosphorylate ADP and generate ATP. So the fact it has a higher energy of hydrolysis, it just makes that transformation thermodynamically accessible. Let's now take a look at the arrow pushing mechanism of how phosphoenolpyruvate can phosphorylate phosphoglycerate mutase.

Here is the phosphoenolpyruvate molecule, and here is the histidine in the active site of the enzyme. Now, as you know, histidine has a pKa of about 6, so an important fraction of the histidine will be protonated at physiological pH. However, for this reaction to work we need the histidine to act as a nucleophile to attack the phosphate. Therefore, we're going to consider it deprotonated.

Now, the reaction starts by assuming there's a base in the active site that's going to deprotonate the histidine, and then it's going to attack the phosphate. And finally, the phosphate group is going to leave with the assistance of a general acid. So these are the products that we obtain. This is the phosphoenzyme with the histidine that now has the phosphate group attached. And this is the enol that is released from the phosphoenolpyruvate, which is the enol form of the pyruvate.

Now, if we evaluate the starting material and the product in terms of their ability to stabilize negative charge, such as the charges on the phosphate, by resonance-- We notice that there is no significant difference. Here we have two negative charges and one set of phosphorus oxygen bonded. The charge can delocalize on this oxygen. We also have this carboxylate group, which we also have here.

So there's not a lot of resonant stabilization between starting materials and products so far. Therefore, this reaction is thermodynamically close to neutral. However, notice the enol form of pyruvate. Now this is in fact a very unstable product. And it likes to tautomerize, basically isomerize in acid base conditions to the keto form of a pyruvate. The mechanism would be, as such, the base can deprotonate the enol. And then, the general acid can protonate CH2 group to generate the keto form of the pyruvate.

It turns out the delta G for this transformation is very negative. Delta G here is approximately minus 40 kilojoules per mole. So that means this reaction is strongly going to the right, and strongly favors the keto form. So that means per ensemble the transformation going from PEP in our histidine in the active site is going to be strongly driven to the right because of this keto equilibrium.

Therefore, the entire process shown here is expected to be thermodynamically very favorable. Now let's take a look at some structural considerations. In order for PEP to phosporylate the enzyme, it has to be able to reach the histidine that's deep in the active site. Notice that the 2-phosphoglycerate is one of the products or substrates of the enzyme. And therefore, it fits very nicely in the active site.

Now phosphoenolpyruvate looks a lot like 2-phosphoglycerate. Going to sketch it here, going to have the phosphate there, and then there's the double bond in this position. So because phosphoenolpyruvate looks a lot like 2-phosphoglycerate it should have no problem fitting inside the active site of the enzyme and reaching the active site histidine.

Therefore, the chemical reaction proposed in this problem is quite reasonable. First of all, the thermodynamics are excellent because the hydrolysis of phosphoenolpyruvate gives a lot of energy. And then the sterics are also favorable because PEP resembles 2-phosphoglycerate, one of the products of the enzyme.

Part 2 of this problem asks us to evaluate the consequences on the major function of glycolysis of this reaction that we just discussed-- of phosphoenolpyruvate with phosphoglycerate mutase. Here is the second half of glycolysis, going from glyceraldehyde phosphate, or GAP, all the way to pyruvate. As you know, the main function of glycolysis is to generate ATP. And for each molecule of glucose, we have a net generation of two molecules of ATP.

Now, ATP is produced in two places. First, at the phosphoglycerate kinase when 1,3-bisphosphoglycerate can phosphorylate ADP to generate ATP. And then the pyruvate kinase where phosphoenolpyruvate phosphorylates ADP to generate ATP. Now since we start glycolysis by investing some ATP, we need two molecules of ATP to phosphorylate glucose. We recover those two molecules of ATP at the phosphoglycerate kinase step.

So all the net production of ATP that we get in glycolysis comes from the pyruvate kinase reaction shown here. Now, if phosphoenolpyruvate is used to phosphorylate phosphoglycerate mutase, basically, it's going to react to give the phosphate group here. It's going to generate pyruvate but without generating ATP, right? So the phosphate group goes to this phosphoglycerate mutase, and it generates pyruvate, but we get no net production of ATP.

Now if PEP is used to phosphorylate phosphoglycerate mutase, it's not going to be available for the pyruvate kinase step. But we do generate pyruvate, so the whole transformation reaches pyruvate, but without producing a net amount of ATP. Of course, this should not be a significant problem, as in glycolysis we only require the enzymes in catalytic amounts. So initially, we're not going to be generating net amount of ATP until we phosphorylate the entire pool of phosphoglycerate mutase.

After that, now we have phosphoglycerate mutase, so PEP is once again available for the pyruvate kinase reaction to generate ATP. I hope you noticed that there is a more subtle question here. If we're going to use PEP to phosphorylate phosphoglycerate mutase, how are we going to get to produce PEP in the first place since we need phosphoglycerate mutase to

go from 3-phosphoglycerate to 2-phosphoglycerate, which then produces PEP. Again, it's kind of like one of these chicken and the egg problems.

As you'll find out, many pathways feed into or intersect with glycolysis. And therefore, phosphoenolpyruvate could in principle be made in other ways. For example, in gluconeogenesis you'll see that pyruvate can lead to oxaloacetate, which then can lead to phosphoenolpyruvate using this enzyme called PEP carboxykinase, or PEPCK. So there are ways to produce phosphoenolpyruvate, which can then, say, phosphorylate phosphoglycerate mutase, which then allows the glycolysis to flow through in the normal way.

Well, that's it for this problem. I hope you found pretty intriguing how phosphoglycerate mutase works. Now remember, this is one of the very few enzymes in biochemistry that utilizes the phosphorylated histidine. And this is one of the few examples of a phosphorus-nitrogen bond we have in biochemistry. Also remember, the phosphoenolpyruvate is the highest high-energy phosphate compound we have in the body. But all that hydrolysis energy really comes from the keto enol tautomerization equilibrium of the pyruvate that gets released upon hydrolysis.