

Enzymes

In today's labs, we are going to be looking at the enzyme invertase. Now, this is an enzyme because it ends in the letter A, S, E, and so all the enzymes whether they are invertase, glucase, emelase, or hydroxi metal (UNCLEAR 00'15'') by big mean, but at the end, you got the A, S, E, so you know that you are looking at an enzyme. Now before you do that, you want to take a few minutes to familiarize yourself with some of the key terms including catalyst enzyme substrate (00'30''), enzyme substrate complex, active sight product, and transition state. In a nutshell, an enzyme is a catalyst. It accelerates in reactions by converting a substrate to a product. As it does so, there is an intermediate where it is neither the substrate nor the product but sort of in between and this is why it is called the enzyme substrate complex. The enzyme works by grapping the enzyme, grapping the substrate, bringing them together in the right way at (01'00'') the active sight. Once the active sight has done its job, it spits out the product and it is now ready to go ahead and attack the next substrate. This is why it is a catalyst, because it accelerates the reaction but itself is not used up. So the way in which enzymes work if you recall from lecture is that they lower the activation energy. They help make the reaction occur easier as a result; they are bringing the substrates together and sort of bending, or holding them in the right way. (01'30'') Specifically, invertase is an enzyme that acts on sucrose. What it does is that it takes the disaccharide sucrose and converts it to fructose and glucose. By doing so, we end up with a reaction where we can use the Benedict's test as it was recently done to check for the activity of the enzyme. What happens here is that if you add a Benedict's reagent to sucrose, we will get a negative reaction. (02'00'')

That is a blue product that will not give you any reaction. However, if you added a fructose and glucose, you would get a positive reaction. So what can happen is that we can use this test as an indicator of invertase activity. If there is a lot of invertase activity, a lot of sucrose is being converted to fructose and glucose and as a result then, there should be more orange color appearing in your Benedict's reaction. You have in your notes an entire page dedicated (02'30") to just looking at the Benedict's reaction. You should take some time to really understand that. What happens then is that the amount of enzyme activity can be looked at as the amount of fructose and glucose that you produce. So if the solution is very green that means there is a little bit of fructose and glucose, that means your enzyme invertase is working a little bit. If your invertase enzyme were working very well, there would be a very large amount of orange present because this means that there are large amounts of fructose and glucose. (03'00")

So what you are going to do is that you are going to carry out a couple of quick tests just to make sure that you are familiar with how to do the Benedict's test. It is really simple. Just a few drops of the solution, a little bit of water, a little bit of the test's reagent, Benedict's reagent, and you boil it for a few minutes. Then what you are going to look at is that you are going to look at these factors of temperature, pH, and enzyme concentration one at a time as you sort of get a handle on what are some of the factors that affect these enzymes (03'30"). So in order to look at the temperature experiment, you want to make sure that you get everything needed for the reaction to go at the same temperature. So put some enzyme in the ice bath, and put a little bit of substrate, that is the sucrose in the ice bath, put a little bit of water in the ice bath. We need to make sure that they are all cold before you ever combined them. Now you combined them and let

them stay for 10 minutes in the ice bath. Then, you quickly (04'00'') remove a little bit of the substance, sucrose and invertase that have been reacting together and see how much reaction has occurred. So you would take some of that enzyme substrate mixture, the invertase, and the sugar mixture. Do a Benedict's test on it and see what the results are. It is very important though that you keep everything at the same temperature all the time until you are ready to do the test. If your lab mate is holding on to the tube (04'30'') with the invertase in it, (UNCLEAR 04'32''), and you will get some inaccurate readings. So for the temperature test, you want to make sure that everything is at the same temperature before you combine them. Keep them at that temperature. Then you will test after a period of time when they had a chance to react. We are going to be doing room temperatures 37°C, 100°C which is boiling, and 0°C which is freezing.

We will also then look at the effects of pH. (05'00'') The enzyme itself usually has a buffer added to it. We will talk more about that at the very end of the lab today but we are going to see what other pH effects invertase activity. So you will be taking and adding the enzyme and substrate together, again the sucrose and the invertase and see what sort of reactivity you get at different pH is. We can try pH 1.0, 4.0, 8.0, and 12. Once you have added all those things together (05'30''), you wait a few minutes and see what the reactivity is and check. So again, if there is a strong orange color at a certain pH that means the enzyme is reacting at this pH. If there is not very much orange color at the pH that means it is not very active at this pH. A last thing you look at is enzyme concentration. You are going to set up a whole bunch of tubes with the same amount of sucrose, the same amount of invertase, buffer, and water, but then you are going to add a little bit different amount of enzyme (06'00'') and some extra water so that in the end, all

the tubes have exactly the same volume but they have slightly different amount of enzyme. Now, this is a very important time to experiment. You want to make sure that you get all the reaction tubes set up and again I will talk about how to set those tubes up as one of the last point. You get all the tubes set up and then you let them function for just ten minutes. You need to make sure that everyone of them gets check at 10 minutes. So you need to have the Benedict's test ready to go and take a little bit (06'30'') of (UNCLEAR) test tube after ten minutes so that you don't wait around because if you wait, the enzyme might be able to catch up. You could imagine a situation where we have a lot of enzyme, and we have very little enzyme. The little bit of enzyme even is going to catch up to the lot of enzyme if you wait long enough. So this is a time sensitive exercise. Just as your earlier exercise with temperature sensitive, this one is time sensitive. So make sure that you do all the experiments in such way that you are ready to take all of your samples after exactly ten minutes (07'00'') of incubation. The reason this is important is because the amount of invertase is an issue. Our enzyme cost about a nickel to do the whole lab so it is not particularly expensive. However, some enzyme cost 10,000 dollars so you want to make sure that you have enough to do the job but you are not wasting enzyme by adding enzyme in excess.

Now that said then, the other activity in lab is that you will see a fermentation demo. The teacher will set up a series of reactions to see if (07'30'') invertase acts on other sugars. So invertase, we predict is going to act on sucrose but does not react on other sugars. So what will happen is that the teacher will set up a series of fermentation tubes and if invertase is working, it is going to change the sugar and sort of help the digestion process. Eventually, as the sugar gets more and more broken down, you will

start to see gas bubbles forming in this water called fermentation tubes. So the appearance of gas in the fermentation tubes is an indicator (08'00") that the enzyme invertase is active. Now you can check about 5 or 6 sugars and you will also use a control. You want to ask yourself what is the control there for? How is that helping you to determine? It also helps you to look at the reaction and see why maybe bubbles are formed naturally by the yeast. Maybe bubbles are coming out of the solution as the tube warms up in the room. So again, focus on the control and see what different substrates the enzyme is working on and you want to check (08'30") that every so often. One of the last things I want to live you with is some general hints on how to work with enzymes. First of, make sure that everything is clean and dry. A little bit of contamination can cause you a lot of problems. Make sure that everything is accurately and completely labeled before you start. Nothing worse than getting all the experiment started and forgetting: "OH! Is that the 5% sucrose or the 10% sucrose?" So again, everything needs to be labeled. Add all of your substances together. (09'00") So for most reaction, you will have some substrate. That is the sucrose, a little bit of enzyme that is the invertase, some water and some buffer. Then you will add the enzyme last. So again, earlier I said: "Add all of your substances together". I might have accidentally mentioned invertase but make sure we are clear on that. Everything gets added first: Water, substrate, a little bit of buffer, and then the enzyme gets added last. The reason is that the enzyme is kind of wimpy and if you add it (09'30") without all of your other things in place you might actually damage the enzyme. Also then make sure that your volume transfers are very accurate. If you are not familiar with how to use a pipette, not sure on the amounts to transfer or how quick, or how accurately to transfer them, do not

wait until the experiment is halfway done to ask your instructor. The instructor will be more than happy to show you how to determine the volumes on the micropipettes. Last but not least, make sure that you give all the reactions a real quick mixing before you start. (10'00'') Sometimes students will add all the chemicals together and not get a reaction. The reason is that the sucrose is very heavy. The sugar solution like syrup sinks to the bottom of tube and maybe on the enzyme at the top of the tube because that is not as near as heavy and the tubes just do not come in contact with one another. So again, get everything a really good thorough mixing and then I think after the result of all that, you get some pretty reasonable results and be able to then graph your results before the effects again of the temperature (10'30''), pH, and enzyme concentration. Then don't forget to have someone in your group spend a few minutes every so often going up and reporting the data for the fermentation experiment.

Now as far as time savers go, a couple of hints that might help you is that if you have a bunch of tests tubes to add water, add buffer, or add sugar to; add water to all of them, then go back and add sugar to all of them, then go right back and add buffer to all of them. That way you sort of get an assembly line process going (11'00''). Also, make sure that when you are recording your results, that if you do have different people doing different activities, they save the results to show you so that they can show you what the temperature results were or they can show you what the concentration results were. As far as time saving events for each one of them, generally speaking, the temperature one takes the longest to run because you have to get everything at the right temperature. Then you have to combine them. Then you have to wait. So get that one started early. The pH enzyme concentration ones work (11'30'') a little more quickly. Just remember

also that with the enzyme concentration ones, you always want to have the enzyme last and the clock is ticking. Do not start the reaction and come back half an hour later because chances are that all of the tubes would have digested the sugar very quickly because in half an hour, they all sort of caught up. So even if you have a little bit of enzyme that is acting slow. Over time, it will catch up to the lot of enzyme that went through all the substrate very quickly. Last but not least and it is very important.

(12'00'') Make absolutely sure that you spend some time going over the processes of “despairing” in that test tube. Review the key terms. What is the enzyme? What is the substrate? What is the Benedict’s test doing? How does that help us to understand enzyme activity? I think that you will find that this lab is very revealing and very timely because chances are that right now or maybe in the past week or so, we will be covering enzymes in lecture. (12'30'')