Experimental Biochemistry
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Lecture – 36
Enzyme Kinetics (by using Enzyme from Apple Juice)

Hello everyone, welcome to Experimental Biochemistry course, in today we are going to study the Enzyme Kinetics. Actually when substrate attach to of an enzyme it is degraded to form products. So, basically enzyme acts on a substrate and substrate attach of the active site of the enzyme it is chemically acted upon by the enzyme the substrate is actually chemical acted upon by the enzyme and is degrade to form a completely different chemically product. So, in today's study we are going to see how this phenomenon takes place in terms of spectrophotometric analysis. Before going into our experiment, I just want to give you a small example which we generally see in everyday life.

I hope all of your acquainted with apple ok. So, when you cut apple into small pieces and keep it open in dry air, actually what you feel that the yellowish part of the apple which of the inner part of the apple gradually gets converted into brown color and with casual progress in time the colored gets darken and deep brown color is formed in the apple. So, why the phenomenon takes place? The general concept lies in the fact that there are various types of phenolic compounds like catechol or other polyphenolic compounds present in the apple which are actually oxidized in presence of air and who oxidize is it generally molecular oxygen cannot carry out this oxidation until and unless and enzymes come in comes into play. The enzyme which comes into play is actually catechol oxidase which is generally present in an apple. So, this is an apple so when we will cut it into pieces we can see that the color turns brownish with gradual exposure in air.

So, this catechol oxidase present in an apple is basically converting the substrate that if catechol into oxidase product which is actually benzoquinone. Now the phenomenon which we see in regular life we want to quantify it and see how the kinetics occur, that is how fast or slow the reaction takes place, for that what we need we need the enzyme, we need it enzyme from this apple. So, we will see how we can carry out the extraction of that enzyme are basically the solution from this apple number two if that we need the

substrate and what is the substrate it is catechol. So, we will actually prepare a solution of catechol and we will add this enzyme obtained from apple to bit and see how the kinetic for how the reaction gradually takes place if using UV spectrophotometric technique. In addition what we can do is that we can add one inhibitor. You have in your you have seen in your theory classes that an inhibitor actually slows down or actually modify the rate of the reaction or in certain cases actually stops the enzymatic reaction.

So, in our case we will applied any one of the enzyme are basically two enzymes actually it is phenylthiourea number one and the other is para hydroxybenzoic acid. So, we can see how this chemical compounds acts on this enzyme and prevent the enzyme reaction. So, let us proceed into our experimental procedures.

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So, the substrate we are going to take is catechol. Here this is catechol, this is the 100 m gram catechol in a bottle. So, we are going to prepare 20 millimolar or 50 ml catechol in phosphate buffer pH 7.4 solution. So, keep in mind that all the solutions we are carrying out in this reaction, we are all the solution of preparing in this reaction we are doing it in phosphate buffer pH 7.4 and the strength of the phosphate buffer you can actually vary from 10 millimolar to 20 millimolar or 25 millimolar in valley all the biological processes are done with this concentration of phosphate buffer.

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Now, we are going to take this spatula and we have keeping in mind that the spatula should be clean, actually you are cleaning this with a tissue paper and visually it is clean. However, we are again cleaning this with a tissue paper and now we are going to take this catechol, now we have calculated it for 20 millimolar and 50 ml catechol solution we required 0.11 gram catechol.

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So, we are taking 0.11 gram catechol. Here if the butter paper we have tear this and it is 00 is showing ensure we are adding a small amount of this catechol powder this were

catechol generally crystals ok. So, we have taken actually 0.1138 and gram of catechol. So, as we have said in the previous lectures, that whatever if the calculated amount the calculated weight you can take a small amount a bit higher then this calculated weight ok.

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Now, you have taken this clean falcon this is the 50 ml falcon tube. So, we have dried it and properly cleaning with double distilled water.

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So, we will transfer these catechol crystals carefully into this falcon. Now, after that what we will do, we will actually take this falcon and make up this volume up to 50ml using phosphate buffer of pH 7.4 and strength either 20 or 10 millimolar.

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Basically here the phosphate buffer we have prepared out here is the 20 millimolar. So, what we can do is that we can take half of this phosphate buffer and fill here up to 25 ml and then make up the rest of the volume with double distilled water; so that the final concentration of phosphate buffer if 10 millimolar. You can actually keep 20 or 25 as per your wish; however, sometimes this concentration might affect the reaction. So, we can try out this reaction with 10 millimolar of phosphate buffer initially.

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Now, fill this is falcon tube and the mouth is quite large. So, we can actually add this one transfer this one carefully, once you have put small amount of this phosphate buffer you can stir it gently. For beginners you can use actually pipette or a like 10 ml pipette which we have seen in the previous experimental classes and here if the graduation we can see, we will pore up to 25 ml here. I have added 25 ml of phosphate buffer and now what we can do? We will actually stir this solution. So, that this catechol devolves in the phosphate buffer. Now, we can see here there is no such crystals floating in this solution so it is completely dissolved.

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Now, to this one we will be adding double distilled water. So, the volume is actually here you can see it is 50 ml. Again what I am suggesting for beginners you can actually use pipette while pouring this double distilled water or buffer into this one. So, here what we have made, we have made actually 20 millimolar of catechol solution. Now one thing if that you could easily have added double distilled water, phosphate buffer and after that this catechol powder out here and what actually problem takes place in that case is that the volume correction may not be present in case where you have added the solid catechol after adding double distilled water.

So, always put the solid crystal so whatever you have taken the solute usually take it in this falcon tube and then add the required solvent.

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Now, we are going to prepare around 10 millimolar and 50 ml of the inhibitor. The inhibitor we are taking here if in phenylthiourea ok. So, this is often referred to as P T U you can see. So, this is actually a 25 gram bottle having solid in phenylthiourea and the molecular weight is also mentioned with the molecular formula. So, we are going to prepare around 10 millimolar and 50 ml phenylthiourea in the same procedure as previously you have done for catechol.

Now, we are going to prepare 10 millimolar of 50 ml phenylthiourea for that we need 0.076 gram of P T U that is phenylthiourea. We have again taken this spatula. The spatula we have used previously, we have cleaned it properly with double distilled_water and

then after that with clean tissue paper you have dried it and then again we are using it to take this phenylthiourea. We have again taken clean butter paper out here please do not reuse the butter paper which we have taken for weighing previous solute.

So, we have tarred it, it is 0.00 gram. Now we are going to take a pinch of this phenylthiourea, now this is 0.02 gram. We have to add a bit more. It is 0.03, 0.06 and now it again increase to 0.09. So, what we have to do we have to take a small pinch of this phenylthiourea out here and transfer it into another butter paper. So, what you do is that you can actually take this excess of solid compound if it is transfer to the butter paper into the same container or otherwise you can actually omitted. Generally in this case, we are not transferring it in this one because some contamination might be there, we are trying to avoid this because this should be pure the actually container now is 0.80, 0.078 it is. Before transferring it, we are again closing this container properly.

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And now again we have taken a new falcon washed it properly with double distilled water and if preferable you can actually reuse this one with phosphate buffer. The phosphate buffer you would be using for making this entire solution. So, fiddlestick previous with phosphate buffer as you can see the water properly spatula droplets are still here out. Now we will take this one phenylthiourea and transferred it carefully. So, here we have taken the solid phenylthiourea as we have you can see here the solid compound is there.

Now, again we will be adding phosphate buffer 25 m1 of 20 millimolar that is pinch 7.4. Now you have to stir it. Here what we can see, that the compound is not properly devolving it here. So, for that we need to add again double distilled water so I have change the concentration and increase the volume. So, although we can see that it is not dissolving properly, but if we actually give it for fornication out here with it will get devolved after fornicating it for some time.

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Now, will give it for fornication in the meantime will prepare another solution that is para hydroxy benzoic acid.

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The next inhibitor we can prepare if para hydroxybenzoic acid as there we can see in this bottle. So, we can use either of the two in inhibitive either PTU that is phenylthiourea or para hydroxy benzoic acid. Now we are going to prepare this one we again going to prepare 10 millimolar of 50 ml of para benzoic para hydroxybenzoic acid for that we need 0.069 gram.

Now, we have again taken this cleaned spatula and a fresh butter paper out here and after tarring we are adding a small pinch of para hydroxybenzoic acid our aim is to get 0.069 or basically 0.7 or 0.07 actually it is 0.03 and if we at a bit more**,** it is 0.053, it is 0.040649, now 0.040649 and small pinch out here again it have increased to 0.08, now will again take another butter paper and we will transfer a bit from here it is 0.074 we can take a small amount now it is 0.0703. We will again close this container and we have taken again falcon clean falcon, this is the falcon in which we will transfer this solid that is para hydroxy benzoic acid we carefully transfer this one.

So, here we can see the para hydroxybenzoic acid is out here and again we will be adding 25 ml phosphate buffer, here if the graduation will put this lead and will stir it here we can see it is almost dissolved in the solution a few particles are there which are actually floating in this falcon tube. So now, if we make up this volume with double distilled water, we can actually see the complete solid dissolves out here. So, make up to 50ml and now it as almost dissolved or other completely dissolved in phosphate buffer.

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Now, we have prepared all the three types of solution number one is catechol, number two is PTU and we have fornicated it a bit and here we can see that the color is almost soluble. Now, we do not seen is such solid particles floating out here in this PTU solution and the 13 we have prepared is para hydroxybenzoic acid. Now, the main thing we required if the apple enzyme.

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So, we will so we will now prepare the apple extract by taking these fresh apples. So, from market you have to just get a fresh apple and let us see how to get the apple extract from here.

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Now, you are going to extract the apple juice or rather the apple extract from this apple for that we need few things number one is the fresh apple this bought from the market, number two a knife, clean knife be careful while using a knife and then number three a mortar pestle out here and number four a beaker where will transfer this apple extract and number five a cloth ok. So, instead of this mortar pestle you can also use mixer grinder, a juice mixer grinder that would be much more easy ok. So, let us proceed for this experiment. So, we have taken this knife we have cleaned it and now we are going to cut this apple carefully.

Now I have taken out this fresh piece of apple, as you can see this one is yellowish color, but when you keep it in air for sometime this color turns into brownish. Now we are going to make these small pieces out here and the small pieces we are going to put it in the mortar pestle**.** Try to make small pieces so that it will be better for grinding. Now we are again taking the previously cut pieces and making smaller fragment out here be careful while cutting this one. Here we can see it has already we started turning into slightly brownish [FL].

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Now, here we can already see that a part of this apple inner part have already started turning a bit brown ok. So, these are regular experience we can see, but many of us might not know the reason the actually reason is that the phenolic compounds are getting oxidized by the catechol oxidase enzyme and the enzymes actually present in the apple.

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Now, what we can do if that, we can basically mix this one, here we can see we have taken a bit large amount of apple out here pieces apple we will first grind this one using a pestle and then will at the remaining pieces.

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So, the pestle what we have taken here we will actually grind this one. It is better to grind with smaller pieces of apple and while you have started grinding, add small amount of water.

Now, once we have made a small amount of this apple extract as we have seen in the video, now we have we can add the remaining pieces one thing which I basically experience while doing this one and I would like to recommend you also basically pill of this skin at first the apples skin. And, basically pill this one of remove this part having the skin of this apple and then makes small pieces and you can add those into the mortar again out here I will removing this skin I have remove the skin of this piece of apple out here, I have remove as much as possible this one is there again I will be making smaller pieces. Now I will again add double distilled water and grind it in previous way.

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Now, what we have got actually is something like this out here, here we can see the apple pieces and the liquid is actually the apple extract with in present in water. Now we have to take out this extract using this cloth. So, let us see how to take out this apple extract from here.

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Now, we have taken this beaker where we will taken the apple extract and a cloth and a piece of cloth out here.

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So, we are keeping this piece of cloth on this beaker out here and in such a way that a part of this cloth go inside make it sure that the you are placing the sample in the mid of this cloth. Now out here we can see here is the apple extract and again add some amount of water and we will transfer this in this cloth along with the entire thing we will transfer out here.

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Now, after that, we will fold this one and here already we can see a small amount of this liquid present there in the beaker and we will heavily press here such that the apple juice

comes out in this way we have to take the apple extract again we can add small amount of water and press it such that we get the apple juice from here.

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In this method what basically happens is that the remaining the solid apple pieces are actually filtered out here and we can get the filtrate that is the apple juice out here we can see here this is actually almost clear solution. So, almost clear solutions the filtrate and here in this cloth we have the solid apple residues which we will not use and we will discard this one and with this filtrate will proceed to the to our analysis. And, we will dilute this filtered a bit because this is highly concentrated will dilute a bit and with this we will proceed to our enzyme kinetics analysis.