University of Ahel AL-Bait
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Practical of Biochemistry

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Enzymes

Enzymes are proteins that act as catalysts for biological reactions. Enzymes, like all catalysts, speed up reactions without being used up themselves. They do this by lowering the activation energy of a reaction. All biochemical reactions are catalyzed by enzymes. Since enzymes are proteins, they can be denatured in a variety of ways, so they are most active under mild conditions. Most enzymes have optimum activity at a neutral pH and at body temperature.

How enzyme works: - Most biologic reactions would therefore occur only very slowly in the absence of catalyst. A substance that increases the rate at which a reaction occurs without itself being changed. Catalyst work by decreasing the energy barrier (ΔG*) between reactants and products, thereby making it easier to reach a transition state. Catalysts do not change the free energy difference (ΔG) between reactants and products and therefore do not affect the outcome of the reaction. Enzymes lower the activation energy and allows a reaction to proceed rapidly.

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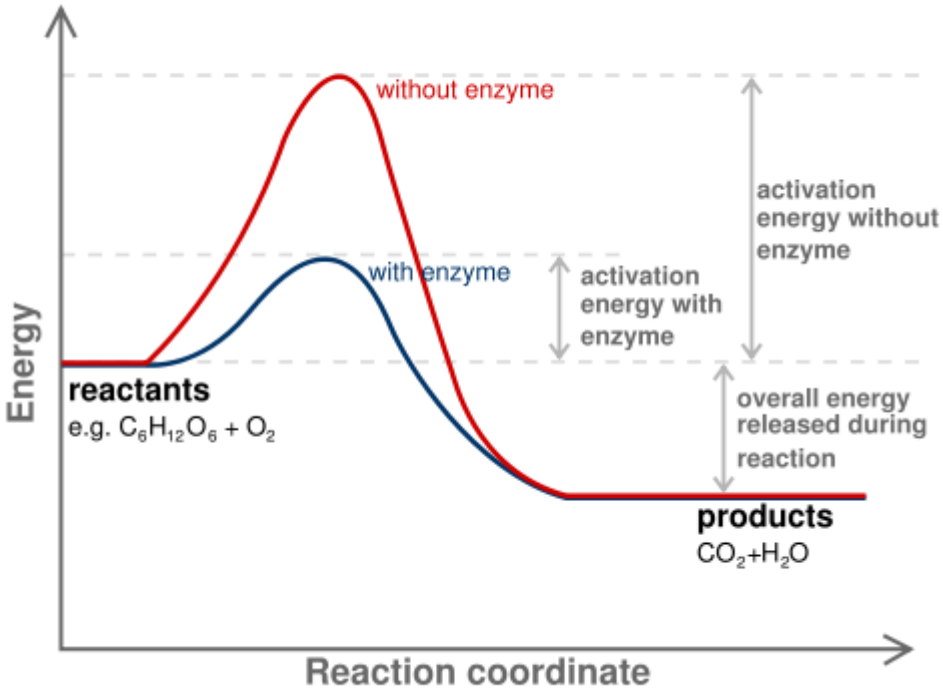

Enzymes are also very specific – they only act on one substrate or one class of related substrate molecules. The reason for this is that the active site of the enzyme is complementary to the shape and polarity of the substrate. Typically, only one kind of substrate will "fit" into the active site.

In this experiment, we will work with the enzyme amylase. This enzyme is responsible for hydrolyzing starch. In the presence of amylase, a sample of starch will be hydrolyzed to shorter polysaccharides, dextrins, maltose, and glucose. The extent of the hydrolysis depends on how long it is allowed to react – if the starch is hydrolyzed completely, the resulting product is glucose.

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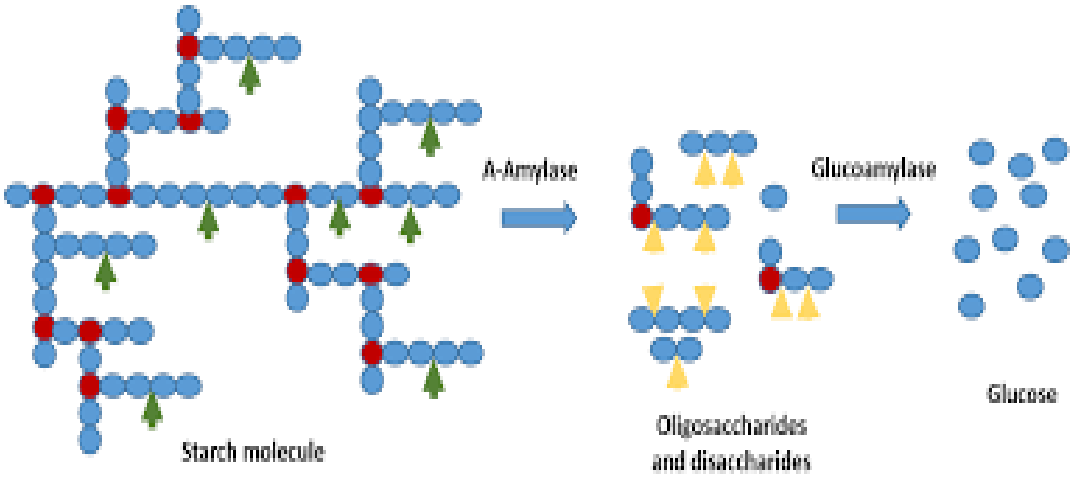


Iodine forms a blue to black complex with starch, but does not react with glucose. If iodine is added to a glucose solution, the only color seen is the red or yellow color of the iodine. Therefore, the faster the blue color of starch is lost, the faster the enzyme amylase is working. If the amylase is inactivated, it can no longer hydrolyze starch, so the blue color of the starch-iodine complex will persist.

You will also test for the presence of glucose in the samples using Benedict's reagent. When a blue solution of Benedict's reagent is added to a glucose solution, the color will change to green (at low glucose concentrations) or reddish-orange (at higher glucose concentrations). Starch will not react with Benedict's reagent, so the solution will remain blue.

Effect of Enzyme Concentration

During catalysis, the first step is the substrate (S) binding to the enzyme (E), giving an enzyme-substrate complex (ES). This is an equilibrium reaction, and will be favored by a high concentration

of enzyme and/or substrate. After the substrate is bound, the reaction takes place, and then the product is released.

E + S ⇔ES ⇔E + P

Effect of Temperature

All reactions are faster at a higher temperature. However, enzyme-catalyzed reactions become slower or stop if the temperature becomes too high, because enzymes become denatured at high temperatures. Therefore, enzymes have an optimum temperature that corresponds to maximum activity. (At higher or lower temperatures, the activity of the enzyme is lower.) The optimum temperature is usually around body temperature (٣٧°C).

Effect of pH

Each enzyme has an optimum pH. Above or below an enzyme's optimum pH, its activity is lower. The optimum pH of a particular enzyme corresponds to the pH of its natural environment. For many enzymes, this corresponds to pH values of around ٧. For pepsin, which is active in the stomach, the optimum pH is ٢ (the pH of the stomach). Trypsin, which is active in the small intestine, has an optimum pH of ٨ that matches the pH of the small intestine.

Effect of Inhibitors

Inhibitors are substances that slow down or stop enzymes. Competitive inhibitors are molecules that are very similar to the

substrate, so they can bind to the enzyme but cannot react. They compete with the substrate for the active site of the enzyme.

Noncompetitive inhibitors are molecules that are not similar to the substrate and therefore do not bind to the active site. They do, however, bind to a different location on the enzyme and change the shape of the active site so that the substrate can no longer bind. Irreversible inhibitors form covalent bonds to the enzyme and therefore cannot be removed.

Examination

Effect of Amylase activity on Starch

In animals, saliva is produced in and secreted from the salivary glands. It is a fluid containing:

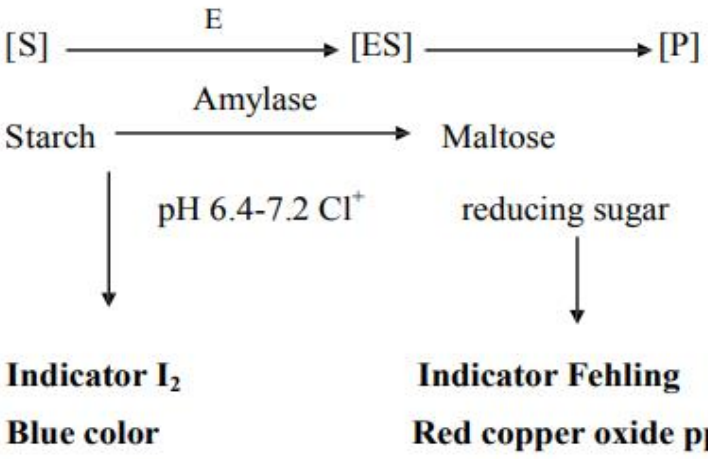
- Electrolytes: (٢-٢١ mmol/L sodium, ١٠-٣٦ mmol/L potassium, ١.٢-٢.٨ mmol/L calcium, ٠.٠٨-٠.٥ mmol/L magnesium, ٥-٤٠ mmol/L cloride, ٢-١٣ mmol/L bicarbonate,١.٤-٣٩ mmol/L phosphate)
- Mucus. Mucus in saliva mainly consists of

mucopolysaccharides and glycoproteins;

- Antibacterial compounds (thiocyanate, hydrogen peroxide, and secretory immunoglobulin A)
- various enzymes. The major enzymes found in human saliva are alpha-amylase, lysozyme, and lingual lipase. Amylase starts the digestion of starch before the food is even swallowed. It has pH optima of ٦.٧-٧.٤. Human saliva contains also salivary acid phosphatases A+B, Nacetylmuramyl-L-alanine amidase, NAD(P)H dehydrogenase-quinone, salivary lactoperoxidase, superoxide dismutase, glutathione transferase, glucose-٦ phosphate isomerase, and tissue protein. The presence of these things causes saliva to sometimes have a foul odor.

Healthy people produce about ١.٥ L of saliva per day

Amylase: found in two forms: ١. α-amylase (in saliva and pancreatic juice) which is endoglycosidase that attack starch randomly. Inactivated by the acidity of the stomach. ٢. β-amylase (from plant origin) which is exoglycosidase cleaves maltose from the non-reducing end to produce β-maltose ٥ Principle: When we want to measure enzyme activity either we measure the decrease in the substrate concentration or the increase in the product concentration.



Other uses of amylase in industry: It is used in clarification of fruit juices. The turbidity present in natural beverages is due primly to the presence of starch and cellulose molecules too large to be completely soluble. Amylase hydrolysis these molecules to glucose which are more water soluble.

Reagents:

- Starch ١٪ solution in ٠.٣٪ aqueous sodium chloride
- Freshly prepared; iodinated potassium iodide solution.
- Amylase

Procedure:

Prepare ٢ test tubes which contain the following:

Test tube	A	B
Amylase	-	١ml

Starch	١ml	١ml
Allow the tubes to stand for ٣٠ min in water bath (٣٧°C - ٤٠°C)		
Iodine solution	١-٢ drops	١-٢ drops