Enzyme

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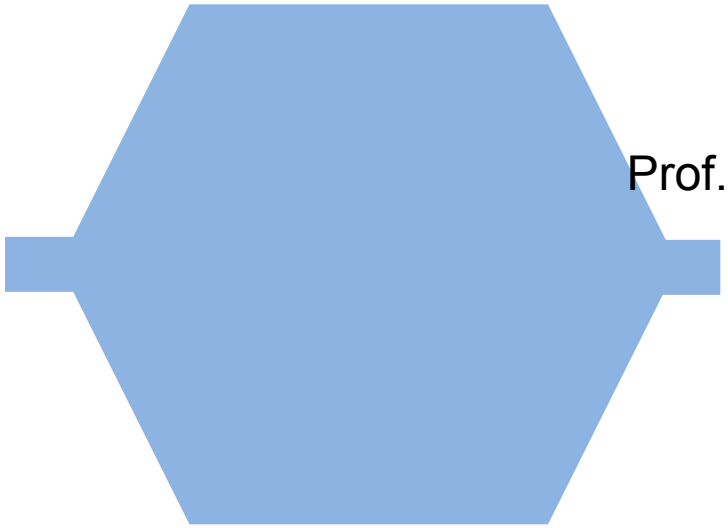
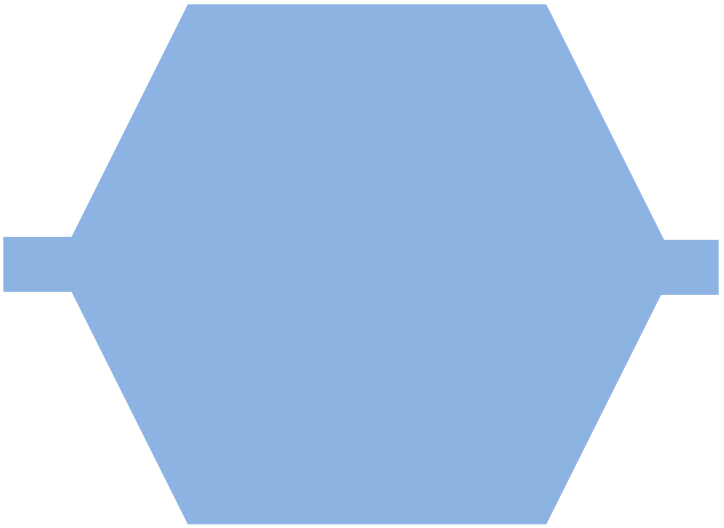




Amylase activity test

Amylase is a hydrolytic enzyme which breaks down .many polysaccharides such as starch

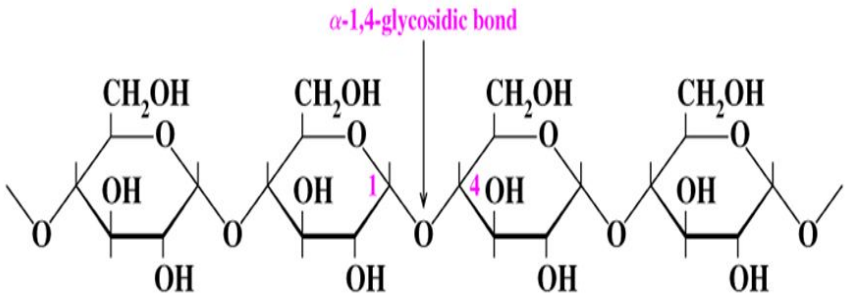
Starch

Is a polymer of D- glucose units linked by α-1, 4 glycosidic .bonds

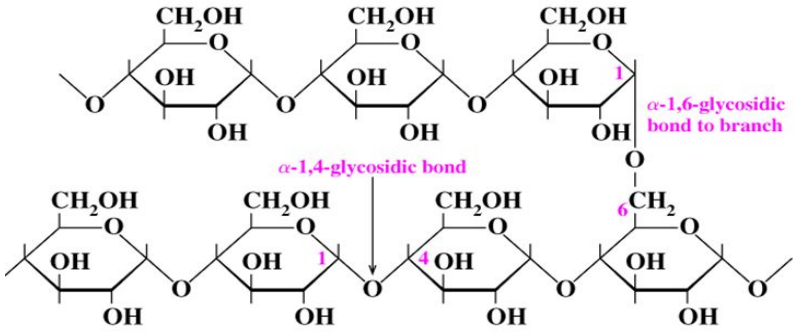
:The starch is made up of two polysaccharides

..Amylopectin (branched-chain polysaccharide)

Amylose (unbranched-chain polysaccharide



(a) Unbranched chain of amylose



Branched chain of amylopectin

The hydrolytic effect of amylase on starch results in yielding maltose (composed of two D-glucose molecules .) as the end product

There are two broad groups of amylases: α and β- .amylases

The β- amylases rapidly hydrolyse the amylose portion of starch to maltose by acting on residues at the non .reducing terminals

They hydrolyse α-1, 4 glycosidic links in polysaccharides so as to remove successive maltose units .from the non-reducing ends of the chains

The α amylases, in contrast to the β- amylases, cause a rapid loss of the capacity of amylase to give a blue colour with iodine, also the rate of appearance of maltose is much slower in the α amylases catalysed .reaction than in the β- amylases catalysed one

:Types of amylase

amylases – human amylase -α

β- amylases – bacteria and plant amylase

:Source of amylase

Pancreas -1

Salivary gland -2

:Secretion

Serum or urine

- **Amylase is a hydrolytic enzyme which hydrolyses starch into maltose/Glucose.**

- **Amylase is involved in the digestion of the polysaccharides of the diet.**

- **It present in saliva and pancreatic juice where it is secreted by parotid glands and pancreas respectively.**

- **The circulating enzyme is excreted by the kidneys into urine. Therefore, only a small amount of amylase in serum normally.**

- **Optimum pH is 7.0 , molecular weight of amylase (55 to 60 kDa).**

- **A normal concentration is in the range .30-110 U/L**

- **Blood serum amylase may be measured for .purposes of medical diagnosis**

- **: CLINICAL SIGNIFICANCE**

- **Assays of Amylase activity in serum and urine are largely of use in the diagnosis of disease of the pancreas and in the investigations of pancreatic function in .acute pancreatitis**

Hyperamylasemia

Increase the secretion of amylase in blood

Some Causes of Hyperamylasemia

Acute pancreatitis, Salivary gland lesions, etc

Saliva

Colorless fluid secreted by 3 glands in the mouth

Sublingual, submandibular, and parotid –

Saliva from parotid glands contain amylases, – enzymes, which aid in the digestion of carbohydrates

Saliva is composed of electrolytes, enzymes, mucus –

Screening for saliva is based on detection of high levels of amylase in the sample

It is not a confirmatory test; amylase is found in – other body fluids

.Serum, urine, sweat, lip mucous, semen, feces, etc •

The concentration of amylase in saliva is variable – among individual; if amylase is not detected in a sample it does not mean saliva is not present

- **UV light can be used to aid in locating saliva stains**

The intensity of the fluorescence can be affected –
by the substrate, concentration of the stain, and other body fluids

Saliva does not fluoresce as intensely as semen –

- Humans have both pancreatic and salivary amylase
- Salivary amylase is a hydrolytic enzyme
- Salivary amylase is also referred to as ptyalin

Saliva α-amylase

α-amylase •

Found in humans elephants, rats, and pigs –

Cleaves starch at its internal bonds (acts on α 1,4 – glucosidic linkages) allowing for compound hydrolysis-- the total breakdown of starch to maltose or glucose and dextrin

β-amylase •

Found in plants –

Cleaves only α 1,4 glycosidic links –

Can't cleave starch at its internal bonds so there – is an incomplete starch breakdown (maltose)

- One of the earliest tests for amylase was the starch-iodine test
- Iodine solutions cause starch to turn a deep blue color
- Amylase is a starch hydrolyzing enzyme
- The presence of amylase causes the disappearance of the blue color (due to hydrolysis of the starch) and can be used an indicator for the presence of amylase

Saliva

- **Both α and β-amylase react with starch – iodine tests**

β-amylase results in a hazy/cloudy clearing due to partial breakdown of starch

α –amylase has full clearing due to complete breakdown

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The Assay

In any enzyme assay, the rate of the reaction can be known by measuring the amount of substrate (s) that is
utilized or the amount of product (s) that is formed in unit
.time, it also called enzyme unit

,In the case of amylase
The substrate is starch (colourless)
The product is maltose (colourless)

They can be converted to coloured products by specific
.chemical reactions

Starch +Amylase → maltose (colourless)

Starch + Amylase + Iodine → blue colour (qualitative)

Procedure

:Principle

- Serum or saliva is incubated with starch substrate, Amylase in the serum or saliva hydrolyzes starch to simpler units which will not react with iodine while it react with starch molecules .that are not hydrolyzed by the amylase

- The iodine-starch complex is blue -violet in color and is measured in the spectrophotometer at 660 nm after the . addition of iodine

- The degree of loss in color is proportional to the amount of starch hydrolyzed and hence to the activity of the amylase in .the serum

: Procedure •

- Pipette 1.0 ml of starch substrate into 2 test tubes. 1 tube for .your sample and 1 tube for a reagent blank

- Place the tubes in a water bath at 37 ^{0}C for 5 minutes to .2 .warm the contents

- Pipette 20 µl of serum or saliva into the sample tube, (No .3 serum is added to the reagent blank) mix and incubate at 37 .^{0}C for exactly 7 minutes and 30 seconds

- After 7 minutes and 30 seconds remove the test tubes from .4 the water bath immediately add 1.0 ml of working iodine solution to each tube (sample and reagent blank) then add 8 .ml of distilled water

- Mix the contents of each tube well then measure the .5 absorbance without delay at 660 nm setting the .spectrometer to zero with distilled water

: Calculation *

- Amylase activity U/L = B-T/B x 1470

- B = absorbance of reagent blank
- T = absorbance of test
- factor to express values in U/L = 1470