

Measuring the optimal pH and temperature of an unknown α -amylase using starch can determine its source

Spring Moffitt, Biology 181 Lab Monday 10am

Introduction

An enzyme is a protein that acts as a catalyst. It changes the rate at which a reaction takes place. In a chemical reaction there is a reactant, (the beginning material), which is converted into a product, (the ending material), when certain conditions are applied to it. In a reaction involving enzymes, a substrate is the label used for the reactant. Each enzyme has unique characteristics that are determined by their shape. The shape of an enzyme governs which substrate the enzyme can affect. Substrates fit into the specially formed groove of an enzyme called an active site into which only one type of substrate can fit. Environmental changes such as temperature or pH heavily affect an enzyme's ability to work. Unfavorable situations can even change the shape of an enzyme rendering it useless. Every enzyme has its own optimal conditions. This holds true even for the same enzyme from different species.

α -Amylase is an enzyme that breaks down polysaccharides, (such as starch and glycogen), into simpler forms of sugar, (such as maltose and glucose). The rate at which α -amylase can break these sugars down is dependent upon the environmental circumstances and how closely they resemble optimal conditions. When α -amylase is added to starch it will begin to break the starch down into maltose. Changing the temperature or pH of the environment will affect how quickly the enzyme is able to break the starch down. By measuring the optimal temperatures and pH of three α -amylase enzymes from unknown sources, the identity of the species from which it originated can be deciphered.

Materials and methods

α -Amylase from three separate sources was obtained: *Bacillus licheniformis* a bacterium, *Aspergillus oryzae* a fungus, and pancreas of a pig. α -Amylase was anonymously labeled as A, B, or C before distribution. Each specimen was tested twice by separate groups for different temperature conditions and for differences in pH environments. Twelve test tubes were used to create the various atmospheres needed to test the enzyme's ability to break starch down.

The first six tested temperature. The tubes were properly labeled as blank, 4° C, 23° C, 37° C, 65° C, and 100° C. 1 ml of a 1% starch solution with a pH of seven was administered to each test tube by use of a pipette. Each tube was then placed in a setting corresponding to the temperature listed on the tube. The 4° C was placed in ice, the blank and 23° C were left at room temperature, while the remaining three were placed in water baths. The tubes were given 10 minutes to acclimate to the desired temperature. In a separate test tube, the unknown α -amylase sample was diluted at a rate of 100 μ l: 9.9 ml of distilled water. It was mixed homogenously through use of vortex then placed in ice. Once the tubes reached the correct temperature 1 ml of diluted α -amylase was

pipetted into each tube with the exception of the blank. To the blank tube, 1 ml of distilled water was added. As soon as the α -amylase was emptied into the first tube, a timer was started for 12 minutes. Each tube was mixed by the vortex method and placed back in the corresponding temperature until the timer finished. At the end of twelve minutes 1 ml of maltose color reagent (0.4 M NaOH) was measured into each of the six tubes in the same order the α -amylase had been put in. The timer was set for 15 minutes and time began as soon as the maltose color reagent entered the first tube. All six tubes were uniformly mixed by use of vortex and situated in boiling water for the remainder of the 15 minutes. The six tubes were then placed in ice to cool to room temperature. Once at room temperature, 9 ml of distilled water was added to each tube evenly mixed with the use of vortex. A spectrophotometer was used to measure the absorbance of each tube. It was set to zero using the blank at 540nm.

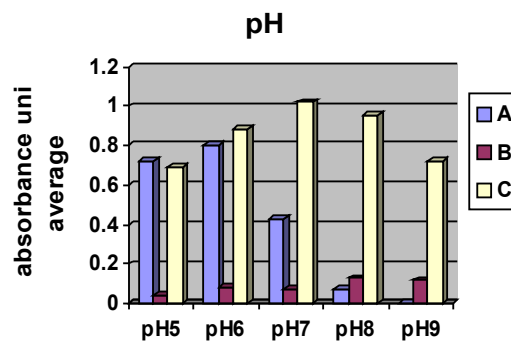
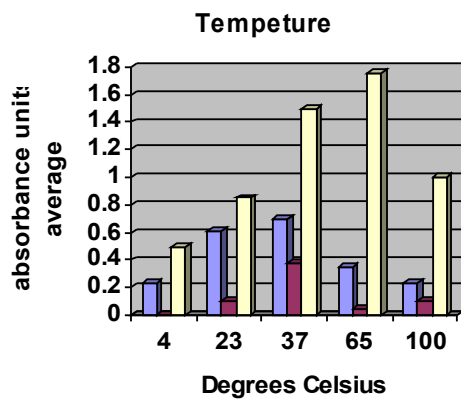
The second six tested pH. The tubes were properly labeled as blank, pH5, pH6, pH7, pH8, and pH9 and 1 ml of matching pH was inserted. 1 ml the 1% starch solution with a pH of seven was dispensed to each test tube by use of a pipette. The unknown diluted α -amylase sample created for temperature testing was used to pipette 1 ml of diluted α -amylase into each tube with the exception of the blank. To the blank tube, 1 ml of distilled water was added. As soon as the α -amylase was emptied into the first tube, a timer was started for 12 minutes. Each tube was mixed by the vortex method. At the end of twelve minutes 1 ml of maltose color reagent was measured into each of the six tubes in the same order the α -amylase had been put in. The timer was set for 15 minutes and time began as soon as the maltose color reagent entered the first tube. All six tubes were uniformly mixed by use of vortex and situated in boiling water for the remainder of the 15 minutes. The six tubes were then placed in ice to cool to room temperature. Once at room temperature, 9 ml of distilled water was added to each tube evenly mixed with the use of vortex. A spectrophotometer was used to measure the absorbance of each tube. It was set to zero using the blank at 540nm.

Results

Absorbance units (540nm)					
Temperature	4°C	23°C	37°C	65°C	100°C
α -amylase	.27	.625	.82	.42	.265
α -amylase	.19	.59	.57	.29	.21
A average	.23	.6075	.695	.355	.2375
α -amylase	0	.1	.36	.06	.01
α -amylase	< 0	.11	.4	.02	.11
B average	0	.105	.38	.04	.105
α -amylase	.5	1.6	1.8	2	2
α -amylase	.5	.1	1.2	1.5	.01
C average	.5	.85	1.5	1.75	1.005

Absorbance units (540nm)

pH	pH5	pH6	pH7	pH8	pH9
α -amylase	.85	.9	.37	.01	0
α -amylase	.6	.7	.49	.13	.01
A average	.725	.8	.43	.07	.005
α -amylase	.09	0	0	.02	.01
α -amylase	0	.165	.14	.24	.23
B average	.045	.0825	.07	.13	.12
α -amylase	.43	.47	.54	.415	.34
α -amylase	.95	1.3	1.5	1.5	1.1
C average	.69	.885	1.02	.9575	.72



<http://userpages.wittenberg.edu/s04.bdoane/>