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Cell Biology
02/10/13

Quantitative Analysis and Identification of Alpha-Amylase

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Abstract:

A critical need of information

Introduction:

Probably important

Methods:

Standard Curve:

Following the outlined laboratory procedure, six 15 mL round test tubes were obtained and labeled 1-6. To each tube, an appropriate volume of each reagent was added following given volumes on Table 1.1. Following the addition of the maltose color reagent, the tubes were vortexed and immersed in a boiling water bath for 15 minutes. The tubes were removed and cooled to room temperature prior to an addition of 9 mL of deionized water to each tube. Using tube 1 as a blank, a Spec-20D was blanked at 540 nm absorbance before absorbances were recorded for each of the five remaining tubes. This data was used to generate a standard curve for maltose.

Table 1.1	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Deionized Water	2.0 mL	1.8 mL	1.6 mL	1.4 mL	1.2 mL	1.0 mL
Maltose (2mg/ml)	0 mL	0.2 mL	0.4 mL	0.6 mL	0.8 mL	1.0 mL
Maltose Color Reagent	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL

Table 1.1 displaying ratios of water and maltose to be added to each of 6 test tubes.

Enzyme Activity:

Enzyme activity was observed in purified *Bacillus licheniformis* following the same laboratory outlines to generate a standard curve for maltose. 40 mL of deionized water was added to a 50 mL test tube kept on ice. 40 μ l of concentrated *B. licheniformis* α -amylase was carefully pipetted into the 50 mL tube to dilute it. Three 15 mL test tubes were labeled: blank, α -amylase A, and α -amylase B. To the blank tube, 1 mL of deionized water was added and using the diluted α -amylase prepared earlier, 1 mL was added to the tubes labeled α -amylase A, and α -amylase B. To each tube, 1 mL of 1% starch, pH 7, was then added, the tubes were vortexed, and left to sit for exactly 12 minutes. 1 mL of maltose color reagent was added to each tube, and the tubes were placed in a boiling water bath for 15 minutes. After boiling, the tubes were cooled to room temperature before 9 mL of deionized water was added to each tube. The tubes were then inverted several times to mix. The Spec-20D was blanked with the blank tube at 540 nm Absorbance and the absorbances for α -amylase A and for α -amylase B were recorded.

Protein Assay:

Following the BioRAD protein assay protocol, a standard curve for α -amylase was produced using 7 test tubes containing 0, 10, 20, 40, 60, 80, and 100 μ l of protein. To each tube, 100, 90, 80, 60, 40, 20, and 0 μ l of deionized water was added, respectively. Finally 5 mL of a 1:4 solution of Coomassie Brilliant Blue G-250 dye was added to each tube. The tubes were vortexed and left to incubate for 15 minutes at room temperature. Prior to reading the absorbances, the Spec-20D was set to 595 nm absorbance and blanked using the tube containing 0 μ l of protein. The data was

collected and used to generate a standard curve. Samples of α -amylase were then assayed using a similar protocol, but using three test tubes, 5, 10, and 20 μ l of protein was added to 95, 90, and 80 μ l of deionized water, respectively. 5 mL of Coomassie Brilliant Blue G-250 dye was added to each tube prior to vortexing and 15 minute incubation. The absorbances at 595 nm were recorded after incubation.

Effect of Temperature on α -amylase activity:

Following the Thiel-Bissen-Lyons protocol, 100 μ l of α -amylase unknown "B" was diluted with 9.9 mL of deionized water to create a stock solution which was kept on ice. Six test tubes were labeled "blank", "4°C", "23°C", "37°C", "65°C", and "100°C". To each test tube, 1 mL of 1% starch solution, pH7, was added and the test tubes were placed in water baths for 10 minutes at their respective temperatures, except the 23°C and Blank test tubes which were left at room temperature for 10 minutes. Following the 10 minute equilibration time, 1 mL of deionized water was added to the "blank" tube and to the remaining five tubes, 1 mL of diluted α -amylase was added. The tubes were vortexed to mix, and placed in their respective environmental temperatures for 12 minutes. After the 12 minute incubation time, 1 mL of maltose color reagent was added to each tube. The tubes were vortexed and placed in a boiling water bath for 15 minutes. After 15 minutes of boiling, the tubes were cooled to room temperature and 9 mL of deionized water was added to each tube, the tubes were then inverted to mix. The absorbance of each tube's solution was read on the Spec-20D. The "blank" tube was used to zero the Spec-20D at 540 nm absorbance.

Effect of pH on α -amylase activity:

Following the Thiel-Bissen-Lyons protocol, 100 μ l of α -amylase unknown "B" was diluted with 9.9 mL of deionized water to create a stock solution which was kept on ice.

Six test tubes were labeled “blank”, “pH 5”, “pH 6”, “pH 7”, “pH 8”, and “pH9”. The pH of the 1% starch solution was altered to the appropriate pH, and 1 mL of the corresponding pH solution was added to each tube (pH 7 was used for the blank tube). Following a similar procedure to the temperature protocol, 1 mL of deionized water was added to the blank tube while 1 mL of stock enzyme solution was used for the remaining 5 tubes. The tubes were vortexed, then allowed to incubate at room temperature for 12 minutes. Following incubation 1 mL of maltose color reagent was added to each tube, the tubes were vortexed and placed in a boiling water bath for exactly 15 minutes. After boiling, the tubes were cooled to room temperature when 9 mL of deionized water was added to each tube. The tubes were inverted to mix before the “blank” tube was used to zero the Spec-20D at 540 nm absorbance. Each of the 5 remaining tubes were read and their absorbances were recorded.

Results:

Using Figure 1.1 and tables 1.2 and 1.3, the average units of enzyme in 1 ml of our test solution, α -amylase B was discovered to be 155.388 units.

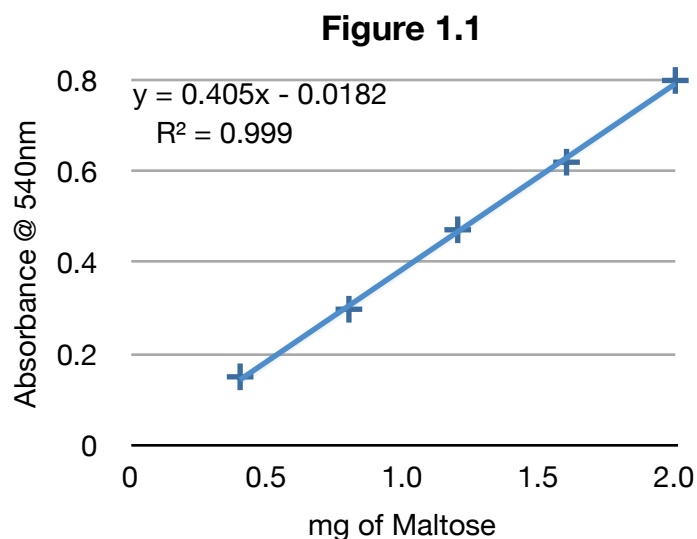


Figure 1.1 displaying the maltose standard curve.

Table 1.2	1	2	3	4	5	6
Absorbance @540nm	0	0.150	0.298	0.472	0.620	0.799
mg of maltose	0	0.4	0.8	1.2	1.6	2

Table 1.2 displaying the observed absorbances at 540 nm compared to the mg of maltose used. This generated the standard curve.

Table 1.3	Alpha-Amylase A	Alpha-Amylase B
Absorbance @540nm	0.712	0.762
mg of maltose in assay	1.8029	1.9264
mg maltose produced by 1 ml starting solution	1802.9	1926.4
Units of enzyme in 1 mL starting test solution	150.241667	160.534979
Average units of enzyme		155.388323

Table 1.3 displaying the observed absorbances of two replicate samples of α -amylase, the mg of maltose in assay derived from the standard curve, the mg of maltose produced by 1 mL starting solution, and the units of α -amylase in 1 mL of starting solution.

From the BioRAD protein assay on α -amylase “B” (Tables 1.4, 1.5, and Figure 1.2), an average of 1.0391 mg of α -amylase “B” was observed in 1 ml of solution.

Table 1.4	Concentration	Absorbance
μl protein	mg/ml	@595nm
0	0.0	0
10	0.148	0.250
20	0.296	0.422
40	0.592	0.720
60	0.888	1.025
80	1.184	1.230
100	1.480	1.520

Table 1.4 displaying μ l of α -amylase, concentration of α -amylase sample, and absorbance @595nm used to generate Figure 1.2.

Table 1.5	mg/ml	Absorbance
μl protein		@595nm
5	0.698	0.123
10	1.236	0.211
20	1.183	0.323

Table 1.5 μl protein	mg/ml	Absorbance @595nm
	1.0391 avg	

Table 1.5 displaying μl of α-amylase, concentration of α-amylase sample, and absorbance @595nm.

Figure 1.2

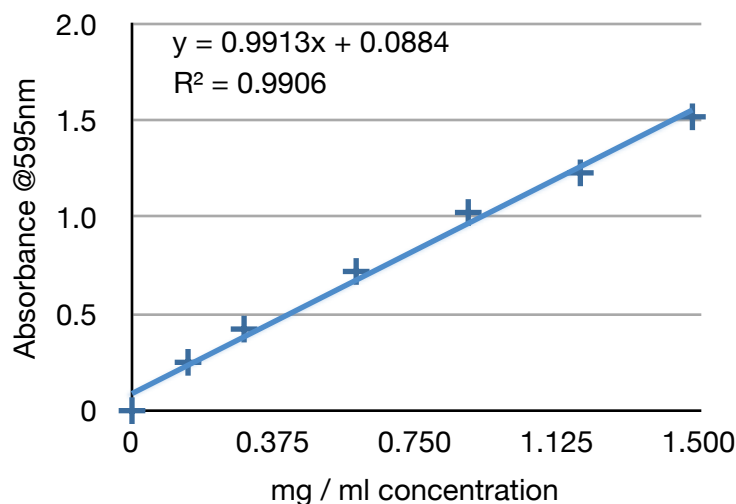


Figure 1.2 displaying the standard curve for α-amylase concentration compared to absorbance @ 595nm

From the derived data in Table 1.3, and the data collected from the BioRAD protein assay (Table 1.5) The specific activity of α-amylase “B” could be observed as 149.541 units per mg. (Table 1.6)

Table 1.6	units/ 1 ml	total mg of α-amylase	specific activity (units/mg)
Pure alpha-amylase	155.388	1.0391	149.541

Table 1.6 displaying the specific activity of α-amylase “B” (units per ml / total mg of α-amylase)

The effect of temperature on α-amylase activity was observed, and the observed data for α-amylase “B” was recorded (Table 2.1) and α-amylase “B”, in addition to samples “A”, and “C”, was put into Table 2.2. Figure 2.1 was generated to compare activity in varying temperatures between samples A, B, and C.

Table 2.1	4°C	23°C	37°C	65°C	100°C
mg maltose	3.304	3.452	3.551	3.600	3.650
A@540nm	1.320	1.380	1.420	1.440	1.460

Table 2.1 displaying the absorbances observed as well as the mg of maltose produced at varying temperatures for sample B.

Table 2.2	4°C	23°C	37°C	65°C	100°C
α-amylase A	0.456	0.940	0.980	1.680	0.283
α-amylase B	1.320	1.380	1.420	1.440	1.460
α-amylase C	0.624	0.688	0.780	0.618	0.227

Table 2.2 displaying the absorbances observed at varying temperatures for samples A, B, and C.

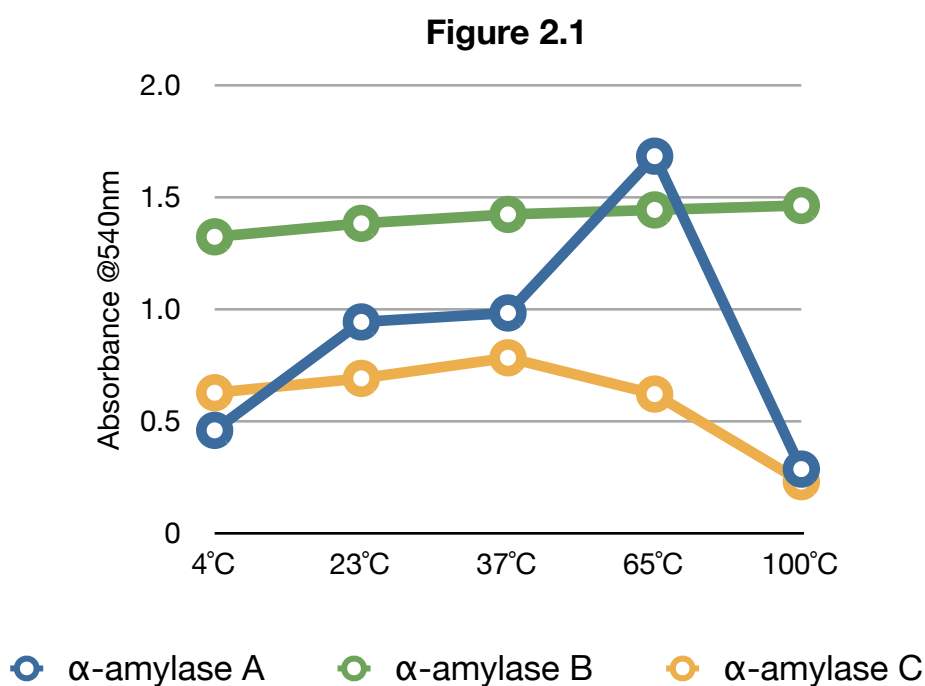


Figure 2.1 comparing the observed absorbencies of samples A, B, and C depending on varying temperatures.

The effect of pH on α-amylase activity was observed, and the observed data for α-amylase “B” was recorded (Table 2.3) and α-amylase “B”, in addition to samples “A”, and “C”, was put into Table 2.4. Figure 2.2 was generated to compare activity in varying pH between samples A, B, and C.

Table 2.3	pH 5	pH 6	pH 7	pH 8	pH 9
mg maltose	4.192	3.255	4.045	2.9338	3.255
A@540nm	1.680	1.300	1.620	1.170	1.300

Table 2.3 displaying the absorbances observed as well as the mg of maltose produced at varying pHs for sample B.

Table 2.4	pH 5	pH 6	pH 7	pH 8	pH 9
α -amylase A	1.900	1.350	1.260	0.341	0.218
α -amylase B	1.680	1.300	1.620	1.170	1.300
α -amylase C	1.680	1.500	1.210	0.464	0.786

Table 2.4 displaying the absorbances observed at varying pH for samples A, B, and C.

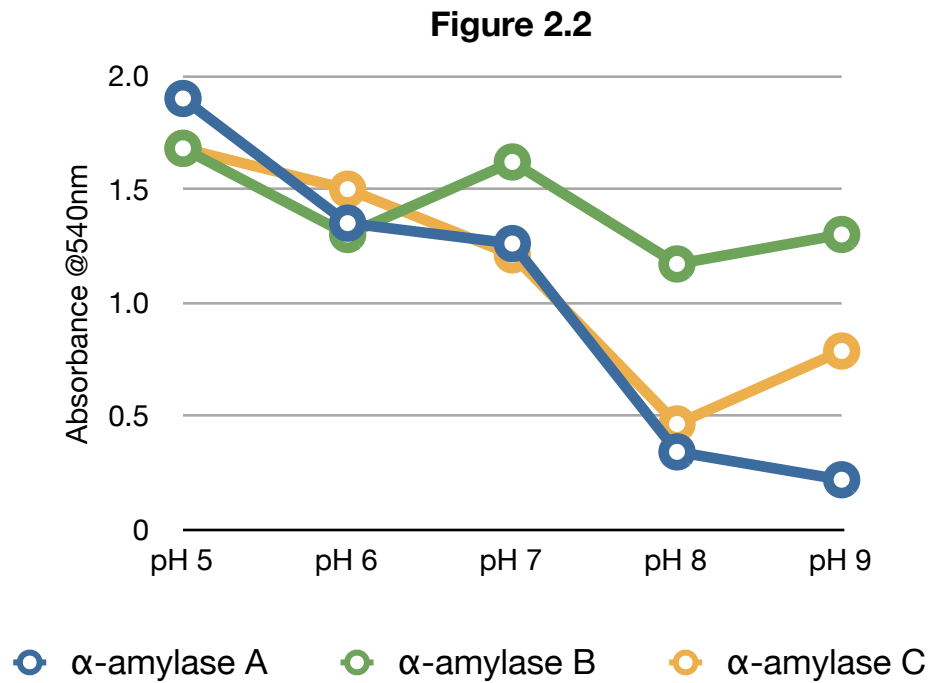


Figure 2.2 comparing the observed absorbencies of samples A, B, and C depending on varying temperatures.

Discussion:

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References:

Something!!