Lieu, Lab Section 54 Fri 10-12:50, Gaentano Romano, 10/9/15

**Examining the Effect of Various Enzyme and Substrate Concentration on the Rate of Product Formation**

**Introduction:**

 Enzymes in cells are very important tothe human body. Enzyme acts as a catalyst that speed up the rate of biochemical reactions that occur in our bodies.The enzyme that will be use in this experiment is Alkaline Phosphatase. The substrate for this enzyme is p-nitrophenyl phosphate, and the product of the overall reaction is p-nitrophenol + Phosphate (PI). The purpose of this experiment is to observe and examine how the reaction rate of product differ when the reaction proceeds with enzyme and without enzyme, with various enzyme concentrations and/or various substrate concentrations. HypothesisThis experiment can be carry out by using the spectrophotometer machine, which will provide the absorbance value needed to create graphs and determine the reaction rate.

**Materials and Method:**

 This section is included in an attached protocol on page

**Data and Results:**

**B. Standard Curve of Reaction product: P-Nitrophenol**

Table 1

|  |  |  |
| --- | --- | --- |
| Tube # | Concentration (mM) | OD at 405nm |
| 1 | 0.0025 | 0.000 |
| 2 | 0.005 | 0.059 |
| 3 | 0.01 | 0.306 |
| 4 | 0.025 | 0.484 |
| 5 | 0.05 | 0.915 |
|  | blank | 0.001 |

Figure 1

 **C. Rate of Reaction of the Hydrolysis of P-Nitrophenyl Phosphate by Serum Alkaline Phosphatase**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube Label | Volume of Buffer (mL) | Volume of Substrate(mL) | Volume of H2O (mL) | Volume ofSerum (mL) |
| 1a | 1.0 | 2.6 | 0.4 | 0.0 |
| 1b | 3.6 | 0.0 | 0.4 | 0.0 |
| 2a | 1.0 | 2.6 | 0.0 | 0.4 |
| 2b | 3.6 | 0.0 | 0.0 | 0.4 |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube Label |  |  |  |  | O.D. | at |  |  |  |  |  |
|  | t=0 | t=1 | t=2 | t=3 | t=4 | t=5 | t=6 | t=7 | t=8 | t=9 | t=10 |
| 1awith no enzyme | 0.066 | 0.066 | 0.067 | 0.068 | 0.065 | 0.066 | 0.065 | 0.065 | 0.065 | 0.065 | 0.065 |
| 1bblank | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 |
| 2awith enzyme | 0.288 | 0.297 | 0.325 | 0.326 | 0.310 | 0.321 | 0.328 | 0.340 | 0.346 | 0.364 | 0.367 |
| 2bblank | 0.205 | 0.192 | 0.192 | 0.173 | 0.170 | 0.166 | 0.147 | 0.145 | 0.147 | 0.166 | 0.150 |

Table 2

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube Label | t=1 | t=2 | t=3 | t=4 | t=5 | t=6 | t=7 | t=8 | t=9 | t=10 |
| 1a-[P]with noenzyme | 0.0068475 | 0.0068972 | 0.0069476 | 0.0067974 | 0.0068475 | 0.0067974 | 0.0067974 | 0.0067974 | 0.0067974 | 0.0067974 |
| 2a-[P]with enzyme | 0.018410 | 0.019811 | 0.019861 | 0.019060 | 0.019611 | 0.019962 | 0.020563 | 0.020863 | .021764 | .021914 |

Figure 2

With Enzyme

Vo = the slope of the initial linear

Slope of the line = enzyme activity or rate of the reaction

Vo = rate of reaction = 0.0003umoles/min/ml

There is no enzyme activity or Vo for sample 1a (no enzyme) because no [P] is produced in which

 the slope is close to zero.

**D. Effect of Serum (Enzyme) Concentration (Volume) on the Rate of Reaction of Hydrolysis of P-Nitrophenyl**

 **Phosphate**

|  |  |  |  |
| --- | --- | --- | --- |
| Tube Label | Volume Buffer (mL) | Volume Substrate (mL) | Volume Serum (mL) |
| 1a | 1.3 | 2.6 | 0.1 |
| 2a | 1.2 | 2.6 | 0.2 |
| 3a | 1.0 | 2.6 | 0.4 |
| 4a | 0.8 | 2.6 | 0.6 |
| 5a | 0.6 | 2.6 | 0.8 |
| 6a | 0.4 | 2.6 | 1.0 |
| 1b | 3.9 | 0.0 | 0.1 |
| 2b | 3.8 | 0.0 | 0.2 |
| 3b | 3.6 | 0.0 | 0.4 |
| 4b | 3.4 | 0.0 | 0.6 |
| 5b | 3.2 | 0.0 | 0.8 |
| 6b | 3.0 | 0.0 | 1.0 |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube Label |  |  |  |  | OD @ | t= |  |  |  |  |  |
|  | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1a | 0.120 | 0.131 | 0.137 | 0.140 | 0.146 | 0.150 | 0.153 | 0.155 | 0.158 | 0.162 | 0.168 |
| 2a | 0.217 | 0.233 | 0.247 | 0.268 | 0.274 | 0.280 | 0.283 | 0.293 | 0.296 | 0.316 | 0.325 |
| 3a | 0.368 | 0.363 | 0.382 | 0.398 | 0.428 | 0.444 | 0.460 | 0.468 | 0.512 | 0.512 | 0.520 |
| 4a | 0.492 | 0.506 | 0.536 | 0.582 | 0.602 | 0.618 | 0.660 | 0.662 | 0.714 | 0.698 | 0.717 |
| 5a | 0.552 | 0.604 | 0.628 | 0.668 | 0.716 | 0.748 | 0.770 | 0.800 | 0.855 | 0.890 | 0.915 |
| 6a | 0.702 | 0.746 | 0.764 | 0.815 | 0.870 | 0.905 | 0.930 | 0.930 | 1.035 | 1.080 | 1.120 |
| 1b | 0.064 | 0.063 | 0.061 | 0.065 | 0.066 | 0.065 | 0.065 | 0.066 | 0.070 | 0.070 | 0.072 |
| 2b | 0.129 | 0.138 | 0.117 | 0.124 | 0.129 | 0.125 | 0.120 | 0.123 | 0.126 | 0.136 | 0.130 |
| 3b | 0.336 | 0.338 | 0.337 | 0.335 | 0.335 | 0.334 | 0.334 | 0.334 | 0.341 | 0.340 | 0.340 |
| 4b | 0.298 | 0.292 | 0.298 | 0.283 | 0.287 | 0.283 | 0.285 | 0.284 | 0.281 | 0.291 | 0.266 |
| 5b | 0.516 | 0.516 | 0.516 | 0.516 | 0.632 | 0.516 | 0.510 | 0.524 | 0.504 | 0.506 | 0.506 |
| 6b | 0.598 | 0.606 | 0.606 | 0.606 | 0.606 | 0.604 | 0.602 | 0.610 | 0.606 | 0.604 | 0.604 |

Table 3

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube Label | t=1 | t=2 | t=3 | t=4 | t=5 | t=6 | t=7 | t=8 | t=9 | t=10 |
| [P]1a | 0.010101 | 0.010401 | 0.010552 | 0.010852 | 0.011052 | 0.011202 | 0.011302 | 0.011456 | 0.011652 | 0.011953 |
| [P]2a | 0.015207 | 0.015907 | 0.016959 | 0.017259 | 0.017559 | 0.017709 | 0.018210 | 0.018360 | 0.019361 | 0.019812 |
| [P]3a | 0.021714 | 0.022665 | 0.023466 | 0.024967 | 0.025768 | 0.026570 | 0.026970 | 0.029172 | 0.029172 | 0.029573 |
| [P]4a | 0.028872 | 0.030373 | 0.032676 | 0.033677 | 0.034478 | 0.036580 | 0.036680 | 0.039283 | 0.038482 | 0.039433 |
| [P]5a | 0.033777 | 0.034978 | 0.037982 | 0.039383 | 0.040985 | 0.042086 | 0.043588 | 0.046341 | 0.048093 | 0.049344 |
| [P]6a | 0.040885 | 0.041786 | 0.044339 | 0.047092 | 0.048844 | 0.050095 | 0.050095 | 0.055351 | 0.057603 | 0.059606 |

Figure 3

[P]1a - enzyme activity = 0.0002umoles/min/ml

[P]2a - enzyme activity = 0.0005umoles/min/ml

[P]3a - enzyme activity =0.0009umoles/min/ml

[P]4a - enzyme activity = 0.0012umoles/min/ml

[P]5a - enzyme activity = 0.0017umoles/min/ml

[P]6a – enzyme activity = 0.0021umoles/min/ml

Table 4

|  |  |  |
| --- | --- | --- |
| Sample | Volume Serum (mL) | V0 |
| 1a | 0.1 | 0.0002 |
| 2a | 0.2 | 0.0005 |
| 3a | 0.4 | 0.0009 |
| 4a | 0.6 | 0.0012 |
| 5a | 0.8 | 0.0017 |
| 6a | 1.0 | 0.0021 |

Figure 4

**E. Effects of Substrate Concentration on the Rate of the Reaction of Hydrolysis of P-Nitrophenyl Phosphate**

 **by Serum Alkaline Phosphatase**

|  |  |  |  |
| --- | --- | --- | --- |
| Tube Label | Volume Buffer (mL) | Volume Substrate (mL) | Volume Serum (mL) |
| Blank | 3.6 |  | 0.4 |
| 1 | 2.6 | 1.0 | 0.4 |
| 2 | 1.6 | 2.0 | 0.4 |
| 3 | 1.0 | 2.6 | 0.4 |
| 4 | 0.8 | 2.8 | 0.4 |
| 5 | 0.6 | 3.0 | 0.4 |
| 6 | 0.4 | 3.2 | 0.4 |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube Label |  |  |  |  | OD @ | t =  |  |  |  |  |  |
|  | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Blank | 0.187 | 0.193 | 0.188 | 0.186 | 0.184 | 0.176 | 0.180 | 0.175 | 0.153 | 0.154 | 0.150 |
| 1 | 0.205 | 0.211 | 0.215 | 0.231 | 0.241 | 0.239 | 0.245 | 0.250 | 0.255 | 0.259 | 0.262 |
| 2 | 0.220 | 0.243 | 0.267 | 0.288 | 0.294 | 0.311 | 0.310 | 0.321 | 0.320 | 0.321 | 0.342 |
| 3 | 0.232 | 0.247 | 0.264 | 0.268 | 0.279 | 0.298 | 0.300 | 0.310 | 0.320 | 0.310 | 0.328 |
| 4 | 0.278 | 0.283 | 0.288 | 0.292 | 0.307 | 0.326 | 0.340 | 0.349 | 0.355 | 0.360 | 0.384 |
| 5 | 0.291 | 0.319 | 0.327 | 0.342 | 0.356 | 0.378 | 0.410 | 0.428 | 0.434 | 0.446 | 0.454 |
| 6 | 0.306 | 0.322 | 0.343 | 0.363 | 0.382 | 0.386 | 0.394 | 0.408 | 0.416 | 0.424 | 0.436 |

Table 5

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube Label | t=1 | t=2 | t=3 | t=4 | t=5 | t=6 | t=7 | t=8 | t=9 | t=10 |
| [P]1 | 0.014106 | 0.014306 | 0.015066 | 0.015607 | 0.015507 | 0.015807 | 0.016058 | 0.016308 | 0.016508 | 0.016658 |
| [P]2 | 0.015707 | 0.016909 | 0.017960 | 0.018260 | 0.019111 | 0.019061 | 0.019612 | 0.019562 | 0.019612 | 0.020663 |
| [P]3 | 0.015907 | 0.016758 | 0.016959 | 0.017509 | 0.018460 | 0.018560 | 0.019061 | 0.019562 | 0.019061 | 0.019962 |
| [P]4 | 0.017710 | 0.017960 | 0.018160 | 0.018912 | 0.019862 | 0.020563 | 0.021031 | 0.021313 | 0.021564 | 0.022765 |
| [P]5 | 0.019511 | 0.019912 | 0.020663 | 0.021364 | 0.022465 | 0.024066 | 0.024967 | 0.025268 | 0.025868 | 0.026269 |
| [P]6 | 0.019662 | 0.020713 | 0.021714 | 0.022665 | 0.022865 | 0.023266 | 0.023966 | 0.024367 | 0.024767 | 0.025368 |

Figure 5

[P]1 – enzyme activity = 0.0003umoles/min/ml

[P]2 – enzyme activity = 0.0004umoles/min/ml

[P]3 – enzyme activity = 0.0005umoles/min/ml

[P]4 – enzyme activity = 0.0006umoles/min/ml

[P]5 – enzyme activity = 0.0008umoles/min/ml

[P]6 – enzyme activity = 0.0006umoles/min/ml

Table 6

|  |  |  |
| --- | --- | --- |
| Tube Label | Substrate Concentration (mM) | V0 |
| 1 | 1.25 | 0.0003 |
| 2 | 2.50 | 0.0004 |
| 3 | 3.25 | 0.0005 |
| 4 | 3.50 | 0.0006 |
| 5 | 3.75 | 0.0008 |
| 6 | 4.00 | 0.0006 |

**Protocol Lab 6: Enzyme Kinetics**

**Objective:**The purpose of this experiment is to observe and examine how the reaction rate of product differ

 when the reaction proceeds with enzyme and without enzyme, with various enzyme concentration

 and/or various substrate concentrations.

**Materials:**

* Sodium carbonate- bicarbonate buffer (0.1M pH 10.0)
* P- Nitrophenol Standard (0.05mM)
* P- Nitrophenylphosphate substrate solution (5mM)
* Serum Alkaline Phosphatase
* Numbered test tubes
* Spectrophotometer (405nm)

**Part B: Standard Curve of Reaction Product: P-nitrophenol**

1. Label 6 tubes: 1-5 and blank
2. Using the 0.05mM p-nitrophenol standard, prepare 5 dilutions in buffer with the following concentrations: 0.0025mM, 0.005 mM, 0.01mM, 0.025mM, 0.05mM. Final volume should the 4mL of each dilution.
3. Blank the instrument at 405nM using 4 mL of buffer
4. Determine the OD of the 5 dilutions on the spectrophotometer at 405nm and record.

**Part C: Rate of Reaction of Hydrolysis of P-Nitrophenyl Phosphate**

**by Serum Phosphatase as compared to water**

**1).** Prepare the solutions in spectrophotometer cuvettes. Use one cuvette for the samples and another

for the blank. Final volume should be 4mL.

**2).** Sample 1a; Without enzyme- add 1.0 mL buffer & 2.6 mL substrate. Mix thoroughly.

**3).** Sample 1b; Blank- add 3.6 mL buffer & 0.4 mL of H2O.

**4).** Use blank sample 1b to zero the instrument att 405nm. Then add 0.4 mL H2O to the sample 1a, mix

 and read at 405 nm to get the time 0 reading. All final volume is 4 mL.

**5).** Sample 2a; with enzyme- add 1.0mL buffer & 2.6 mL substrate.

**6).** Sample 2b; Blank; add 3.6mL buffer & 0.4 mL serum.

**7).** Blank the instrument at 405nm. Add 0.4mL serum to sample 2a cuvette, mix and read at 405nm to

 get the time 0 reading. Final volume is 4mL.

**8).** Read sample 2a at 1 minute intervals for 10 minutes and record. Re-blank the instrument after the

 final reading.

**Part D: Effect of Serum (Enzyme) Concentration (volume) on the Rate of Reaction of Hydrolysis of**

**P- Nitrophenyl Phosphate by Serum Alkaline Phosphatase**

1. Label 12 test tubes 1a-6a(samples) and 1b-6b(blanks).
2. Samples (1a -6a) – 2.6mL substrate in each tube and 1.3, 1.2, 1.0, 0.8, 0.6, &0.4 mL buffer in each tube respectively.
3. Blanks (1b-6b) – 3.9, 3.8, 3.6, 3.4, 3.2, & 3.0 mL buffer in each tube and respectively 0.1, 0.2, 0.4, 0.8 & 1.0mL serum in each tube.
4. Run each tube separately- place blank 1b containing 3.9mL buffer and 0.1mL of serum into a cuvette and blank the instrument at 405nm.
5. Take sample 1a, add 0.1 mL serum, mix and read the OD at 405nM to get 0 time reading. Final volume of both sample and blank is 4mL.
6. Read sample 1a at 1 minute intervals for 10 minutes and record. Re-blank the instrument at the end of the course of the readings.
7. Run the other pair of tubes (blank and experimental) in the same manner. Do not add enzyme to a sample cuvette until ready to start the reaction.

**Part E: Effect of Substrate Concentration (volume) on the Rate of Reaction of the Hydrolysis of P-**

**Nitrophenyl Phosphate by Serum Alkaline Phosphatase**

1. Label 7 test tubes 1-6 experimental and 1 test tube for blank
2. Samples (1-6)- 1.0, 2.0, 2.6, 2.8, 3.0, & 3.2mL substrate in tubes 1-6 and respectively 2.6, 1.6, 1.0, 0.8, 0.6, 0.4 mL of buffer in tubes 1-6.
3. Blank- 3.6 mL buffer, 0.4 mL serum. Total volume is 4mL.
4. Blank the instrument at 405 nm. Run each tube separately. Place the sample containing 1.0 mL substrate and 2.6 mL buffer in a cuvette. Add 0.4 mL serum to the sample cuvette, mix and record OD at 405nm to get time 0 reading.
5. Read the sample at 1 minute intervals for 10 minutes and record the results. Re-blank the instrument at the end of the course of these readings.
6. Run the other 5 sample tubes in the same manner. Do not add enzyme until ready to start the reaction.