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Lab section: _____

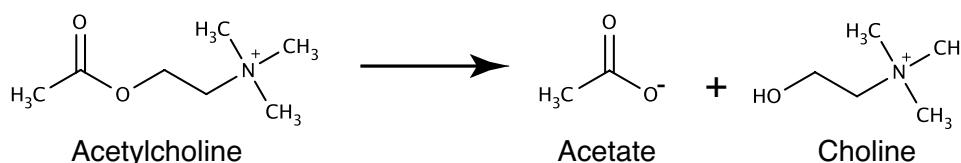
Biology 3515/Chemistry 3515
Biological Chemistry Laboratory
Spring Semester 2016
Quiz 2 - 10 March 2016

Please write your name on each page.

Be sure to show your work and include correct units in all of your answers!

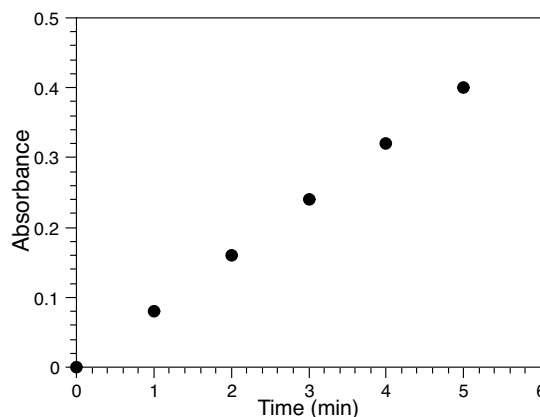
25 points total.

1. After graduation, you have been hired by the giant pharmaceutical company Proteins-R-Us and have been put to work in a group developing reversible inhibitors of acetylcholinesterase (AChE). As mentioned in class, AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine:



The mechanism of acetylcholine esterase (AChE) is very similar to that of the serine proteases, and some irreversible inhibitors of serine proteases are also potent inhibitors of AChE, making them very toxic compounds. But, weaker inhibitors of AChE have been found to be therapeutic for conditions such as Alzheimer's disease. AChE is normally found as an integral membrane protein, but the genetic engineers at Proteins-R-Us have constructed a gene that allows production of a soluble form of the enzyme that is more convenient for biochemical experiments. The engineered enzyme has a molecular weight of 53,000 Da.

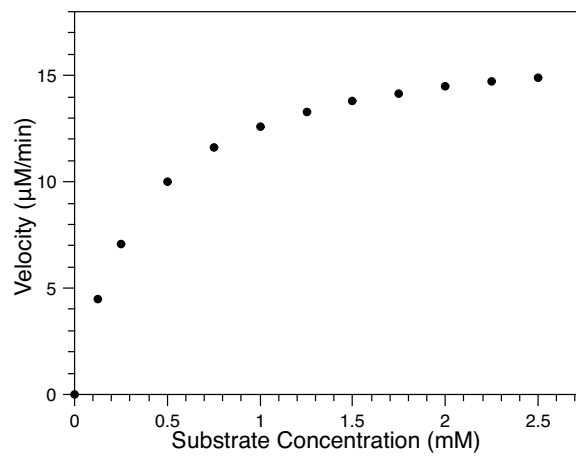
- (a) (5 pts.) You have been given a sample of purified soluble AChE and told to identify conditions under which its enzymatic activity can be conveniently studied. After some work, you have identified a synthetic substrate that produces a colored compound when hydrolyzed. The extinction coefficient of the product is $8,000 \text{ cm}^{-1} \text{ M}^{-1}$. In one of your experiments, you measure absorbance as a function of time, using a 1-cm cuvette and obtain the results shown below:



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From the graph on the previous page, calculate the reaction velocity, in units of $\mu\text{M}/\text{min}$.

- (b) (5 pts.) You then do an experiment to measure reaction velocity as a function of substrate concentration and obtain the results shown below.

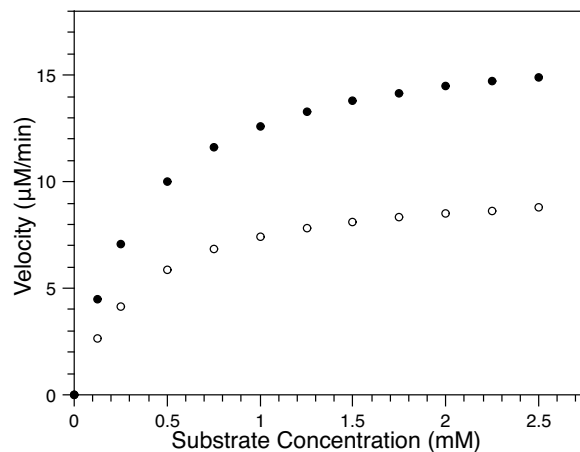


From this graph, estimate K_m and V_{\max} , with appropriate units.

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- (c) (5 pts.) The velocity versus substrate concentration experiment was performed using an enzyme concentration of $3 \mu\text{g}/\text{mL}$. Using this information, calculate k_{cat} for this reaction. Be sure to give your answer with appropriate units.

2. The synthetic chemistry group at Proteins-R-Us has created a molecule designed to act as a competitive inhibitor of AChE. To test this compound, you compare the velocity of the reaction with and without the inhibitor, at different substrate concentrations. In the graph shown below, the filled symbols represent the velocity in the absence of inhibitor, and the open symbols represent the velocity in the presence of $50 \mu\text{M}$ inhibitor.



- (a) (5 points) From your data, do you think that the compound is, as intended, a competitive inhibitor of AChE? Briefly explain why or why not. (There is additional room on the following page.)

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(b) (5 points) Do you think that this inhibitor binds to the free enzyme, the enzyme-substrate complex or both? Again, briefly explain your reasoning.