## Enzyme inhibition

Threonine is an essential amino acid for birds and mammals. There is much interest in the industrial synthesis of this amino acid, largely by conversion of Aspartate via a multi-step pathway in Escherichia coli. Your goal is to investigate the effects of inhibitors, which may be present in feed waters, on the last metabolic step in which O-phospho-homoserine (OPH) is converted to Threonine by the enzyme threonine synthase (TS):

Aspartate

Threonine

enzyme (TS)



You examine the effects of two inhibitors: cadmium and 2-amino-5-phosphonovaleric acid (APB).

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| --- | --- | --- |
|  | *KI* (nM) | inhibition mechanism |
| cadmium | 100 | non-competitive |
| APB | 100 | competitive |

Assume standard conditions of:

* OPH is present at a concentration of 1 mM.
* *K*m for the interaction of OPH with TS is 0.5 mM.
* At this concentration of OPH, the reaction proceeds at a rate of 300nM/min in the absence of inhibitor.
* These questions explore two of the enzyme kinetics inhibition equations:

Competitive: V = Vmax\*s / (Km\*(1 + i/Ki) + s)

Non-competitive: V = Vmax / (1 + i/Ki) \* s / (Km + s)

Find:

1) The maximum reaction rate (Vmax) associated with this reaction.

* Vmax=(300nM/min)\*(1mM+0.5mM)/(1mM)=450nM/min

2) The reaction rate expected if cadmium OR APB are present at 200 nM.

* Cadmium: non-competitive, V = Vmax / (1 + i/Ki) \* s / (Km + s) =100nM/min
* APB: competitive: V = Vmax\*s / (Km\*(1 + i/Ki) + s) = 180nM/min

3) The concentration of cadmium that reduces the reaction rate by 90%.

* It comes down to the multiplicative term in the non-competitive case, 1/(1+i/Ki)  
  set that equal to 0.1 (90% inhibition). 0.1 = 1/(1+i/100 nM). Then i = 900 nM

4) One day, you find that Threonine production has been decreased by 10% (to 270 nM/min). Analysis of the feed waters indicates the presence of APB. Your goal is to bring the production rate back to 300 nM /min by increasing the concentration of OPH. What concentration should you raise OPH to?

* Find the concentration of APB that would produce that drop in reaction, using the rate equation for competitive inhibition. i = 1/3 KI = 33.33 nM.
* Now, find the concentration of s that would bring the rate back to 300 nM. Use the same competitive inhibition rate equation, with i=33.33 nM and V = 300 nM/min. With that, [OPH] = 1.33 mM.