Biochemistry tutorial 4: enzyme kinetics

**Question 1**

Figure 1. Lineweaver-Burke plot of the reciprocal of the velocity of the enzyme (in absorbance units per minute) versus the reciprocal of the substrate concentration in (in mmol-1.L-1).

Inhibitor A is a competitive inhibitor (Figure 1). This is evident by the plot of the uninhibited enzyme intersecting with the plot of the enzyme inhibited by A on the y-axis (indicating a similar 1/Vmax value, and therefore similar Vmax).

Inhibitor B is a non-competitive (mixed) inhibitor (Figure 1). The plot of the enzyme inhibited by B intersects with the uninhibited enzyme on the x-intercept (indicating the same or similar 1/km value, and therefore same km).

**Question 2**

a)

Figure 2. Primary Lineweaver-Burke plot of the reciprocal of substrate concentration versus the reciprocal of initial velocity for the inhibited and uninhibited enzyme.

Table 1. Calculation of the Km and Vmax for the uninhibited enzyme and the enzyme inhibited by 1mmol inhibitor

|  |  |  |  |
| --- | --- | --- | --- |
| uninhibited enzyme equation | y=1521.2x+0.0753 | |  |
| (-1/Km) | -4.95004E-05 | Km | 20201.86 |
| 1/Vmax | 0.0753 | Vmax | 13.28021 |
| inhibited enzyme equation | y=2168.2x + 0.107 | |  |
| (-1/Km) | -4.93497E-05 | Km, apparent | 20263.55 |
| 1/Vmax) | 0.107 | Vmax, apparent | 9.345794 |

There is a non-competitive (mixed) inhibitor. The Lineweaver Burke plots of reciprocal initial velocity versus reciprocal substrate concentration for the uninhibited and inhibited enzyme cross on the x-axis (Figure 2). This assessment allows for some experimental error —the plots appear to cross on the x-axis but their x-intercepts are not exactly equal (.-4.95004E-05 versus -4.93497E-05). The similar Km values indicate that the inhibitor is non-competitive.

2b)

calculate the values of Vmax’ and Km’ (these are the Vmax and Km values when

there’s an inhibitor present) in the presence of 3.0 mmol l-1

inhibitor (5 marks).

Figure 3. A and B.

Figure 4. Dixon plot

Ki value is approximately -2.2 mmol.L inhibitor

For a 3 mmol.L noncompetitive inhibitor:

Km’

Km’ would be between 20263.5514 mmol.L-1 and 20201.86 mmol.L-1. The inhibitor is non-competitive, therefore the Km apparent is the same as the Km value of the uninhibited enzyme.

Vmax’

1/Vmax, app = 1/Vmax(1+[I]/Ki)

Based on the secondary Lineweaver-Burke plots, the Ki is between 2.351159 and 2.375394322 (assume 2.4).

1/Vmax, app = 0.0753(1+3mmol.L/2.4)

1/vmax, app= 0.169425

Vmax,app = 1/0.169425

Vmax, app = 5.902316659