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 |

Figure 2 shows that the inhibitor is a non-competitive (mixed) inhibitor. The primary-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 (Figure 1). The similar Km values indicate that the inhibitor is non-competitive.

2b)

Figure 3. Secondary Lineweaver Burke plots of (A) slope (or aKm/Vmax from the primary Lineweaver-Burke plot) versus inhibitor concentration (mmol per litre) and (B) y-intercept (or a’/Vmax from the primary Lineweaver-Burke plot) versus inhibitor concentration (mmol per litre).

Figure 4. Dixon plot of inhibitor concentration (mmol per litre) versus reciprocal of initial velocity of the enzyme for each substrate concentration.

Table 2. Calculation of the Ki value for the inhibitor

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Equation | x-intercept | Ki | Average |
| 2° LB A | y=647x+1521.2 | -2.35116 | 2.351159 |  |
| 2° LB B | y = 0.0317x + 0.0753 | -2.37539 | 2.375394 |
| Dixon |  | between -2.2 and -2.4 | |

The Dixon plot seems to confirm that the inhibitor is non-competitive as the line graphs for each substrate concentration appear to meet on the x-axis, as is expected. However, the graph line for substrate concentration 6.67 mmol-1 did not cross with the others in the same place. This will be assumed to be a case of experimental error.

The Dixon plot suggests that the Ki value (- x-intercept) is between -2.2 and -2.4 mmol.L of the inhibitor (Figure 4, Table 2). Based on the secondary Lineweaver-Burke plots, the Ki is between 2351.159 and 2375.394322 (assume 2400 mmol.L-1) (Figure 3, Table 2). The Ki value will be rounded off to 2.4 mmol.L-1 for the calculations of Vmax’.

For a 3 mmol.L-1 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’

From the equation for y-intercepts of a non-competitive inhibitor: 1/Vmax, app = 1/Vmax(1+[I]/Ki)

From the primary Lineweaver-Burke plot (Figure 2) Vmax=0.0753 mmol.L-1.sec-1, [I] given is 3 mmol.L-1, Ki calculated to be 2.4 mmol.L-1.

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

1/vmax, app= 0.169425

Vmax,app = 1/0.169425

Vmax, app = 5.902316659