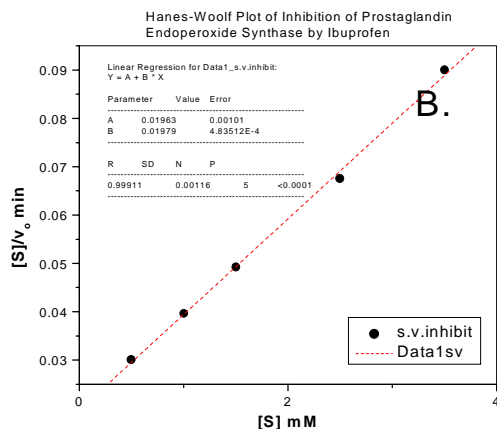
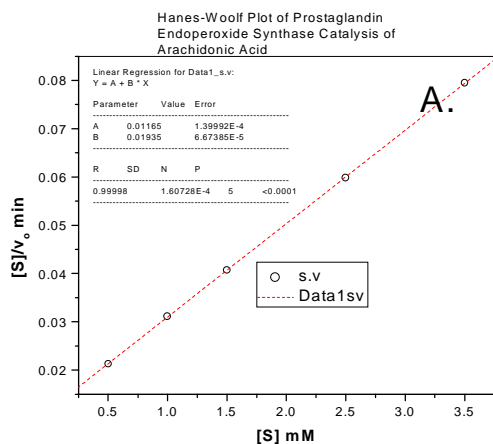


Chem 4540 Enzymology Winter 2001
Problem Set #3 ANSWERS

- At a pH midway between the two pK_a values (pH 5.2), the side-chain carboxyl group of Asp⁵², with the lower pK_a (4.5), is mainly deprotonated (--COO⁻), whereas Glu³⁵, with the higher pK_a (5.9; the stronger base) is protonated (--COOH). At pH values below 5.2, Asp⁵² becomes protonated and the activity decreases. Similarly, at pH values above 5.2, Glu³⁵ becomes deprotonated and the activity also decreases. The pH-activity profile suggests that maximum catalytic activity occurs at a pH midway between the pK_a values of the two acidic groups, when Glu³⁵ is protonated and Asp⁵² is deprotonated.
- (a) Calculate the data according to the Hanes-Woolf plot to determine the kinetic parameters from the linear fit of the plotted data. The transformed data are in parentheses in the table below.

Rate of Formation of PGG ₂ Arachidonic Acid			Rate of Formation of PGG ₂ with 10 mg/mL ibuprofen	
(mM)	(mM/min)	([S]/v _o , min)	(mM/min)	([S]/v _o , min)
0.5	23.5	(0.0213)	16.67	(0.0300)
1.0	32.2	(0.0311)	25.25	(0.0396)
1.5	36.9	(0.0407)	30.49	(0.0492)
2.5	41.8	(0.0598)	37.04	(0.0675)
3.5	44.0	(0.0795)	38.91	(0.0900)



From the graph A: $Y = 0.01165 + 0.01935X \therefore 1/V_{\max} = 0.01935$ and $V_{\max} = 51.68$

mM/min and $K_M/V_{max} = 0.01165$ so $K_M/51.68 = 0.01165$ and $K_M = 0.602$ mM.

From graph B: $Y = 0.01963 + 0.01979X$ $\therefore 1/V_{max} = 0.01979$ and $V_{max} = 50.53$ mM/min. Thus, the V_{max} is essentially unchanged by the inhibitor. For calculation of K_M , $K_M/V_{max} = 0.01963$ so $K_M/50.53 = 0.01963$ and $K_M = 0.992$ mM. Thus, the K_M has been significantly increased by the presence of the inhibitor, indicating that ibuprofen is a competitive inhibitor of arachidonic acid (an increase in K_M but no change in V_{max}).

- Curve A shows competitive inhibition. V_{max} for A is the same as for the normal curve, as seen by the identical intercepts on the v_o axis. For every value of $[S]$ (until maximal velocity is reached at saturating substrate levels), v_o is lower for curve A than for the normal curve, indicating competitive inhibition. Note that as $[S]$ increases, $v_o/[S]$ decreases, so that V_{max} —that is, the v_o at the highest (saturating) $[S]$ —is found at the intersection of the curve at the y-axis. Curve B has a different y-intercept (V_{max}) than the uninhibited curve (bold line) and therefore can not be competitive inhibition. Curve C, while also having an identical V_{max} , shows higher v_o values for every $[S]$ (and for every $v_o/[S]$) than for the normal reaction, which is not indicative of inhibition.
- An equivalency exists:
 $0.342 \times 10^{-3} \text{ mmol} = (1.0 \text{ mg/mL})(10 \text{ mL})/(\text{minimum } M_r) (\text{mg/mmol})$
Thus, the minimum $M_r = (1.0 \text{ mg/mL})(10 \text{ mL})/(0.342 \times 10^{-3} \text{ mmol}) = 29,240 \text{ mg/mmol} = 29,240 \text{ g/mol}$.
This calculation assumes that the enzyme contains only one titratable $-SH$ group per molecule. If there were two $-SH$ groups per enzyme molecule then the M_r would be $2 \times 29,240 \text{ g/mol} = 58,480 \text{ g/mol}$ and so forth.
- First measure the *total* acid phosphatase activity in a blood sample in units of μmol of phosphate ester hydrolysed per mL of serum. Then remeasure this activity in the presence of tartrate ion at a concentration sufficient to completely inhibit the enzyme from the prostate gland. The difference between the two activities represents the activity of acid phosphatase from the prostate gland.
- The graph gives us several pieces of information. First, the inhibitor prevents the enzyme from achieving the same V_{max} as in the absence of inhibitor. Second, the overall shape of the two curves is very similar: at any $[S]$ the ratio of the two velocities (\pm inhibitor) is the same. Third, the velocity does not change very much above $[S] = 1$ mM, so at much higher $[S]$ the observed velocity is essentially V_{max} for each curve. Fourth, if we estimate the $[S]$ at which $1/2V_{max}$ is achieved, this value is nearly identical for both curves. Noncompetitive inhibition, a special form of mixed inhibition, alters the V_{max} of enzymes but leaves K_M unchanged. Thus, acetazolamide acts as a non-competitive inhibitor (mixed) inhibitor of carbonic anhydrase.
- A competitive inhibitor changes the K_M but not the V_{max} for M-M kinetics. Let v_i represent the velocity in the presence of the inhibitor.

$$\frac{v_i}{V_{\max}} = \frac{[S]}{K_M \left(1 + \frac{[I]}{K_i} + [S]\right)} = \frac{[S]}{K_M + [S]} \text{ where } K_{M'} = K_M(1 + [I]/K_i)$$

$$\frac{v_i}{55 \mu\text{molL}^{-1} \text{min}^{-1}} = \frac{2 \cdot 10^{-4} M}{2 \cdot 10^{-4} M \left(1 + \frac{2.5 \cdot 10^{-3} M}{2.5 \cdot 10^{-3} M}\right) + 2.5 \cdot 10^{-4} M}$$

$$v_i = \frac{55 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-4} M)}{(2 \cdot 10^{-4} M)(1 + 1) + 2 \cdot 10^{-4} M} = 18.35 \mu\text{molL}^{-1} \text{min}^{-1}$$

In general: when $[S] = K_M$ and $[I] = K_i$ (competitive inhibition) then $v_i = 1/3 V_{\max}$.

8. Again, $\frac{v_i}{V_{\max}} = \frac{[S]}{K_{M'} + [S]}$ where $K_{M'} = K_M(1 + [I]/K_i)$

$$\frac{1.5 \mu\text{molL}^{-1} \text{min}^{-1}}{300 \mu\text{molL}^{-1} \text{min}^{-1}} = \frac{2 \cdot 10^{-5} M}{K_{M'} + 2 \cdot 10^{-5} M};$$

$$1.5 \mu\text{molL}^{-1} \text{min}^{-1} (K_{M'}) + 1.5 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-5} M) = 300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-5} M)$$

$$1.5 \mu\text{molL}^{-1} \text{min}^{-1} K_{M'} = 597 \cdot 10^{-5} \mu\text{molL}^{-1} \text{min}^{-1} (M); K_{M'} = 398 \cdot 10^{-5} M$$

Now solve for K_i where $K_{M'} = K_M(1 + [I]/K_i)$

$$398 \cdot 10^{-5} M = 6.7 \cdot 10^{-4} M \left(1 + \frac{1 \cdot 10^{-5} M}{K_i}\right)$$

$$398 \cdot 10^{-5} M = 6.7 \cdot 10^{-4} M + \frac{6.7 \cdot 10^{-9} M^2}{K_i}$$

$$K_i = \frac{6.7 \cdot 10^{-9} M^2}{33.1 \cdot 10^{-4} M} = 0.202 \cdot 10^{-5} M = 2.02 \cdot 10^{-6} M.$$

9. (a) $v_o = \frac{V_{\max} [S]}{K_M + [S]}$ (calculate v_o at $2 \cdot 10^{-5} M$ in absence of inhibitor)

$$v_o = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-5} M)}{(6.7 \cdot 10^{-4} M + 2 \cdot 10^{-5} M)} =$$

$$\frac{600 \cdot 10^{-5} \mu\text{molL}^{-1} \text{min}^{-1} M}{69 \cdot 10^{-5} M} = 8.7 \mu\text{molL}^{-1} \text{min}^{-1}.$$

V_i from Question #8 = $1.5 \mu\text{molL}^{-1} \text{min}^{-1}$ in the presence of $10^{-5} M [I]$

$$\therefore v_i/v_o = 1.5 \mu\text{molL}^{-1} \text{min}^{-1} / 8.7 \mu\text{molL}^{-1} \text{min}^{-1} \times 100\% = 17.25\%$$

$$\therefore \text{D.O.I. (degree of inhibition)} = 100\% - 17.25\% = 82.75\%$$

(b) If $[S]$ increases 10 fold then $[S] = 2 \times 10^{-4} M$ and using $v_o = \frac{V_{\max} [S]}{K_M + [S]}$ then

$$V_o = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-4} \text{M})}{(6.7 \cdot 10^{-4} \text{M} + 2 \cdot 10^{-4} \text{M})} = \frac{600 \cdot 10^{-4} \mu\text{molL}^{-1} \text{min}^{-1} \text{M}}{8.7 \cdot 10^{-4} \text{M}} = 69 \mu\text{molL}^{-1} \text{min}^{-1}.$$

In the presence of inhibitor: $v_i/V_{\max} = \frac{[S]}{K_M + [S]}$

$$V_i = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-4} \text{M})}{(39.8 \cdot 10^{-4} \text{M} + 2 \cdot 10^{-4} \text{M})} = \frac{600 \cdot 10^{-4} \mu\text{molL}^{-1} \text{min}^{-1} \text{M}}{41.8 \cdot 10^{-4} \text{M}} = 14.35 \mu\text{molL}^{-1} \text{min}^{-1}.$$

$$\text{Relative velocity} = v_i/v_o = \frac{14.35 \mu\text{molL}^{-1} \text{min}^{-1}}{69 \mu\text{molL}^{-1} \text{min}^{-1}} \times 100\% = 20.8\%$$

$$\text{D.O.I.} = 100\% - 20.8\% = 79.2\%.$$

(c) If [S] increases by 100 fold then $[S] = 2 \cdot 10^{-3} \text{M}$

$$V_o = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-3} \text{M})}{(6.7 \cdot 10^{-4} \text{M} + 2 \cdot 10^{-3} \text{M})} = \frac{600 \cdot 10^{-3} \mu\text{molL}^{-1} \text{min}^{-1} \text{M}}{2.67 \cdot 10^{-3} \text{M}} = 224.5 \mu\text{molL}^{-1} \text{min}^{-1}.$$

$$V_i = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-3} \text{M})}{(39.8 \cdot 10^{-4} \text{M} + 2 \cdot 10^{-3} \text{M})} = \frac{600 \cdot 10^{-3} \mu\text{molL}^{-1} \text{min}^{-1} \text{M}}{5.98 \cdot 10^{-3} \text{M}} = 100 \mu\text{molL}^{-1} \text{min}^{-1}.$$

$$\text{Relative velocity} = v_i/v_o = \frac{100 \mu\text{molL}^{-1} \text{min}^{-1}}{224.5 \mu\text{molL}^{-1} \text{min}^{-1}} \times 100\% = 44.5\%$$

$$\text{D.O.I.} = 100\% - 44.5\% = 55.5\%.$$

(d) If $[S] = 2 \cdot 10^{-5} \text{M}$ and $[I] = 10^{-6} \text{M}$ then K_M changes when $[I]$ changes so must calculate new K_M value.

$$K_M' = K_M(1 + [I]/K_i) = (6.7 \cdot 10^{-4} \text{M})(1 + \frac{1 \cdot 10^{-6} \text{M}}{2.02 \cdot 10^{-6} \text{M}}) = 1.0 \cdot 10^{-3} \text{M}$$

Now calculate v_i :

$$V_i = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-5} \text{M})}{(1.0 \cdot 10^{-3} \text{M} + 2 \cdot 10^{-5} \text{M})} = \frac{600 \cdot 10^{-5} \mu\text{molL}^{-1} \text{min}^{-1} \text{M}}{102 \cdot 10^{-5} \text{M}} = 5.88 \mu\text{molL}^{-1} \text{min}^{-1}.$$

$$\text{Relative velocity} = v_i/v_o = \frac{5.88 \mu\text{molL}^{-1} \text{min}^{-1}}{8.7 \mu\text{molL}^{-1} \text{min}^{-1}} \times 100\% = 67.5\%$$

$$\text{D.O.I.} = 100\% - 67.5\% = 32.5\%.$$

(e) If K_i is increased by 20 fold ($K_i = 20(2.02 \cdot 10^{-6} \text{M}) = 4.04 \cdot 10^{-5} \text{M}$)

$$\text{Calculate new } K_M': K_M' = K_M(1 + [I]/K_i) = (6.7 \cdot 10^{-4} \text{M})(1 + \frac{1 \cdot 10^{-6} \text{M}}{4.04 \cdot 10^{-5} \text{M}}) = 8.36 \cdot 10^{-4} \text{M}$$

$$V_i = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-5} M)}{(8.36 \cdot 10^{-4} M + 2 \cdot 10^{-5} M)} = \frac{600 \cdot 10^{-5} \mu\text{molL}^{-1} \text{min}^{-1} M}{85.6 \cdot 10^{-5} M} = 7.01 \mu\text{molL}^{-1} \text{min}^{-1}$$

$$\text{Relative velocity} = v_i/v_o = \frac{7.01 \mu\text{molL}^{-1} \text{min}^{-1}}{8.7 \mu\text{molL}^{-1} \text{min}^{-1}} \times 100\% = 80.5\%$$

$$\text{D.O.I.} = 100\% - 80.5\% = 19.5\%$$

(f) If $[S] = 2 \cdot 10^{-4} M$ and $[I] = 1 \cdot 10^{-4} M$ then

$$V_i = \frac{300 \mu\text{molL}^{-1} \text{min}^{-1} (2 \cdot 10^{-4} M)}{(6.7 \cdot 10^{-4} M + (1 + \frac{1 \cdot 10^{-4} M}{2.02 \cdot 10^{-6} M}) + 2 \cdot 10^{-4} M)} = \frac{600 \cdot 10^{-4} \mu\text{molL}^{-1} \text{min}^{-1} M}{340 \cdot 10^{-4} M}$$

$$= 1.765 \mu\text{molL}^{-1} \text{min}^{-1}$$

From part (b): $v_o = 69 \mu\text{molL}^{-1} \text{min}^{-1}$

$$\therefore \text{Relative velocity} = v_i/v_o = 1.765 \mu\text{molL}^{-1} \text{min}^{-1} / 69 \mu\text{molL}^{-1} \text{min}^{-1} \times 100\% = 2.56\%$$

$$\text{D.O.I.} = 100\% - 2.56\% = 97.44\%$$

10. (a) The velocity at $3.5 \cdot 10^{-5} M [S]$ in the absence of inhibitor is (v_o at $0.03 M [S] = V_{\max}$ = $295 \mu\text{molL}^{-1} \text{min}^{-1}$):

$$v_o = \frac{V_{\max} [S]}{K_M + [S]}; v_o = \frac{295 \mu\text{molL}^{-1} \text{min}^{-1} (3.5 \cdot 10^{-5} M)}{(2 \cdot 10^{-4} M + 3.5 \cdot 10^{-5} M)}$$

$$v_o = 1030 \cdot 10^{-5} \mu\text{molL}^{-1} \text{min}^{-1} M / 23.5 \cdot 10^{-5} M = 44 \mu\text{molL}^{-1} \text{min}^{-1}$$

$$\text{For noncompetitive inhibition: } v_i = \frac{V_{\max} [S]}{K_M + [S]} \text{ where } V_{\max}' = \frac{V_{\max}}{1 + \frac{[I]}{K_i}}$$

In order to ratio v_i and v_o then ratio the above equation with the equation for v_o where v_o

$$= \frac{V_{\max} [S]}{K_M + [S]}$$

$$\text{Then: } v_i/v_o = \frac{(\frac{V_{\max}}{1 + \frac{[I]}{K_i}})[S]}{\frac{K_M + [S]}{V_{\max} [S]}} = \left(\frac{V_{\max} [S]}{1 + \frac{[I]}{K_i}}\right) \left(\frac{K_M + [S]}{V_{\max} [S]}\right) \left(\frac{1}{K_M + [S]}\right) =$$

$$\left(\frac{1}{1 + \frac{[I]}{K_i}}\right) \left(\frac{K_i}{K_i}\right) = \frac{K_i}{K_i + [I]}; \text{ Thus for noncompetitive inhibition: } \frac{v_i}{v_o} = \frac{K_i}{K_i + [I]}$$

$$\text{Now apply: } v_i = \frac{K_i}{K_i + [I]} v_o$$

$$v_i = \frac{2 \cdot 10^{-5} M}{(2 \cdot 10^{-5} M + 4 \cdot 10^{-5} M)} (44 \mu\text{molL}^{-1} \text{min}^{-1}) = \frac{2 \cdot 10^{-5} M}{6 \cdot 10^{-5} M} (44 \mu\text{molL}^{-1} \text{min}^{-1}) = 14.65 \mu\text{molL}^{-1} \text{min}^{-1}$$

$$(b) \text{ Relative velocity} = \frac{v_i}{v_o} = \frac{14.65 \mu\text{molL}^{-1} \text{min}^{-1}}{44 \mu\text{molL}^{-1} \text{min}^{-1}} \times 100\% = 33.3\%$$

$$\text{D.O.I.} = 100\% - 33.3\% = 66.7\%.$$

$$11. \quad \frac{v_i}{v_o} = \frac{K_i}{K_i + [I]}$$

$$\text{D.O.I.} = 65\% \therefore \text{relative velocity} = 35\%$$

So $v_i/v_o = 0.35$ so can substitute into the formula:

$$0.35 = \frac{4 \cdot 10^{-6} M}{4 \cdot 10^{-6} M + [I]} \quad \text{so, } [I] = 7.43 \cdot 10^{-6} M.$$

12. MW for $\text{CH}_3\text{OH} = 32 \text{ g/mol}$ then 1.0 mL CH_3OH in 6L of bodily solution has a concentration:

$$[\text{CH}_3\text{OH}] = \frac{1.0 \text{ mL} \cdot 0.70 \text{ g/mL}}{32 \text{ g/mol} \cdot 6 \text{ L}} = 4.11 \cdot 10^{-3} M$$

$\text{CH}_3\text{CH}_2\text{OH}$ is a competitive inhibitor of CH_3OH with LADH \therefore

$$v_o = \frac{V_{\max} [S]}{K_M + [S]} \quad \text{where } K_M = K_M \left(\frac{1 + [I]}{K_i} \right) \quad \text{and } K_M(\text{CH}_3\text{OH}) = 1.7 \cdot 10^{-2} M$$

If reduce the LADH activity to 4% of original then $v_i/v_o = 0.04v_o$

$$\text{At 96\% inhibition, } v_o = \frac{V_{\max} (4.11 \cdot 10^{-3} M)}{(1.7 \cdot 10^{-2} M + 4.11 \cdot 10^{-3} M)}$$

$$V_o = 0.195 V_{\max} \therefore v_i = 0.04 (0.195) V_{\max} = 7.8 \cdot 10^{-3} V_{\max} = 7.8 \cdot 10^{-3} V_{\max}$$

$$\text{So, } v_i/V_{\max} = 7.8 \cdot 10^{-3}$$

$$K_i = 0.2(1.3 \cdot 10^{-3} M) = 2.6 \cdot 10^{-4} M$$

$$\frac{v_i}{v_o} = \frac{[S]}{K_M \left(1 + \frac{[I]}{K_i} \right) + [S]}$$

$$7.8 \cdot 10^{-3} = \frac{4.11 \cdot 10^{-3} M}{(1.7 \cdot 10^{-2} M) \left(1 + \frac{[I]}{2.6 \cdot 10^{-4} M} \right) + (4.11 \cdot 10^{-3} M)}$$

$$4.11 \cdot 10^{-3} M = 1.33 \cdot 10^{-4} M + 0.51 [I] + 3.21 \cdot 10^{-5} M$$

$$3.94 \cdot 10^{-3} \text{M} = 0.51[\text{I}]$$
$$[\text{I}] = 7.73 \cdot 10^{-3} \text{M}$$

$$\text{MW CH}_3\text{CH}_2\text{OH} = 46 \text{ g/mol}$$

$$7.73 \times 10^{-3} \text{ mol/L} \times 6\text{L} \times 46\text{g/mol} = 2.13 \text{ g CH}_3\text{CH}_2\text{OH}$$

$$\text{Volume of pure CH}_3\text{CH}_2\text{OH required: } 2.13 \text{ g} / 0.79 \text{ g mL}^{-1}$$
$$= 2.70 \text{ mL CH}_3\text{CH}_2\text{OH}$$

Now, since Molson Canadian is 5% $\text{CH}_3\text{CH}_2\text{OH}$ by volume then the volume of beer required to provide 2.70 mL $\text{CH}_3\text{CH}_2\text{OH}$ is: $2.70 \text{ mL} / 0.05 = \mathbf{54 \text{ mL}}$ of beer.

I would hope that after being saved from the throws of death by Molson's ale that the child would holler a mighty Canadian Rant!