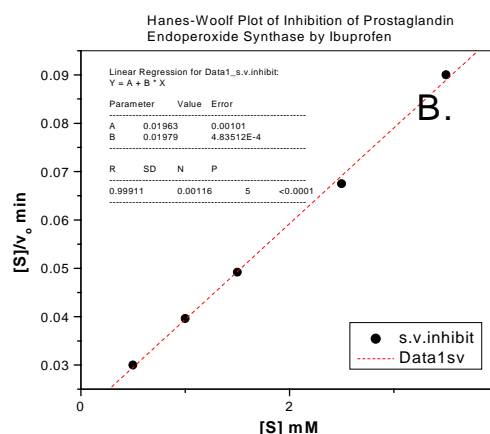
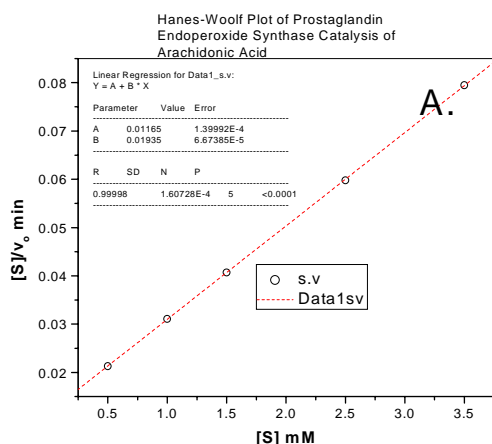


Chem 4540 Enzymology Winter 2005
Problem Set #3 ANSWERS

- 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 $V_{\max} = 50.53 \text{ mM/min}$. 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 $K_M = 0.992 \text{ mM}$. 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}).

3. 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.
4. 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.
5. 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.
6. 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 $[S] = 1 \text{ mM}$, 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.
7. 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 ($K_i = 20(2.02 \cdot 10^{-6} \text{M}) = 4.04 \cdot 10^{-5} \text{M}$)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$$\left(\frac{V_{\text{max}}}{1 + \frac{[I]}{K_i}} \right) [S]$$

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

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

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

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

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

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

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

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

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 $v_i/v_o = 0.04v_o$

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

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

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

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

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

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

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

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

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

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

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

Volume of pure $\text{CH}_3\text{CH}_2\text{OH}$ 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!