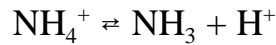


## *pH, Protein-Ligand Interactions, Enzyme Kinetics*

### 1. pH

- a. Henderson-Hasselbalch: Derive the HH equation for the following reaction involving the protonation of ammonia to ammonium



*For the reaction:*



$$K_a = [\text{H}^+][\text{A}^-] / [\text{HA}]$$

$$K_a = [\text{H}^+][\text{NH}_3] / [\text{NH}_4^+]$$

$$\log_{10} K_a = \log_{10} [\text{H}^+] + \log_{10} ([\text{NH}_3] / [\text{NH}_4^+])$$

$$-\log_{10} [\text{H}^+] = -\log_{10} K_a + \log_{10} ([\text{NH}_3] / [\text{NH}_4^+])$$

*substituting the definitions of pH and pK<sub>a</sub> (pH = -log<sub>10</sub> [H<sup>+</sup>], and pK<sub>a</sub> = -log<sub>10</sub> K<sub>a</sub>), then*

$$\text{pH} = \text{pK}_a + \log_{10} ([\text{NH}_3] / [\text{NH}_4^+])$$

*Rearrange this to a more convenient form:*

$$10^{(\text{pH} - \text{pK}_a)} = \text{ratio}$$

*where ratio is dissociated/undissociated*

- b. Ammonia is sometimes produced by bacteria that reside in large stones in the kidney. Assuming that a patient forms one liter of urine, and that the urine pH is 7.3, what % of the total ammonia would be in the NH<sub>4</sub><sup>+</sup> form? (pK = 9.3)

$$10^{(\text{pH} - \text{pK}_a)} = \text{ratio}$$

$$10^{-2} = 1/100 = \text{ratio} = [\text{NH}_3] / [\text{NH}_4^+]$$

therefore most of the ammonia is in the ammonium form

- c. Three different acids, each with their own  $K_a$  dissociate into  $\text{H}^+$  and their respective anions. Fill in the following table (TABLE 1) which relates the  $K_a$ , pH and percent dissociated (ionized). Use a calculator with an inverse log function!

These you can calculate from the above described relationships. Remember: the ratio must be converted to % dissociated;

$$\text{ratio} = \text{A}^- / \text{HA}$$

$$\% \text{ dissociated} = \text{A}^- / \text{total}$$

$$\% \text{ dissociated} = \text{A}^- / \{\text{A}^- + \text{HA}\}$$

**Table 1**

pH	pKa	% dissociated
7.4	?	75%
?	6.5	25%
6.8	8.2	?

- d. Do these in your head (TABLE 2)!

- e. Sodium lactate ( $\text{Na}^+\text{Lac}^-$ ) is in a commonly used intravenous fluid *Ringers Lactate*. Given that the pK of the dissociation of lactic acid is 3.9, and the patient's blood pH is 7.4, what would be the immediate effects on the blood pH of infusing sodium lactate, and why? What would be the immediate effects on the stomach pH if the patient ingested the sodium lactate (assume pH of stomach is 2.0).

**Table 2**

pH	pKa	ratio A/HA
7.4	?	10:1
?	6.5	1:100
7.0	8.0	?

The patient's pH will change only if  $\text{Lac}^-$  picks up and buffers a proton. What is the likelihood? The Henderson-Hasselbalch relationship indicates that at a pH of 7.4, most of the  $\text{Lac}^-$  will remain as  $\text{Lac}^-$

$$\text{pH} = \text{pK}_a + \log_{10} ([\text{Lac}^-] / [\text{Hlac}])$$

$$\text{ratio} = 10^{7.4-3.9} = \sim 300:1$$

Thus, almost no  $\text{Lac}^-$  will buffer a proton.

This is not true in the stomach where the pH is much lower. Most of the  $\text{Lac}^-$  will be in the H $\text{Lac}$  form.

## 2. Protein-Ligand Interactions

A study of the binding of insulin to its receptor was carried out (Table 3). Adipocytes were bathed in insulin, whose concentration ranged from 300 nM to 2000 nM. The amount of insulin per mg of tissue was measured.

- What is the approximate  $K_d$  of insulin and its receptor?
- What is the approximate maximal number of insulin receptors per gram of adipose tissue?
- Is binding cooperative?

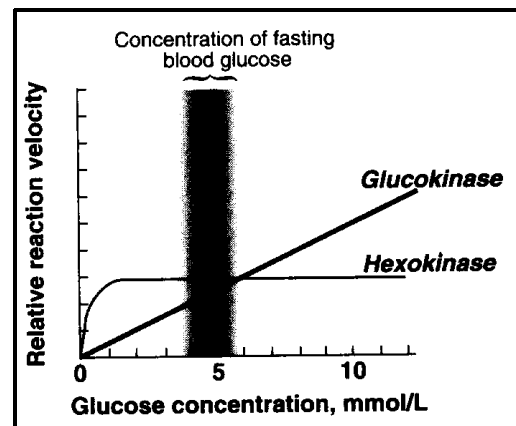
**Table 3** Binding

Bath Insulin (nM)	Bound Insulin (nmol/mg)
300	190
500	300
900	470
1300	580
2000	690
3000	760

Draw the curve of “bound” (y axis) versus bath insulin (x axis). Guesstimate where the curve saturates at a maximal value and locate the concentration at which binding is half-maximal; this is the  $K_D$ .

## 3. Enzyme Kinetics

- Transport proteins are often characterized as being either “high capacity, low affinity” or “low capacity, high affinity.” Some enzymes may be described in much the same way. Figure 1 demonstrates the relationship between glucose concentration and the activity of two intracellular enzymes which phosphorylate glucose during the first



**Figure 1** Activity of glucokinase and hexokinase

step of glycolysis.

i. Which is the “low capacity, high affinity” enzyme?

*The capacities are quite different; glucokinase  $\gg$  hexokinase. The affinity of hexokinase is much higher than glucokinase; can not even tell what the affinity of glucokinase is because there appears to be no saturation to pin a maximal rate on.*

ii. What are the approximate  $K_m$ 's of each of the two enzymes?

*You can tell that of hexokinase ( $<1$  mM), but not of glucokinase.*

b. The kinetics of an enzyme are measured as a function of substrate concentration in the presence and absence of 2 mM inhibitor,  $I$  (TABLE 4)

i. What are the approximate values of  $V_{\max}$  and  $K_m$  in the absence and presence of the inhibitor?

ii. What type of inhibition is this?

**Table 4** Enzyme Problem

$[S], \mu M$	Velocities ( $\mu mol/min$ )	
	$[I] = 0$	$[I] = 2 mM$
3	10.4	4.1
5	14.5	6.4
10	22.5	11.3
30	33.8	22.6
90	40.5	33.8

*You really need to plot a double-reciprocal with and without the inhibitor. But this is inconvenient! Plot the rate of the reaction (y axis) versus  $[S]$  (x axis) in the absence of the inhibitor and guess at the maximal rate. Go to the half-way point and see what the approximate  $K_m$  is. Do the same for the results in the presence of the inhibitor. Even using this silly and imprecise method, it should be clear that the affinity was lowered greatly by the presence of the inhibitor. This is classic competitive inhibition.*