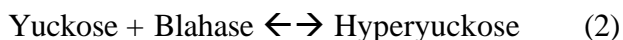


1. A pharmaceutical company is investigating a new lead for a drug known as Cureall. The drug binds to the active site of enzyme Blahase, which tends to make sick people feel lousy if it is not inhibited. The binding equilibrium is known to be:



The substrate for Blahase is the carbohydrate Yuckose. Cureall is a competitive inhibitor of Yuckose and prevents the formation of Hyperlyckose in the reaction:



A chemist reports that the association constant for Cureall is greater than that for Yuckose and that it has a high binding enthalpy. You are an analyst for the investment firm Smartmoney, Inc. and you are asked to examine the thermodynamic data. To determine whether Cureall will be a success in clinical trials you should do the following:

- Determine the binding (association) enthalpy for Cureall and Yuckose, respectively, with Blahase.  
 $\Delta H^\circ (1) = \underline{\hspace{2cm}}$ .  $\Delta H^\circ (2) = \underline{\hspace{2cm}}$ .
- Determine the binding (association) entropy for Cureall and Yuckose, respectively, with Blahase.  
 $\Delta S^\circ (1) = \underline{\hspace{2cm}}$ .  $\Delta S^\circ (2) = \underline{\hspace{2cm}}$ .
- Cureall is a mimic for a carbohydrate, but it is a floppy molecule (i.e. it has many ether linkages and there are many possible conformations of the drug in solution). Your boss asks you to explain the sign of the entropy of binding of the drug.
- Ascertain whether the drug binds more tightly than the native substrate Yuckose at body temperature.

Temperature (K)	$K_a$ for (1) $10^9 \text{ M}^{-1}$	$K_a$ for (2) $10^9 \text{ M}^{-1}$
280	5.08	2.15
290	1.55	1.02
300	0.51	0.51
310	0.18	0.27
320	0.069	0.15

- The pharmaceutical company states that the concentration of the drug Cureall can be as high as  $10^{-8} \text{ M}$  while the native substrate has a concentration of  $10^{-6} \text{ M}$  (1 micromolar). Assuming these concentrations and an enzyme concentration of  $10^{-6} \text{ M}$  determine the effect of inhibitor on the enzyme kinetics at 290 K. The enzyme turnover number (i.e. the rate constant  $k_b$ ) is  $1000 \text{ s}^{-1}$  at 290 K. The binding half-life for the substrate Yuckose

is 69.3 milliseconds. NOTE: The binding constants above are association constants.  $K_I$  for inhibition is usually reported as a dissociation constant ( $K_I = 1/K_a$  for 1).

$$K_M = \text{_____}. \quad V_{\max} = \text{_____}.$$

$$V = \text{_____} \text{ for } [S] = 10^{-6} \text{ M}.$$

$$V_I = \text{_____} \text{ for } [S] = 10^{-6} \text{ M and for } [I] = 10^{-8} \text{ M}.$$

The rate slows by a factor of:

$$V / V_I = \text{_____}.$$

f.) Assuming the same concentrations as above determine the effect of inhibitor on the enzyme kinetics at 310 K. At this temperature the enzyme turnover number (i.e. the rate constant  $k_b$ ) is unchanged at  $10^3 \text{ s}^{-1}$  and the binding half-life for the substrate Yuckose is 6.93 milliseconds.

$$K_M = \text{_____}. \quad V_{\max} = \text{_____}.$$

$$V = \text{_____} \text{ for } [S] = 10^{-6} \text{ M}.$$

$$V_I = \text{_____} \text{ for } [S] = 10^{-6} \text{ M and for } [I] = 10^{-8} \text{ M}.$$

$$V / V_I = \text{_____}.$$

g.) Experts suggest that an inhibitor should lower the enzyme rate by at least a factor 100 to be an effective drug. Should Smartmoney, Inc. invest in Cureall?

2. Write down rate constant corresponding to the following description.
  - a. The first-order fluorescence lifetime is 12 nanoseconds.
  - b. The half-life is 3 milliseconds (first-order process).
  - c. The half-life is 40 microseconds for reagents at 1 micromolar concentration (second-order)
  - d. The observed decay time is 150 picoseconds.
  
3. The quantum yield for the isomerization of retinal is 0.67. The observed isomerization time is 240 femtoseconds. Determine the rate constant  $k_{\text{iso}}$  and  $k_{\text{back}}$  depicted on the scheme below.

