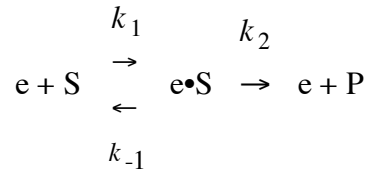


Biochemistry 704
Problem Set 3
Due 03/04/2008

1. Consider the following simplified scheme for the enzymatic conversion of a substrate, S, to a product, P:



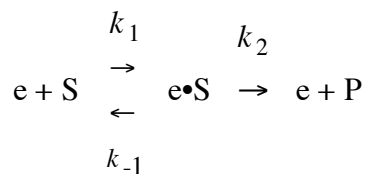
- a. Using the steady-state approximation (that is, $d[e \cdot S]/dt = 0$), show that the reaction velocity v is given by the Michaelis–Menten equation:

$$v = \frac{d[P]}{dt} = \frac{k_{\text{cat}}[e]_{\text{T}}[S]}{K_{\text{M}} + [S]}$$

where $[e]_{\text{T}}$ = concentration of all enzyme forms, $k_{\text{cat}} = k_2$, and $K_{\text{M}} = (k_{-1} + k_2)/k_1$.

- b. What is the observed rate constant when $[S] \ll K_M$? (These conditions are referred to as “sub-saturating” or “second-order”.)
- c. What is the observed rate constant when $[S] \gg K_M$? (These conditions are referred to as “saturating” or “first-order”, and the rate constant is V_{\max} .)
- d. What is the observed rate constant when $[S] = K_M$?

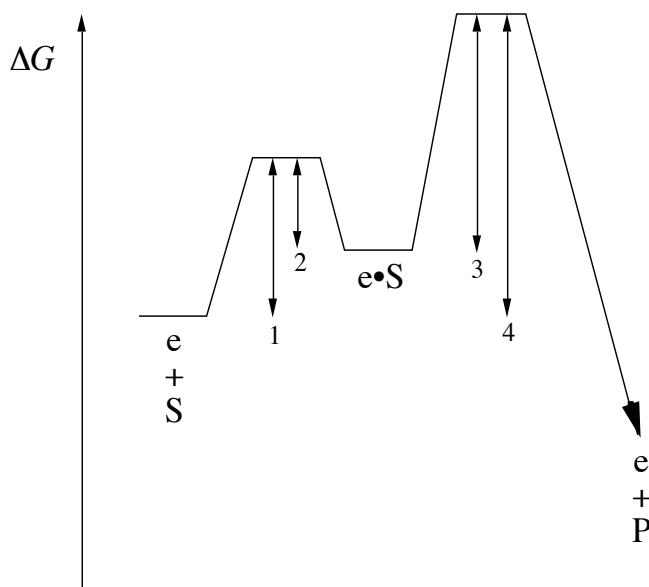
2. The free energy profile (which is actually a “kinetic barrier” diagram) on the next page describes the reaction:



which is catalyzed by an enzyme, e, in the presence of a standard-state concentration of substrate, [S]. The four labeled barriers (1–4) correspond to free energy differences and are calculated using the equation:

$$\Delta G^\ddagger = -RT \ln \left(\frac{k_r h}{k_B T} \right)$$

where k_r is the first-order rate constant for the step, h is the Planck constant, and k_B is the Boltzmann constant. The binding of e and S , which is a second-order process, is described by a barrier that corresponds to $k_1[S]$. (Note also that barrier 3 is much larger than barrier 2.)



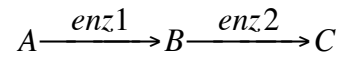
- Which free energy barrier(s) corresponds to k_{cat} , the rate constant if $[S] \gg K_M$?
- Which barrier(s) corresponds to $(k_{\text{cat}}/K_M)[S]_0$, the rate constant if $[S] \ll K_M$?
- What would be the effect on each of the free energy barriers (1–4) if the viscosity of the solution is increased?

d. What would be the effect on each of the free energy barriers if $[S]_o$ is increased?

e. What would be the effect on each of the free energy barriers if a residue in e is replaced leading to weaker binding of the transition state for the chemical step: $e \cdot S \rightarrow e + P$?

3. a. An enzyme catalyzes a reaction that has an overall equilibrium constant of K_{eq} . Use the definitions of k_{cat} and K_{M} in Question 1 to derive a simple mathematical relationship between K_{eq} , $(k_{\text{cat}}/K_{\text{M}})_{\text{f}}$ (which is for the reaction in the forward direction) and $(k_{\text{cat}}/K_{\text{M}})_{\text{r}}$ (which is for the reaction in the reverse direction).
- b. The enzyme has $(k_{\text{cat}})_{\text{f}} = 1200 \text{ s}^{-1}$, $(K_{\text{M}})_{\text{f}} = 1.2 \text{ mM}$, and $(K_{\text{M}})_{\text{r}} = 0.15 \text{ mM}$. What is the value of $(k_{\text{cat}})_{\text{r}}$?

4. An enzyme converts A into B, and another enzyme converts B into C. The maximum velocities for the two enzymes are 10 mM/min and 15 mM/min, respectively. Assume that the reverse reactions and product inhibition are unimportant, so that:



- a. If the concentration of A is *saturating*, what is the maximum concentration of B (relative to K_M^B) that can be present?
- b. What concentration of A (relative to K_M^A) will produce half of this concentration of B?