Chapter 5 Odd Solutions

1. Residues acting as general acids have to be able to donate a proton to their substrate. They therefore have to be at least as acidic as the substrate, or in other words they need to have a low pK_a. Typical general acids are therefore glutamate and aspartate, and often histidine, which has a pK_a close to 7 and is therefore readily protonated and deprotonated at pH 7. Lysine and tyrosine normally have much higher pK_a (around 10; see Table 1.1) and therefore require their pK_a to be lowered by at least 3 pH units by their context within the enzyme if they are to function as general acid catalysts. Although this is perfectly possible, it is unusual.

3. K_m is a measure of the substrate concentration required for effective catalysis to occur. An enzyme with a high K_m requires a high substrate concentration to achieve a given velocity and is therefore to that extent a poor enzyme.

k_{cat} gives a direct measure of the catalytic production of the product under optimum conditions (saturated enzyme). It measures the number of substrate molecules turned over per enzyme per second. It therefore measures the rate of the enzyme-catalyzed reaction and is closer to what we want; however, it is not ideal because if a higher rate is achieved but at the expense of a much higher substrate concentration, then it can hardly be called a better enzyme.

k_{cat}/K_m is a measure of enzyme efficiency. It shows what the enzyme and substrate can accomplish when abundant enzyme sites are available and it allows direct comparison of the effectiveness of an enzyme toward different substrates. Thus, in an enzyme-catalyzed reaction this is the number to optimize.

5. The main reason is that eukaryotic genomes encode a much larger number of genes for regulatory and signaling purposes. The number of enzymes encoded is not all that different, but because the number of non-enzymatic signaling and regulatory proteins is so much higher, the proportion of enzymes is smaller.

7. Magnesium is used mainly for binding to phosphates such as ADP and ATP. The divalent positive charge on magnesium helps neutralize the negative charges on the phosphates, and therefore makes them more reactive and easier to handle. It has a well-defined coordination geometry but can bind and dissociate rapidly, which makes it useful in a biological context (unlike chromium, for example, which is much more kinetically inert). It also does not bind too tightly, which is a useful feature. Zinc is largely similar, but is somewhat 'harder,' and binds more tightly both to sulfur and to nitrogen. In particular it is a good Lewis acid, which makes it a useful electrophilic catalyst, and it is used in two main situations: either as a Lewis acid or electrophile (for example in thermolysin) or more simply as a structural cofactor, for example in zinc fingers. Its reasonably strong affinity for both sulfur (in cysteine) and nitrogen (in histidine) combined with its well-defined octahedral geometry makes it ideal as a structural

element. However, it can form a range of coordination geometries without any large energetic penalty, which makes it very useful as an electrophilic catalyst, where it is often required to change its coordination geometry during the course of a reaction, for example to gain or lose a coordinating water molecule. Iron is used mainly because it has two stable oxidation states and is therefore a useful electron carrier, for example in heme and iron–sulfur clusters. In a free state, iron is rather poorly behaved; for example, it tends to form large aggregates of Fe_2O_3 (rust). But when coordinated within a protein, it can be kept stable.

By contrast, aluminum does not have good properties in aqueous solution: it forms disordered and dynamic hydroxides which readily precipitate, and its small size and high charge give it a high charge density, which is difficult for biological systems to handle. Silicon has different problems, mainly the kinetic stability of silicon–oxygen bonds, which make it not useful. In the early biosphere, zinc would have been unavailable because it would have been mainly present as zinc sulfides, hence making magnesium an obvious choice. If you want more detail on this topic, look at J.J.R. Fraústo da Silva and R.J.P. Williams [*The Biological Chemistry of the Elements: the Inorganic Chemistry of Life*. Oxford: Oxford University Press, 2001] or several other books by these authors.

N1. There are lots of ways to answer this question, which are equivalent in their outcome but have different appearances. The following is one of the more elegant.

From Section 5.1.1, $k = Ae^{-E_a/RT}$, where E_a is the activation energy and A is not known. We can write similar equations for the two temperatures, with the same values of A:

$k_1 = Ae^{-E_a/RT_1}$ and $k_2 = Ae^{-E_a/RT_2}$

Therefore the ratio of one to the other cancels out the unknown A:

$k_1/k_2 = e^{-E_a/RT_1}/e^{-E_a/RT_2}$

Which can be rewritten as

$$k_1/k_2 = \exp\left(\frac{E_a}{RT_2} - \frac{E_a}{RT_1}\right) = \exp\left(\frac{E_a.RT_1}{RT_2.RT_1} - \frac{E_a.RT_2}{RT_2.RT_1}\right) = \exp\left(\frac{E_aR\ T_1 - T_2}{RT_2.RT_1}\right) = \exp\left(\frac{10E_a}{RT_1.T_2}\right)$$

the number 10 in the final equation arising from the fact that the difference in temperature is 10K.

Substituting $E_1 = 5 \times 10^4$, $R = 8.31$, $T_1 = 298$, $T_2 = 308$ gives $k_1/k_2 = e^{0.66}$, or just less than 2: in other words, the rate will go up by slightly less than a factor of 2 as a result of a 10K rise in temperature. This is a typical figure for a wide range of reactions, both catalyzed and uncatalyzed.

N3. (a) 1/2; (b) 1/3; (c) 1/11.

N5. This is a similar sort of calculation to that in Problem N1, and we can approach it in a similar way. We can write the two rates k_1 and k_2 (catalyzed and uncatalyzed, respectively) as

$$k_1 = Ae^{-E_a{}^1/RT} \text{ and } k_2 = Ae^{-E_a{}^2/RT}$$

And therefore

$$k_1/k_2 = e^{-E_a{}^1/RT}/e^{-E_a{}^2/RT}$$

Which can be rewritten as

$$k_1/k_2 = \exp\left(\frac{E_a{}^2}{RT} - \frac{E_a{}^1}{RT}\right) = \exp\left(\frac{E_a{}^2 - E_a{}^1}{RT}\right)$$

The difference in the activation energies is $4\,\text{kJ mol}^{-1} = 4000\,\text{J mol}^{-1}$; $R = 8.31$ and $T = 298$, so $k_1/k_2 = \exp(4000/[8.31 \times 298]) = 5.0$. In other words, a very modest lowering of the activation energy already increases the rate by a factor of 5. This justifies the comment in Chapter 5 that it is much more effective to change the activation energy than to change the fraction of enzyme that is bound, and hence most enzymes work with substrate concentrations below K_m.