4 Calculations in the molecular biosciences

Part 2: Characterization of biological processes

This chapter continues the approach of Chapter 3, and deals with the analysis of some important types of biological processes. The first two sections deal with the energetics of processes (how far and how fast will they proceed?). These sections are followed by a detailed discussion of binding equilibria; this includes the kinetics of enzyme-catalysed reactions since many of the equations involved are similar. A final section deals with radioactivity, which is widely used to track specific compounds. As with Chapter 3, there are several problems at the end of each section and also at the end of the chapter to allow you to check your understanding of the material covered.

4.1 The energetics of processes: thermodynamics

KEY CONCEPTS

- Defining the terms ΔH, ΔS, and ΔG for a process
- Defining the standard state and biochemical standard state
- Knowing the relationship between ΔG^0 and the equilibrium constant
- Defining the mass action ratio (MAR) for a process

In biological systems we deal with two main types of processes, namely (a) chemical reactions in which covalent bonds are broken and made, so that one or more new compounds (products) are made from the starting compounds (reactants or substrates), and (b) complex formation in which two (or more) compounds can associate reversibly with one another. The latter types of processes almost always involve weak non-covalent interactions (see Chapter 1, section 1.7).

Both types of processes will eventually reach a position of equilibrium where there is no further tendency to change with time; this is, however, a dynamic state, rather than a static one. The forward and backward reactions are both proceeding, but at equal rates.

The science of thermodynamics is concerned with the overall changes in energy when processes occur; it can be used to predict how far a process will proceed, i.e. does the equilibrium lie more towards the side of the products or the reactants?

The energy changes that occur during processes can be described using different parameters.

ΔH is the *change in enthalpy* (change in heat content) of a system. This is a measure of the energy changes in breaking and making of bonds (either covalent or non-covalent) in a process. An exothermic reaction or process is one in which heat is given out by the system to its surroundings; this has a negative ΔH. An endothermic reaction or process has a positive ΔH and therefore absorbs heat from the surroundings. The units of ΔH are energy mol^{-1}, usually kJ mol^{-1}.

ΔS is the *change in entropy*. This is a measure of the change in the extent of randomness (or disorder) of a system. Thus, a process in which the number of molecules increases would be expected to have a positive ΔS. Similarly the transition from a solid to a liquid or a liquid to a gas would have a positive ΔS. The units of ΔS are energy degrees $Kelvin^{-1}$ mol^{-1}, e.g. kJ K^{-1} mol^{-1} (or because these values are usually small, J K^{-1} mol^{-1}).

Note that we use the absolute or Kelvin scale of temperature in energy calculations. 0°C is equivalent to 273 K (more precisely 273.15 K).

ΔG is the *changes in (Gibbs) free energy*. This is a measure of the work or usable energy that can be obtained from a process. A process in which free energy is liberated (exergonic) has a negative ΔG. An endergonic process has a positive ΔG. The units of ΔG are energy mol^{-1}, usually kJ mol^{-1}.

The terms enthalpy, entropy and free energy are dealt with in greater detail in many standard textbooks of chemistry and biochemistry, such as those referred to in Chapter 1.

A negative value of ΔG indicates that the process has a tendency to proceed from left to right. Although the value of ΔG will give no indication about the rate at which the process may proceed, it should be noted that in the case of the vast majority of biochemical reactions, the presence of appropriate enzymes normally ensures that reactions that are feasible energetically will proceed at a suitable rate to meet the needs of the organism concerned. The change in free energy for a process is defined by eqn. 4.1:

The rates at which processes proceed are dealt with in the section on kinetics (section 4.2).

$$\Delta G = \Delta H - T\Delta S \qquad 4.1$$

where T is the absolute temperature (degrees Kelvin, K). Thus, the change in free energy is the change in enthalpy minus the energy associated with an increase in entropy.

The values of ΔG, ΔH, and ΔS can be measured under any set of conditions. However, to provide a reference point for measurements and calculations, it is necessary to define a standard state, and the values under these conditions are then denoted by a superscript zero, i.e. ΔG^0, ΔH^0, and ΔS^0. The *standard state of a substance* refers to the substance under 1 atm pressure at the temperature in question. For almost all biochemical purposes we are dealing with solutions and the standard state of a solute is a 1 M solution of that solute. (For a gas, the standard state is the gas at 1 atm; for a solvent, it is the pure solvent). Under standard state conditions, eqn. 4.1 would then be written $\Delta G^0 = \Delta H^0 - T\Delta S^0$.

In the special case of biochemical processes where H^+ is either a reactant or a product of the reaction, it is useful to define a new biochemical standard state, where the standard states of all solutes is 1 M, but that of H^+ is 10^{-7} M (i.e. pH 7). The values of the quantities in the biochemical standard state are denoted by a superscript dash, i.e. $\Delta G^{0\prime}$, $\Delta H^{0\prime}$, and $\Delta S^{0\prime}$.

For most processes either the ΔH term or the ΔS term (strictly speaking the $T\Delta S$ term) makes the dominant contribution to the overall ΔG.

WORKED EXAMPLE

For the combustion of glucose ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$) the values of ΔG^0 and ΔH^0 at 25°C are −2872 and −2822 kJ mol^{-1}, respectively. Calculate the value of ΔS^0 and comment on its sign. Which term makes the dominant contribution to ΔG^0?

STRATEGY

This is an application of eqn. 4.1 using the standard state values of the thermodynamic parameters.

SOLUTION

We can rearrange eqn. 4.1 to give $\Delta S^0 = (\Delta H^0 - \Delta G^0)/T$. Putting $T = 298$ K, we find that $\Delta S^0 = (-2822 + 2872)/298$ kJ K^{-1} mol^{-1} = 0.168 kJ K^{-1} mol^{-1}, or 168 J K^{-1} mol^{-1}. The positive value of ΔS^0 reflects the greater number of molecules of products (12) compared with reactants (7). In this reaction, the enthalpy term makes the dominant contribution to the free energy change.

The derivation of eqn. 4.2 involves application of the laws of thermodynamics and the ideal gas law and is described in the textbook by Price *et al.* (2001).

For the process $A + B \rightleftharpoons C + D$, it is possible to derive the general eqn. 4.2 which applies under any set of conditions:

$$\Delta G - \Delta G^0 = RT \ln \left(\frac{[C] \times [D]}{[A] \times [B]} \right) \qquad 4.2$$

where ΔG is the free energy change under the given set of conditions, ΔG^0 is the free energy change when A and B in their standard states are converted to C and D in their standard states, R is the gas constant (8.31 J K^{-1} mol^{-1}) and ln is the natural logarithm.

It should be noted that the terms [*C*], etc. in eqn. 4.2 are usually taken to be concentrations; in fact each represents a ratio of the concentration under the given conditions to the concentration in the standard state, which for a solute is a 1 M solution under 1 atmosphere pressure. Hence, the term $\left(\frac{[C] \times [D]}{[A] \times [B]} \right)$ (or $\left(\frac{[C]}{[A] \times [B]} \right)$ for the process $A + B \rightleftharpoons C$) is dimensionless, i.e. a pure number – indeed if it were not, it would not be possible to take logarithms. However, values

of equilibrium constants (K_{eq}) or MAR (Γ) (see below) are usually given in molar units to indicate that the standard state of the each component (solute) is a 1 M solution.

The term $\left(\frac{[C]\times[D]}{[A]\times[B]}\right)$ is often known as the MAR and given the symbol Γ. It can be used to calculate the actual free energy change in a process under any given set of conditions. Thus, eqn. 4.2 can be rewritten as eqn. 4.3:

$$\Delta G - \Delta G^0 = RT \ln \Gamma \qquad 4.3$$

Γ is the Greek capital letter gamma.

A special case arises when the process is at equilibrium, i.e. when there is no overall tendency for it to proceed in either direction, although the forward and backward processes will occur. In this case, $\Delta G = 0$, and the values $[A]$, $[B]$, etc. are their equilibrium values ($[A]_{eq}$, $[B]_{eq}$, etc.). Thus, eqn. 4.2 can be rewritten as eqn. 4.4:

$$-\Delta G^0 = RT \ln \left(\frac{[C]_{eq}\times[D]_{eq}}{[A]_{eq}\times[B]_{eq}}\right) = RT \ln K_{eq} \qquad 4.4$$

where K_{eq} is the equilibrium constant for the process.

The formation and dissociation of a protein–ligand complex, PL, is discussed in detail in section 4.3.1.

We can use eqn. (4.4) to calculate K_{eq} (and hence know on which side the equilibrium lies) from the value of ΔG^0 for a process and vice versa. However, it is a very good practice to make an estimate of the value of K_{eq} you would expect before using the calculator.

4.1.1 Key relationships

In Chapter 2, section 2.3.6 we mentioned that at 310 K (37°C) each change of approximately 6 kJ mol^{-1} leads to a 10-fold change in the value of K_{eq}. So we can proceed as follows.

- If the value of ΔG^0 is positive, then the process is unfavourable as written and the equilibrium will lie on the side of the reactants, (i.e. K_{eq} will be less than 1)
- If ΔG^0 is negative, the equilibrium will lie towards the products, i.e. K_{eq} will be greater than 1
- If $\Delta G^0 = 0$, then K_{eq} would equal 1 and the process would be balanced between reactants and products).

A worked example will illustrate these principles.

WORKED EXAMPLE

The ΔG^0 for the reaction catalysed by pyruvate kinase is -31.4 kJ mol^{-1} at 310 K.

phosphoenolpyruvate + ADP $\rightleftharpoons$ pyruvate + ATP

Estimate the equilibrium constant for the reaction.

STRATEGY

This is an application of the general principles stated above.

SOLUTION

Because ΔG^0 is negative, we know K_{eq} will be greater than 1. The value of ΔG^0 represents about five multiples of 6 kJ mol^{-1}. From this we can estimate that K_{eq} should equal about 1×10^5 (M). The accurate answer can be obtained from a calculator using eqn. 4.4., i.e. $-(-31\,400) = 8.31 \times 310 \times \ln K_{eq}$. Hence $\ln K_{eq} = 12.19$, i.e. $K_{eq} = 1.97 \times 10^5$ (M). (Note that we have expressed ΔG^0 in terms of J, since the value of R is given in J.)

Biological systems are not at overall equilibrium with their surroundings (otherwise they would be dead!), although a significant proportion of the several thousand metabolic reactions will be at or very close to equilibrium; this provides a justification for the study of equilibrium thermodynamics. Thus, the value of ΔG^0 for a reaction may not give a very reliable guide to the actual tendency (ΔG, driving force) of the reaction to proceed under the conditions prevailing in the living cell.

The term 'steady state' refers to the situation when the concentrations of intermediates in a series of reactions remains constant (i.e. in each case, the rate of formation equals the rate of breakdown). In living organisms, this would apply to the concentrations of metabolic intermediates in pathways such as glycolysis, the tricarboxylic acid cycle, etc.

Another branch of thermodynamics has been developed to describe and analyse systems that are not at equilibrium. However, non-equilibrium thermodynamics (in which there is an emphasis on the steady state of a system) is beyond the scope of this book.

WORKED EXAMPLE

Phosphofructokinase catalyses the reaction:

fructose-6-phosphate + ATP $\rightleftharpoons$ fructose-1,6-bisphosphate + ADP

for which the $\Delta G^{0\prime} = -17.7$ kJ mol^{-1} at 308 K. In a study of perfused rat heart, the following concentrations of metabolites were found: fructose-6-phosphate (F6P), 60 μM; ATP, 5.3 mM, fructose-1,6-bisphosphate (FBP), 9 μM; ADP, 1.1 mM. Is the reaction at equilibrium in the perfused heart and, if not, what is the value of $\Delta G'$? In the same tissue, the concentration of AMP was found to be 95 μM. Is the reaction catalysed by adenylate kinase (2ADP $\rightleftharpoons$ ATP + AMP) at equilibrium ($\Delta G^{0\prime} = 2.1$ kJ mol^{-1})?

STRATEGY

This problem depends on the application of eqn. 4.3, making sure that the concentrations are quoted in molar terms, e.g. $[ATP] = 5.3 \times 10^{-3}$ M.

SOLUTION

The MAR (Γ) for the phosphofructokinase reaction, i.e.

$([FBP] \times [ADP])/([F6P] \times [ATP]) = (9 \times 10^{-6} \times 1.1 \times 10^{-3})/(6 \times 10^{-5} \times 5.3 \times 10^{-3}) = 0.031$

Hence, from eqn. 4.3 ($\Delta G' - \Delta G^{0\prime} = RT \ln \Gamma$). $\Delta G'$ can be calculated as:

$$\Delta G' = -17\,700 - 8890 \text{ J mol}^{-1} = -26\,600 \text{ J mol}^{-1} = -26.6 \text{ kJ mol}^{-1}$$

Hence, the reaction is clearly not at equilibrium. For the adenylate kinase reaction, the MAR is $([ATP]\,[AMP])/[ADP]^2 = 0.416$. Hence $\Delta G' = 2.1 - 2.24$ kJ mol^{-1}, i.e. -0.14 kJ mol^{-1}. Since $\Delta G'$ is very small, this latter reaction is very close indeed to equilibrium.

SELF TEST ?

Check that you have mastered the key concepts at the start of the section by attempting the following questions.

ST 4.1 For the hydrolysis of ATP to give ADP and phosphate (P_i) at 37°C, $\Delta H^{0\prime}$ and $\Delta S^{0\prime}$ are -20.5 kJ mol^{-1} and 30.6 J K^{-1} mol^{-1}, respectively. What is $\Delta G^{0\prime}$ for the reaction?

ST 4.2 The ΔG^0 for the reaction catalysed by lactate dehydrogenase (lactate + $NAD^+ \rightleftharpoons$ pyruvate + NADH) is +23.5 kJ mol^{-1} at 37°C. Estimate the value of the equilibrium constant.

In ST 4.2 use the key relationships to link the values of K_{eq} and ΔG^0.

ST 4.3 The enzyme aldolase catalyses the reaction FBP $\rightleftharpoons$ G3P + DHAP (where FBP is fructose-1,6-bisphosphate, G3P is glyceraldehyde-3-phosphate and DHAP is dihydroxyacetone phosphate). The $\Delta G^{0\prime}$ for this reaction at 37°C is 23.5 kJ mol^{-1}. The concentrations of FBP, G3P, and DHAP in resting muscle were measured as 30, 20, and 110 µM, respectively. What is the value of K_{eq} for the aldolase reaction? What is the value of $\Delta G'$ for this reaction in muscle?

Answers

ST 4.1 The $\Delta G^{0\prime}$ for the reaction is -30.0 kJ mol^{-1}.

ST 4.2 The estimate of K_{eq} is about 1×10^{-4} (M); the accurate answer is 1.09×10^{-4} (M).

ST 4.3 The values of K_{eq} and $\Delta G'$ are 1.09×10^{-4} (M) and -1.0 kJ mol^{-1}, respectively.

Attempt Problems 4.1–4.4 at the end of the chapter.

4.2 The rates of reactions: kinetics

KEY CONCEPTS

- Illustrating the energy profile for a reaction indicating the following terms: energy of reaction, activation energy, transition state, intermediate
- Distinguishing the order and molecularity of a reaction
- Stating the rate laws for zeroth, first, pseudo-first, and second-order reactions
- Deducing the units of rate constants for different orders of reactions
- Describing the dependence of reaction rate on temperature

Thermodynamics deals with the energy changes in processes and reactions and can thus indicate which reactions are feasible. However, it says nothing about the rates at which they might occur; this is the subject of kinetics. (In this section, we shall use the term 'reaction' to include chemical reactions as well as the formation of complexes between molecules, unless otherwise indicated).

The energy changes which occur during a typical chemical reaction can be depicted by an energy profile (Fig. 4.1).

In order to react, the reactants have to possess sufficient energy (the activation energy) to reach the transition state of the reaction, the state of highest energy in the profile. If the profile involves a local minimum, this would represent an

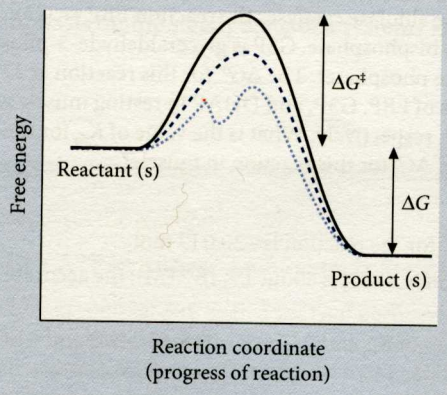

Fig. 4.1 Energy profile for a reaction. The solid line represents a typical energy profile, in which an activation energy barrier ($\Delta G^{\ddagger}$) must be surmounted. The highest point in the profile is the transition state in the reaction. The dashed and dotted lines represent possible profiles for catalysed reactions in which smaller energy barriers must be surmounted. In the case of the dotted line there is a local minimum before the transition state is reached; this represents an intermediate in the reaction. The overall free energy change in the reaction is represented by ΔG.

intermediate. The stability of the intermediate will depend on the depth of this minimum relative to the thermal energy; under favourable circumstances it may be possible to isolate and characterize the intermediate. (It will be easier to isolate the intermediate by working at lower temperatures. The lower thermal energy of the intermediate will trap it in its 'energy well').

Formation or dissociation of a complex also involves surmounting an activation energy barrier, but this will usually be much smaller than for a chemical reaction where strong covalent bonds are broken and made.

As shown in Fig. 4.1, catalysis of the reaction will occur when a new pathway of lower activation energy is available to the reactants.

There is no necessary relationship between the overall energy change for a reaction and the rate at which it will occur (which depends on the magnitude of the activation energy). Thus, a solution of ATP (which is often referred to as a high-energy compound, with a ΔG° of -30 kJ mol^{-1}) is stable for weeks at pH 7.0. However, in the cell there are enzymes which will catalyse reactions involving the transfer of the terminal (γ) phosphate group of ATP to a variety of acceptor molecules so that reactions occur on the time scale required by the demands of the organism.

ATP is often referred to as the 'energy currency' of the cell. There are reactions and processes which generate sufficient energy to drive the synthesis of ATP from ADP plus phosphate. Other reactions and processes can be driven towards completion by being coupled to the hydrolysis of ATP. Problems 4.2 and 4.3 at the end of the chapter deal with this concept in more detail.

Kinetics is concerned with measuring the rate of a reaction under a variety of conditions, changing parameters such as the concentrations of reactants, pH, temperature, etc. From the data acquired, we aim to formulate the rate law (i.e. determine the order of the reaction) and use this and other information (such as attempting to isolate intermediates) to propose a mechanism. The mechanism is a description at the atomic level of the elementary steps of bond breaking and making as the reaction proceeds. Knowledge of the mechanisms of chemical reactions is important to understand the basis of enzyme catalysis.

Rates can be measured in a continuous fashion (in which a property which changes during the reaction, such as absorbance or fluorescence, is monitored) or in a discontinuous fashion (in which samples are taken from the mixture at stated times, the reaction is stopped (quenched) and analysis is undertaken to determine the extent of the reaction). In addition to being much more convenient experimentally, continuous monitoring of a reaction (if it is feasible) provides many more data points for subsequent mathematical analysis of the kinetics.

4.2.1 The order of a reaction

The order of a reaction is defined as the power to which the concentration of a reactant is raised in the rate law. Determination of the order of a reaction is an important part of investigation of its mechanism; any proposed mechanism must generate a rate law consistent with the experimental observations.

Thus, for a hypothetical reaction in which x moles of A and y moles of B react to form products:

$$x\mathrm{A} + y\mathrm{B} \rightarrow \text{products}$$

The rate of the reaction (i.e. the rate of formation of products, or the rate of disappearance of reactants) might be given by eqn. 4.5: