

6 Figures, 2 Appendices, 0 Highlighted Boxes, 2 Footnotes

Chapter 3. Chemical Reactions, Enzyme Catalysts and Stable Isotopes

A corollary to this law shows how the chemical equilibrium varies with temperature - namely, how, as the temperature increases, more of the one compound is formed at the expense of the other, or vice versa. This corollary can be stated as follows: At low temperature the greater yield is always of that product whose formation is accompanied by evolution of heat.

Jacobus H. van't Hoff

Nobel Prize Lecture, 1901

In explaining the relation between temperature and chemical reactions in his *Etude de Dynamique chimique* in 1884, Jacobus van't Hoff built a conceptual bridge between the concepts of thermodynamics, which had emerged in cogent fashion during the second half of the 19th Century, and chemical equilibrium, which had emerged from studies on reversible chemical reactions by Claude Berthollet earlier in that century. In the quote from his Nobel Prize lecture that we have referenced above, van't Hoff refers to the concept of exothermic chemical reactions as being the spontaneous outcome of a chemical interaction which can be predicted by thermodynamic laws. Fluxes between ecosystems and the atmosphere are often the result of biochemistry that occurs in living cells, or physical chemistry that occurs as substances move among alternative thermodynamic states (e.g., the water flux associated with ecosystem evaporation). In both cases, the chemical and physical conversions of compounds adhere to thermodynamic constraint. Thus, a full understanding of ecosystem-atmosphere fluxes requires that we probe the interface between thermodynamics and chemical equilibrium. As we begin this chapter, we will build a foundation from concepts that stretch from the last chapter on thermodynamic principles to the next chapter on biochemistry and metabolism.

Chemistry requires the interaction among particles of mass; either as individual atoms or as atomic components of molecules. As units of mass interact, subatomic particles will rearrange themselves into relations with one another that satisfy the most stable thermodynamic state. In some cases, this results in the actual exchange of one or more electrons, the most common subatomic particle to participate in chemical reactions. In other cases, electrons may move to a more stable, shared association with two nuclei, forming a covalent bond. The result of these

chemical interactions is often reversible, and the probability of reversal can be predicted from thermodynamic knowledge about the free energy state of reactants and products. We begin this chapter with a discussion of chemical equilibrium, reaction kinetics and reversibility. From this discussion, we move into the topic of oxidation-reduction reactions; those reactions involving the exchange of electrons, and reactions that are common to biogeochemical transitions among compounds. We next take up the topic of enzyme catalysis. Most biochemical reactions would not occur at rates sufficient to sustain life, at the temperatures that typically exist on earth, if not for the existence of biological catalysts known as enzymes. Knowledge about enzymes, and their kinetic effect on reaction rates, is crucial to understanding control over the metabolic fluxes that we consider in the next chapter. Finally, we begin a discussion about stable isotopes and their role in biogeochemical studies. As we proceed through subsequent chapters, we will return to the topic of stable isotopes, but applied at progressively larger scales. Knowledge about changes in the abundance of specific isotopic forms of an element is informative about the kinetics of biogeochemical processes. In fact, the study of stable isotope biogeochemistry has become a dominant theme in most national and international scientific symposia within which the topic of earth system sciences is considered. In the interest of educating students about the enormous potential to use stable isotopes as a research tool, we will spend a lot of time on this topic.

3.A Reaction Kinetics, Equilibrium and Steady-State

The *kinetic properties* of a reaction are defined as those factors that affect reaction rate. Some of the most important kinetic properties include temperature, energy of activation, and the concentrations of reactants (the effect of reactant concentration on reaction rate is sometimes referred to as *mass action*). To illustrate how kinetic properties control the rate of a reaction, consider the hypothetical, reversible reaction whereby reactants A + B are transformed into products C + D, and the reverse reaction that transforms reactants C + D into products A + B (i.e., $A + B \leftrightarrow C + D$). The rate of the reaction in both directions will depend on the relative numbers of reactant molecules that carry sufficient energy to exceed an energy threshold during collision with other reactant molecules. The energy threshold is referred to as the *energy of activation* (E_a ; Fig. 3.1). If the collision energy of reactant molecules exceeds E_a , the reactants are forced into the *transition state*, an unstable state that leads spontaneously to the formation of

product. A higher value of E_a means that a smaller fraction of collisions will result in formation of the transition state, and the rate of reaction will be slower. The presence of a catalyst will speed up the reaction by facilitating more ‘collisions’ that exceed E_a (i.e., reactant interactions are organized by the catalyst such that they occur with sufficient energy and orientation to facilitate formation of the transition state).

Any given pool of reactant molecules will exist across a range of different kinetic energies. Some molecules will exist at lower energy and some at higher energy. It is not possible to know the exact number of molecules at each level of energy, and thus predict the number of molecules that will collide with enough energy to exceed E_a . Instead, we rely on statistical models to predict the probability of energy distribution. The most commonly-used model is known as the *Maxwell-Boltzmann energy distribution*. At a given temperature, the statistical distribution of kinetic energy across the pool of reactant molecules participating in a reaction can be described as:

$$n_E = n_T A e^{-E/RT} \quad (3.1)$$

where n_E is the moles of reactant molecules that have energy that exceeds a given energy level (E , with units J mol^{-1}), n_T is the total moles of reactants, A is a synthetic coefficient that scales proportionally with E , R is the universal gas constant (with units $\text{J mol}^{-1} \text{K}^{-1}$), and T is Kelvin temperature.¹ The important point to make in evaluating the relations contained in Equation 3.1 is that as E initially increases, and A increases in proportion to it, n_E will also increase to a maximum; thereafter, the inverse exponential effect of $e^{-E/RT}$ will take over and drive n_E down again (Fig. 3.2). It is also clear from Equation 3.1 that an increase in temperature shifts the distribution of kinetic energies toward the high end of the distribution. As the temperature of a reaction is increased, the average kinetic energy per mole of reactants will also increase, and more reactant molecules will exist at the high end of the energy distribution. Whereas the proportion of molecules possessing higher kinetic energy increases as a function of temperature, the *rate of increase* actually decreases. This is an important distinction to recognize because it will help explain the response of certain metabolic fluxes, such as those associated with respiration, to changes in temperature. To emphasize once again: as temperature increases the fraction of reactants at the high end of the energy distribution also increases; but the rate at

which this fraction increases decreases as temperature progressively increases.

In addition to energetics, the rate of a chemical reaction is controlled by mass action. According to the law of mass action, at a fixed temperature, the rate of a chemical reaction will be directly proportional to the concentration of reactants. Mass action is typically modeled through simple, linear relationships. Returning to our hypothetical reaction whereby reactants A + B are converted to products C + D, the velocity (v_1) of this 'forward' reaction can be modeled as a rate law, with the form of the law being dependent on whether v_1 is determined by the concentration of both reactants or only one. When v_1 is dependent on one reactant the rate law is expressed as:

$$v_1 = k_1 [A] \text{ or } v_1 = k_2 [B] \quad (3.2)$$

where k_1 is the proportionality constant, or reaction rate constant (with units s^{-1}), relating reactant concentration (with units mol) to velocity (with units mol s^{-1}). The reaction is characterized as first order when its rate is dependent on the concentration of one reactant. The reaction is characterized as second order when its rate is dependent on the concentrations of both reactants. First-order dependence would occur, for example, if B is present at a high enough concentration that the reaction rate is saturated with respect to [B]. Second-order dependence would occur, for example, if A and B are both present in rate-limiting concentrations. A second order reaction would be characterized by a rate law with the form:

$$v_1 = k_1 [A] [B] \quad (3.3)$$

and in this case k_1 would carry units of $\text{mol}^{-1} \text{s}^{-1}$. In a first-order reaction, a doubling of the concentration of the limiting reactant will cause a doubling of v . In a second-order reaction, a doubling of the concentrations of both reactants will cause a quadrupling of v . Using similar concepts the velocity (v_2) of the reverse reaction, whereby reactants C + D are converted to products A + B, can be expressed as:

$$\begin{array}{ll} v_2 = k_2 [C] \text{ or } v_2 = k_2 [D] & \text{(first order)} \\ v_2 = k_2 [C][D] & \text{(second order)} \end{array} \quad (3.4)$$

The state of chemical equilibrium is defined as $v_1 = v_2$. At equilibrium, and assuming second-order dependence for completeness, we can write:

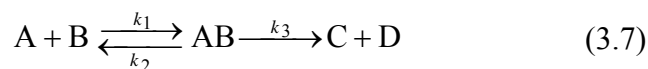
$$k_1 [A] [B] = k_2 [C] [D] \quad (3.5)$$

and after rearrangement:

$$\frac{k_1}{k_2} = \frac{[C][D]}{[A][B]} = K_e \quad (3.6)$$

where K_e is the *equilibrium constant*.

Chemical equilibrium is the potential endpoint for reactions in a closed system; i.e., a system where new reactant molecules are not introduced from outside the reaction boundaries and product molecules are not removed to beyond the reaction boundaries. At equilibrium, the concentrations of reactants and products will not change over time; i.e., $d[A]/dt = d[B]/dt = 0$ and $d[C]/dt = d[D]/dt = 0$. Equilibrium, however, does not imply cessation of the reactions; it's just that the opposing reactions occur at equal rates. Many chemical reactions occur as open systems. In open systems there is a one-way flow through the reaction from reactants to products. These reactions can also reach an apparent state of constancy, referred to as *steady-state*. In order to compare and contrast the concepts of chemical equilibrium and steady-state consider a reaction in which a stable intermediate is formed, which must overcome its own energy barrier to proceed to product(s). A reaction involving a stable intermediate would be modeled with three reaction coefficients:



If the reaction from AB to C + D is irreversible, and if the reaction is allowed to proceed without perturbation (i.e., if the supply of A and B is unlimited), then the concentration of AB will approach a constant value; i.e., $d[AB]/dt = 0$. This is referred to as steady state. At steady state, and assuming second-order dependence of reaction rate on reactant concentration on the left side

of the reaction, we can write:

$$v_1 = k_1 [A] [B], \quad v_2 = k_2 [AB], \quad v_3 = k_3 [AB] \quad (3.8)$$

In this case, k_1 carries units of $\text{mol}^{-1} \text{s}^{-1}$, whereas k_2 and k_3 carry units of s^{-1} . Taking the necessary condition that at steady state the rates of formation and breakdown of AB must be equal, we can write:

$$k_1 [A] [B] = (k_2 + k_3) [AB] \quad (3.9)$$

Rearranging to isolate [AB], we obtain:

$$[AB] = \frac{k_1}{k_2 + k_3} [A][B] = K_s [A][B] \quad (3.10)$$

where K_s is the steady-state constant (with units mol^{-1}) and represents the combined effects of all three rate constants.

It's worth taking some time to appreciate the relationship between the rate constant for a reaction (k) and the energy of activation (E_a). In reactions with a higher E_a , the reaction rate will increase less in response to an increase in reactant concentration, than in reactions with a lower E_a . This is because a smaller fraction of collisions will have the energy capable of forming the transition state in reactions with higher E_a . Thus, in reactions with a high E_a , k , will be correspondingly small. In fact, the fraction of collisions that possess the energy required to exceed the energy barrier and form the transition state decreases exponentially as E_a increases. This conclusion is derived from the Maxwell-Boltzmann energy distribution. A model that relates k to E_a and temperature is known as the Arrhenius equation:

$$k = C e^{-E_a/RT} \quad (3.11)$$

where k is the rate constant with units s^{-1} , C is a reaction-specific constant (also called the 'frequency factor') with units s^{-1} , E_a is the energy of activation with units J mol^{-1} , T is absolute

temperature with units K, and R is the universal gas constant ($8.31 \text{ J mol}^{-1} \text{ K}^{-1}$). The Arrhenius equation is one of the most widely used relations for predicting the response of biochemical reaction rate to temperature; it forms the foundation for deriving the Q_{10} , a commonly-used metric of temperature sensitivity (see Appendix 3.1). As an empirical tool, the Arrhenius equation can be used to derive E_a from the relationship between $\ln k/C$ and $1/T$ at a fixed reactant concentration. The linear slope of such a relationship will equal $-E_a/R$.

Now that we have developed the conceptual foundations of chemical reactions, let's return to a discussion of thermodynamics and work. A system at equilibrium has no potential to do work. In other words, in a system at equilibrium, $\Delta G = 0$. We can derive a standard term for the free energy of a reaction (ΔG^0) which, in turn, can be used to relate free energy to chemical equilibrium:

$$\Delta G^0 = -RT \ln K_e = -2.3 RT \log K_e \quad (3.12)$$

where R is the gas constant ($8.31 \text{ J mol}^{-1} \text{ K}^{-1}$) and K_e is the unitless equilibrium constant of the reaction; thus, ΔG^0 carries units of J mol^{-1} . Following the relations of Equation 3.12, ΔG^0 relates the molar ratio of products to reactants at equilibrium to the inherent free energy of the system. For a hypothetical reaction at equilibrium in which the molar ratio of products to reactants is 1, the ΔG^0 would equal 0. In a reaction in which equilibrium is characterized with the concentration of products greater than reactants, Equation 3.12 solves to a ΔG^0 that is negative in sign and *exergonic*. Conversely, a reaction in which equilibrium is characterized with the concentration of reactants greater than products, Equation 3.12 solves to a ΔG^0 that is positive in sign and *endergonic*. The actual ΔG of a reaction will only be equal to ΔG^0 at equilibrium with the ratio of products to reactants equal to 1. In a reaction at disequilibrium, ΔG will be different from ΔG^0 , and the magnitude of the difference will reflect the amount of disequilibrium. As discussed in Chapter 2, a system at disequilibrium has the potential to do work.

Exergonic reactions occur *spontaneously*, in the thermodynamic sense. In other words, exergonic reactions can occur without extracting energy from the surroundings. Endergonic reactions do not occur spontaneously, but rather must be supported through the addition of free energy. The additional free energy required to drive endergonic reactions can come through

coupling to an exergonic reaction or through the removal of product through 'downstream' exergonic reactions. Coupling to an exergonic reaction often occurs in biological metabolism through the transfer of 'high energy' phosphate bonds from an intermediate compound, such as adenosine triphosphate (ATP), to the reactant of an otherwise endergonic reaction. The energy of the transferred phosphate bond can then be added to the energetics of the reaction, forcing the overall change in free energy to be negative. In the case of product removal, an otherwise endergonic reaction, in which the reverse reaction, from products to reactants, is thermodynamically favored at equilibrium, is driven forward by the sustained removal of product and accompanied mitigation of the reverse reaction. Endergonic reactions occur frequently in living cells, and are especially represented in anabolic metabolism, in which macromolecules are synthesized from simpler precursors. Exergonic reactions tend to be most frequently represented in catabolic metabolism in which chemical energy is extracted from the oxidation of energy-rich molecules.

Relying on Equation 3.12 to define the standard condition, and recognizing the definition of K_e according to Equation 3.6, we can write:

$$\Delta G = \Delta G^0 + RT \ln \frac{[C][D]}{[A][B]} \quad (3.13)$$

Using Equation 3.13, we can deduce that a large and negative ΔG reflects a reaction state that is highly displaced from equilibrium and capable of doing work on the surroundings. A large and positive ΔG reflects a reaction state that is also highly displaced from equilibrium, but requires that work be done to it in order to proceed. A system with a large and negative ΔG is often described as having a high *chemical potential*; that is, it is capable of doing a large amount of chemical work. In fact, the chemical potential of a reaction is defined in exactly the same terms as free energy, and therefore is also described by Equation 3.13.

3.B Reduction-Oxidation Coupling

Coupled reactions in which the oxidation states of two elements or compounds are changed are called reduction-oxidation reactions, or *redox reactions*. The *oxidation state* of an element or compound defines the degree to which it can gain or lose electrons. Stated another way,

oxidation state describes the electrical charge that an atom would have if it only participated in ionic bonds with other atoms. The oxidation state is a term of convenience; it does not describe a conserved property of an element. The oxidation state of an element changes depending on the other elements with which it forms bonds. For example, in their pure elemental forms, hydrogen and oxygen have oxidation states of zero (as do all elements in pure form). However, when two atoms of hydrogen are bonded to an atom of oxygen to form a water molecule, the hydrogen atoms each have an oxidation state of +1 and the oxygen atom has an oxidation state of -2. This means that the oxygen atom is more *electronegative* than the hydrogen atoms, and electrons that are shared in the covalent bonds of the molecule spend more time associated with the oxygen nucleus, than the hydrogen nuclei. If, hypothetically, the hydrogen atoms were to give up their shared electrons to the oxygen atom, and exist as ionically bonded entities, they would carry a charge of +1 and the oxygen would carry a charge of -2.

The concept of oxidation state is most easily applied in those cases where electrons are in fact transferred from the atoms of one element to those of another. The removal of electrons from an atom through chemical reaction will increase its oxidation state, and we refer to such reactions as *oxidation reactions*; the atom has been *oxidized*. The addition of electrons tends to reduce an atom's oxidation state, and we refer to such reactions as *reduction reactions*; the atom has been *reduced*. Oxidation and reduction reactions must, by nature, be coupled; one atom gains the electron lost from another. In biological systems, reduction is usually associated with reactions that not only add electrons, but also potential energy to an atom or molecule. Potential energy content increases when an electron is moved to an orbital at a greater distance from its associated nucleus (thus moving to an atomic zone of less attractive influence by the positively charged nucleus). In this energized state, electrons have more potential to participate in redox reactions. In other words, they have the more potential to do chemical work.

An example of energy transfer during redox reactions is found in the process of photosynthesis. Solar energy is harvested for purpose of driving chemical work through photosynthetic reactions. During photosynthesis, solar photons are captured when an electron in a specialized photosynthetic molecule is excited and 'jumps' to an atomic orbital at a higher energy level, further from its associated nucleus. In this excited state, the electron is more susceptible to being lost to a redox reaction. There are other possible fates for the excited electron, including falling back to its original ground state in one large step and emitting its

energy as secondary photons (fluorescence), or falling back in smaller steps and emitting its energy as heat (radiationless transfer). When the electron is lost through a redox reaction, however, it moves its association to a different nucleus, in this case at the photosystem reaction center. The primary electron acceptor in plant chloroplasts is a chlorophyll derivative known as pheophytin. The reaction center molecule will replenish its lost electron through another set of redox reactions as it extracts an electron from an electron donor compound (e.g., H_2O , H_2S or NH_4^+). The extracted electron will exist at the ground state energy level until photons of light are channeled once again to the reaction center.

Photosynthesis exemplifies an overall 'reductive' process in which electrons are removed from inorganic donors in a state of lower chemical potential and transferred, along with energy, to organic acceptors with higher chemical potential. Respiration, in contrast, exemplifies an overall 'oxidative' process in which electrons are removed from organic substrates in a state of higher chemical potential and transferred to inorganic electron acceptors in a state of lower chemical potential. Respiration can occur in the presence or absence of O_2 ; aerobic and anaerobic respiration, respectively. During aerobic respiration electron transfers occur in a series of steps, each catalyzed by a different protein (often a metalloprotein specialized for electron transfers). The proteins are collectively organized into large electron transfer complexes embedded in the membranes of cellular mitochondria. Ultimately, the energy from the sequential redox reactions is channeled into phosphate-containing organic compounds such as adenosine triphosphate (ATP), the energy currency of cellular metabolism. The channeling of electrons through the series of respiratory redox reactions is maintained by progressive irreversibility of the chain of reactions. Each step in the electron transport chain exists at a slightly lower potential energy level than the previous, so according to the thermodynamic limitations, the redox sequence can only proceed in one direction (Fig. 3.3).

The *reduction potential* (sometimes called redox potential) describes the affinity of an element or compound for electrons, and therefore, its tendency to be reduced when participating in redox reactions. The reduction potential is an *electrochemical potential*, combining the thermodynamic potentials to do both chemical and electrical work. Energy is stored in the form of an electrical potential when unlike charges are separated. In order to convince yourself of this fact, imagine the electrical work that can be done when particles of unlike charge are attracted and move spontaneously toward each other, releasing free energy. The reduction potential (E°)

of a compound can be related to free energy as:

$$E^{\circ} = -\Delta G / nF \quad (3.14)$$

where n is the molar equivalent of electrons involved in the reduction and F is Faraday's constant ($V \text{ mol}^{-1}$). Thus, E° carries units of volts. The negative sign in Equation 3.14 forces the reduction potential to become positive for exergonic reactions. Those compounds that have the highest affinity for electrons, and are most readily reduced in redox reactions, will have the most positive reduction potential values.

Reduction potential forms a central concept in the oxidation of organic and inorganic matter by microorganisms in soils. Soil organic matter represents the debris from living organisms, and thus the carbon contained in the organic matter exists in a relatively reduced state with high potential energy. Microorganisms use soil organic matter as a respiratory substrate, progressively oxidizing the carbon, and passing electrons from the oxidation process to chains of metalloprotein electron carriers, while simultaneously extracting free energy. The ultimate electron acceptor is typically a metal compound or highly electronegative compound (i.e., with a high affinity for electrons). It is clear that O_2 is the preferred ultimate electron acceptor, if present, as it has high reduction potential. When O_2 is present at low concentrations, such as in water-saturated soils, then soil microorganisms will use alternative electron acceptors, favoring the acceptor at the highest reduction potential.

The reduction potential only defines the electrical component of the electrochemical potential. The full electrochemical potential (E) can be calculated using the *Nernst equation*:

$$E = E^{\circ} - \frac{RT}{nF} \ln \frac{[\text{red}]}{[\text{ox}]} \quad (3.15)$$

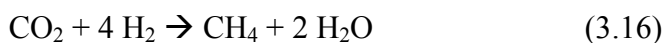
Term I Term II

where 'red' and 'ox' refer to 'reduced' and 'oxidized' compounds, respectively. The Nernst equation combines the concepts of electrical and chemical disequilibrium, and thus defines the full thermodynamic potential for a redox reaction to do chemical work. An examination of Equation 3.15, shows that the electrochemical potential represents the summed thermodynamic

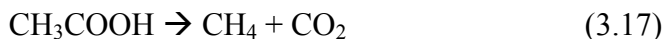
potential due to reduction potential (Term I) and chemical disequilibrium (Term II). The highest electrochemical potential will occur for a reaction in which the oxidized member of a redox pair has a high reduction potential *and* the oxidized member is present in high concentration relative to the reduced member. It is also clear from Equation 3.15, that redox pairs characterized by a negative reduction potential (e.g., the $\text{CO}_2 \rightarrow \text{CH}_4$ redox pair in soils, see Figure 3.4) will only react spontaneously when the concentration ratio of oxidized (in this case CO_2) to reduced (in this case CH_4) compounds in the pair is relatively low.

Biological redox reactions are responsible for producing some of the most important constituents of the atmosphere. For example, nearly the entire atmospheric reservoir of O_2 is the product of photosynthetic oxidation of H_2O . Photosynthetic O_2 began to accumulate in the atmosphere approximately 2-3 billion years ago, following some early epochs during which it was drawn out of the atmosphere by geochemical sinks, such as during the production of iron oxides (Nisbet et al. 2007). Soil redox reactions are also quite important for the production of atmospheric constituents, especially those that are radiatively active and involved in climate warming. For example, methane (CH_4) and nitrous oxide (N_2O) are both produced from soil redox chemistry. Methane has an atmospheric lifetime of 8-9 years and nitrous oxide has a lifetime of 120 years (Monson and Holland 2001). Both of these compounds have higher global warming potentials than CO_2 and have been increasing in their atmospheric concentrations over the past several decades (IPCC Fourth Assessment Report, 2007). As for CO_2 , microbial and root respiration rates in global soils are high enough to annually offset most of the mass of CO_2 taken out of the atmosphere by global gross photosynthesis (approximately 80 Pg C yr^{-1} released in soil respiration and approximately $120 \text{ Pg C per yr}^{-1}$ taken up by global gross photosynthesis).

Methane is produced in soils in which all possible electron acceptors at higher reduction potential (e.g., O_2 , nitrate, iron(III) and sulfate) have been used up, leaving CO_2 as the remaining available option. Thus, the overall redox potential of soils that produce CH_4 (the process of *methanogenesis*) is extremely low. Such low redox potentials are usually restricted to soils that have been flooded or waterlogged for long periods, causing severe O_2 limitations. Electrons are extracted from organic matter and passed to CO_2 to form CH_4 by methanogenic archaea. The overall reaction of methanogenesis can be represented as:



Electron acceptors other than CO_2 can also be used. The alternative acceptors are typically small organic compounds, such as acetate:



Nitrous oxide (N_2O) is produced by both archaea and bacteria as they oxidize NH_4^+ in the process of *nitrification*. Nitrification is the biogeochemical process whereby nitrite, then nitrate, is produced by sequential oxidation processes beginning from NH_4^+ . In general, microorganisms that participate in nitrification are known as nitrifiers. Nitrification is an aerobic process, with O_2 being the usual electron acceptor. However, in situations when O_2 becomes limiting, such as in flooded soils, nitrifying bacteria will use nitrite (NO_2^-) as an electron acceptor, reducing it to nitric oxide (NO) or N_2O . Generally, NO is produced at approximately 10 times the rate of N_2O by anaerobic nitrifying bacteria (Davidson and Verchot 2000), but N_2O has a much longer lifetime in the atmosphere. A second source of N_2O is the process of denitrification, whereby NO_3^- is used as an alternative electron acceptor in soils with depleted O_2 , eventually being reduced to N_2 through the reaction sequence:



The principal denitrifiers in soils are heterotrophic bacteria. In the process of denitrification, some of the N_2O that is produced leaks from the metabolic sequence into the atmosphere. The production of CH_4 and N_2O can be considered a type of anaerobic respiration.

3.C Enzyme Catalysis

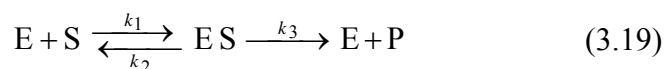
Enzymes are protein catalysts.² As catalysts, enzymes are capable of increasing the rate of metabolic reactions by 10^{10} - 10^{23} fold over non-catalyzed rates at equivalent temperatures (Kraut et al. 2003). This represents an astonishing capacity for catalysis and it is crucial to sustain the energy and biomass transformations of organisms on a planet with surface temperatures that would otherwise not support such metabolism. The metabolic reactions catalyzed by an enzyme are determined by its structure, which in turn is determined ultimately by an organism's genetic

code. Protein enzymes are constructed as chains of amino acid molecules that interact with each other through various hydrophobic and hydrophilic forces, and consequently form a three-dimensional shape with various pockets, clefts and tunnels distributed across the protein surface. Enzymatic catalysis occurs on a specific part of the protein surface called the *active site*. Although the entire enzyme may contain hundreds or thousands of amino acids, typically only 10-20 are found within the active site domain. Interactions between the substrate and active site are highly specific, and are determined by complementary relationships involving shape and chemical characteristics. Research into the structural attributes of proteins have revealed that a relatively small number of characteristic folds and shapes exist at the active sites of enzymes across the eukaryotic and prokaryotic kingdoms, and these have been conserved, co-opted and recycled during the evolution of a broad diversity of protein functions (Hrmova and Fincher 2001).

Various hypotheses have been proposed to explain the catalytic mechanism of enzymes, all of which focus on how the active site facilitates transformation of the substrate to the transition state (Fig. 3.5). In the *strain hypothesis*, catalysis results when the structural integrity of the substrate is strained after binding to the active site. Strain occurs because the active site complements the structural and chemical nature of the transition state more than the original form of the substrate. Strain allows for the rearrangement of electron orbitals, forcing the substrate through its transition state and on to formation of product. In the *induced-fit hypothesis*, the active site complements the structural and chemical nature of the substrate, but loosely so. When the substrate binds to the active site, polarity and charge interactions force the enzyme to ‘flex’ and more tightly ‘envelope’ the substrate. Flexing of the enzyme aligns critical catalytic groups that complement the transition state, forcing the form of the substrate to distort toward that of the transition state. In both hypotheses, the binding energy that goes into the enzyme-substrate interaction is channeled into distortion of the substrate to the point that it passes through the transition state and, spontaneously, on to product formation. In energetic terms, the binding energy is used to lower the activation energy. This is the essence of catalysis – the conversion of catalyst-substrate binding energy into the energy required to reach the transition state and form product.

3.C.1 The Michaelis-Menten and Briggs-Haldane models of enzyme kinetics

The rate at which an enzyme-catalyzed reaction occurs is determined by the combined effects of how tightly the enzyme's active site binds the substrate and how rapidly the active site releases product. Consider the simplest of the enzyme-catalyzed reactions – one in which a single substrate (S) is catalytically converted with the aid of an enzyme (E) to a single product (P). The reaction and its relevant reaction constants can be represented as:



where ES represents the enzyme-substrate complex which is formed according to rate constant k_1 , and can either produce the transition state followed by dissociation to form product and free enzyme (according to rate constant k_3), or decompose back into the original substrate and free enzyme (according to rate constant k_2). The Michaelis-Menten model of enzyme kinetics is built on the assumption that the concentrations of E, S and ES reach equilibrium at a rate that is rapid relative to the rate at which ES dissociates to form E+P. The Michaelis-Menten model is often referred to as the *rapid equilibrium model*. The model takes the form:

$$v = \frac{V_{\max} [S]}{K_m + [S]} \quad (3.20)$$

where v is the velocity of the reaction (mol s^{-1}), V_{\max} is the maximum velocity that the reaction is capable of reaching (i.e., at a saturating concentration of substrate) (mol s^{-1}), $[S]$ is substrate concentration (mol), and K_m is the Michaelis-Menten constant (mol). A derivation of Equation 3.20 is presented in Appendix 2.2. Graphically, Equation 3.20 describes a rectangular hyperbola (Fig. 3.6). Under conditions when $[S] \ll K_m$, $[S]$ can be ignored in the denominator on the right side of Equation 3.20, and $v \approx (V_{\max}/K_m) [S]$. Thus, at low $[S]$, the relation between v and $[S]$ approaches first-order kinetics, with a first-order reaction coefficient equal to V_{\max}/K_m . As $[S]$ increases to the point where $[S] \gg K_m$, the K_m can be ignored and Equation 3.20 reduces to $v \approx V_{\max}$. Thus, at high $[S]$, v approaches zero-order dependence on $[S]$ and is approximated by an asymptotic limit represented by V_{\max} .

We can gain a deeper perspective on the V_{\max} and K_m parameters by placing Equation 3.20 within the context of the reaction sequence depicted in Equation 3.19. At saturating substrate concentration, the concentration of free enzyme molecules ($[E]$) will be negligible since nearly all of the enzyme should be bound to substrate as ES. Under these conditions, $[ES] \approx [E_t]$, where $[E_t]$ is the total concentration of enzyme. Recognizing that by definition V_{\max} occurs at saturating $[S]$, V_{\max} will reflect the product between $[E_t]$ and k_3 . The rate constant k_3 is also known as k_{cat} , the *catalytic rate constant* or the *enzyme turnover number*. The enzyme turnover number can be viewed as the rate at which the active site is capable of converting a unit quantity of ES to E + P and carries measurement units of s^{-1} .

An examination of Equation 3.20 also reveals further insight into the nature of K_m . Resolving the equation for the condition $K_m = [S]$, yields the relation, $v = 0.5 V_{\max}$. Thus, by definition the K_m is the substrate concentration that yields half the maximum catalyzed velocity. An enzyme with a lower K_m for a specific substrate is capable of reaching half its V_{\max} at a lower substrate concentration, compared to an enzyme with higher K_m for that substrate. From that conclusion, we can in turn state that the lower the K_m , the less the tendency for ES to dissociate back to E and S. In other words, an enzyme with a lower K_m for a specific substrate, will reflect a higher ratio of k_1/k_2 , and thus higher $[ES]$ compared to $[E]$ and $[S]$ at steady state, compared to an enzyme with a higher K_m for that same substrate. Using this logic, the K_m for an enzyme reflects the apparent affinity of the enzyme for its substrate. The *lower* the value of the K_m , the tighter is the association between enzyme and substrate in the ES state, and the *higher* is the affinity of the active site for its substrate.

As enzyme-substrate interactions have evolved over time, catalytic constraints have emerged that limit the amount of divergence that is possible between the K_m and V_{\max} values. In theory, the evolution of greater affinity toward a substrate (i.e, reflecting lower K_m), will also require that an active site turn over its substrate at a slower rate (i.e., reflect a lower V_{\max}). The requisite relations between an active site and substrate, and the fact that efficient enzyme catalysis requires the antagonistic interactions of tight binding and fast release, prevent evolutionary trajectories that favor independent optimization of K_m and V_{\max} ; i.e., their evolutionary fates are ‘intertwined’.

The Michaelis-Menten assumption of rapid equilibrium among $[E]$, $[S]$ and $[ES]$, is not always valid. For the assumption to be valid, k_3 must be considerably less than k_2 . In some

enzyme-catalyzed reactions, k_3 is of similar magnitude as k_2 , meaning that Equation 3.19 approaches a steady state with respect to [ES], rather than an equilibrium with respect to [E], [S] and [ES]. In the case of steady state, a different kinetic model is required. The Briggs-Haldane model takes the same form as Equation 3.20 except that K_m is defined as $K_m = (k_2 + k_3)/k_1$, rather than $K_m = k_2/k_1$. (Recall that this same relationship between equilibrium and steady-state reaction coefficients was discussed in Section 3.A within the context of chemical reactions in general.)

3.C.2 Modification of enzyme activity

Enzymes are subject to catalytic modification from a number of different environmental and biological factors. Two of the most important factors are temperature and pH. Temperature affects enzyme-catalyzed reactions just as it does all chemical reactions, by increasing in the reaction system the fractions of substrate and enzyme molecules that have kinetic energy exceeding the energy of activation. Enzyme-catalyzed reactions exhibit an exponential dependence of reaction rate on temperature, as reflected in the Arrhenius model (see Section 3.A and Appendix 3.1). At extremely high temperatures, cohesive bonding interactions in the protein break down, causing deformation of the active site, denaturation of the enzyme and, eventually, loss of catalytic function. Cellular pH affects the catalytic efficiency of enzymes by influencing electrical charge interactions within the structural framework of the protein and between the active site and substrates. Depending on the prevailing pH, certain amino acid groups in a protein can be protonated or non-protonated, affecting their interaction with other polar or charged groups in the protein or on the substrate surface. These electrical interactions have the potential to affect the shape of the active site and enzyme-substrate binding affinity. Enzymes typically exhibit maximal activity within a narrow range of pH.

In addition to environmental influences, enzyme activity is often modified by the binding of alternative substrates to the active site (e.g., competitive inhibition), or the binding of effector ions or molecules to *allosteric sites*. Allosteric sites are alternative binding sites that, when engaged, cause the protein to change its shape or electrical properties and, in doing so, change the enzyme-substrate binding affinity. Allosteric modification can cause changes to the K_m and/or V_{\max} of the active site with either a negative or positive influence on catalytic efficiency.

In a further example of complex regulation, enzymes can have multiple active sites that function in a cooperative manner. This is common for enzymes with multiple protein subunits. *Cooperativity* involves a change in the catalytic properties of one active site as the result of substrate binding to a different active site. Cooperativity is usually expressed through changes in the shape or charge properties of the protein. Finally, enzymes can be modified by covalent modification, including attachment of specific effectors, such as inorganic phosphate molecules or adenylate phosphate molecules (e.g., ADP or AMP), or cleavage of certain amino acids or amino acid side groups from the protein.

3.D Stable isotopes and isotope effects

The reservoir of chemical compounds in the earth system is composed of both common and rare isotopic forms of elements. For example, the element carbon exists as three naturally-occurring isotopes, including the most common, ^{12}C , and the rarer forms ^{13}C and ^{14}C . Isotopes of the same element possess the same numbers of electrons and protons, but different numbers of neutrons; thus, isotopes differ in atomic mass. In the case of the isotopes of carbon, ^{12}C has 6 protons and 6 neutrons in its nucleus, giving it a total atomic mass of 12; the other two carbon isotopes, ^{13}C and ^{14}C , have 7 and 8 neutrons, respectively. The atmospheric CO_2 reservoir reflects approximately 98.9% $^{12}\text{CO}_2$, 1.1% $^{13}\text{CO}_2$ and a minute trace of $^{14}\text{CO}_2$. *Stable isotopes* refer to those forms that have not been observed to break down through the emission of high-energy particles, and are thus stable over time. With regard to carbon, the ^{12}C and ^{13}C forms are stable, but the ^{14}C form is unstable (or 'radioactive').

The abundance of an isotope is characterized by the *isotope abundance ratio*, which is formally defined as:

$$R = \frac{[\text{rare isotope}]}{[\text{abundant isotope}]} \quad (3.21)$$

A distinction must be made between the isotope abundance ratio and the isotope concentration; the latter is defined as the ratio of the rare isotope to *all* isotopic forms of the element. It is common to express the isotope abundance ratio in terms of *delta notation*, in which the ratio of isotopes in an observed sample is related to the ratio of isotopes in a standard material:

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (3.22)$$

Delta notation reflects a ratio of ratios. In the case of the stable isotopes of carbon, for example, R_{standard} is typically taken as the $^{13}\text{C}/^{12}\text{C}$ obtained in a specific belemnite limestone formation (Pee Dee) found in South Carolina, USA (Craig 1957). The units in δ notation are expressed as parts per thousand (‰), or 'per mil', which, once again, does not reflect a unit measure, but rather a unitless ratio.

Isotopes of the same element participate in the same types of chemical reactions, but their differences in atomic mass affect their reaction rates, as well as physical properties such as diffusive mobility. Differences in reaction rate or mobility due to differences in atomic mass are called *isotope effects*. Isotope effects have the potential to alter the relative abundances of isotopes in a system. Thus, by measuring ratios of isotopes we can gain insight into the kinetics of biochemical and biophysical fluxes that may have influenced the state of mass in a biogeochemical system. We can quantify an isotope effect (α) as:

$$\alpha = \frac{R_a}{R_b} \quad (3.23)$$

where R_a and R_b refer to the molar isotope abundance ratios of a source or reactant (a) and a sink or product (b). One way to describe an isotope effect is that it results in 'discrimination against' or 'fractionation of' certain isotopes. The terms *fractionation* and *discrimination* are often used to describe isotope effects. Discrimination (Δ) is formally defined as:

$$\Delta = (\alpha - 1) * 1000 = \left(\frac{R_a}{R_b} - 1 \right) * 1000 \quad (3.24)$$

Note that discrimination is scaled similarly to that for δ notation, but that Δ is not dependent on the isotopic ratio of a measurement standard. Discrimination can be expressed in terms of δ notation as:

$$\Delta = \frac{\delta_a - \delta_b}{1 + \delta_b/1000} \quad (3.25)$$

where (δ_a) reflects the reactant (or source) isotope ratio and (δ_b) reflects the product (or sink) isotope ratio, with both referenced to a measurement standard.

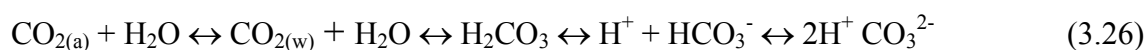
In order to illustrate the relationship between Δ and δ with an example, let's take the case of CO_2 assimilation by plants that possess the C_3 photosynthetic pathway. The C_3 photosynthetic pathway uses CO_2 from the atmosphere as substrate, which has a $^{13}\text{C}/^{12}\text{C}$ that is ~ 8 ‰ lower than that in the Pee Dee limestone standard. We will consider CO_2 in the atmosphere as our reactant, such that $\delta_a = -8$ ‰. The bulk biomass of C_3 plants has a $^{13}\text{C}/^{12}\text{C}$ ratio that is ~ 27 ‰ lower than the limestone standard. We will consider plant biomass as our product, such that $\delta_b = -27$ ‰. When we put these values into Equation 3.25, we calculate $\Delta = 19.5$ ‰. It is important to note that for work with ^{13}C and ^{12}C related to photosynthetic CO_2 assimilation, δ values for atmospheric CO_2 and plant tissues are typically negative in sign, whereas Δ for the process of photosynthetic CO_2 assimilation is positive in sign.

Isotope effects that occur in transport processes (e.g., diffusion) due to the differential mobility of isotopic forms are directly proportional to differences in mass; larger isotopes have relatively less mobility than lighter isotopes. Differences in the chemical reaction rates of isotopes are also a direct result of differences in atomic mass. Chemical reactions require the breaking and forming of bonds between atoms. In the case of covalent bonds, the participating atoms are separated by a distance that is not fixed, but rather changes as the atoms vibrate back and forth along the axis of bonding. The vibrations are caused by a dynamic equilibrium between the attractive and repulsive forces that result from atomic interactions between the positively-charged nucleus of one atom and negatively-charged electron cloud of the neighboring atom (in the case of the attractive force), and between the two negatively-charged electron clouds of the neighboring atoms (in the case of the repulsive force). The mass of the nucleus influences this dynamic equilibrium, such that heavier isotopes tend to exhibit lower vibrational frequencies, which translates to shorter bond lengths, stronger bonds, and a higher activation energy for bond cleavage; the weaker bonding energies of atoms from lighter isotopes facilitates higher reaction rates.

There are two types of isotope effects; thermodynamic and kinetic. *Thermodynamic*

isotope effects occur when differential isotopic distributions exist between reactants and products within a reaction system that is at equilibrium. *Kinetic isotope effects* reflect differences in rate constants for a reaction when different isotopes take part in the reaction; kinetic effects occur when there is continuous production of a product from reactants of different isotopic form, rather than equilibration of isotopic forms within different fractions of a system at equilibrium.

Thermodynamic isotope effects are typically smaller than kinetic effects. An example of a thermodynamic isotope effect involves the dissolution of CO₂ from air (CO_{2(a)}) into water (to form CO_{2(w)}), and subsequent reaction with water to form carbonic acid and forms of carbonate:



In tracing the steps through this equilibrium, molecules of CO₂ will dissolve in water with a thermodynamic isotope effect that favors ¹²CO₂, $\alpha = 1.0011$ ($\Delta = 1.1$ ‰). The actual reaction of CO₂ with H₂O to form HCO₃⁻ (the predominant compound at pH 6-9) favors ¹³C concentrated in HCO₃⁻ and ¹²C concentrated in CO_{2(w)}. This is because the heavier ¹³C isotope achieves greatest stability in this equilibrium series in the form most strongly bonded to water molecules (i.e., as covalently bonded HCO₃⁻, rather than as CO_{2(w)}). Thus, the equilibrium between CO_{2(w)} and HCO₃⁻ exhibits an isotope effect less than 1, $\alpha = 0.991$ ($\Delta = -9$ ‰). The overall influence of these effects is to favor ¹³C in HCO₃⁻ and ¹²C in CO_{2(a)} at $\Delta = 1.1 + -9.0 = -7.9$ ‰, when CO_{2(a)} is at equilibrium with HCO₃⁻.

In order to illustrate the kinetic isotope effect, we can turn once again to the process of CO₂ assimilation in plants with the C₃ photosynthetic pathway. The assimilation of CO₂ is catalyzed by the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which favors the assimilation of ¹²CO₂ relative to ¹³CO₂ with $\alpha = 1.030$ ($\Delta = 30$ ‰). The fractionation in this reaction is kinetic, and is due to the differences in the potential for ¹³CO₂ versus ¹²CO₂ to form a bond with a second substrate, ribulose 1,5 bisphosphate (RuBP). For kinetic isotope effects, we can re-write Equation 3.23 to reflect reaction rates, rather than isotope abundance ratios:

$$\alpha = \frac{k_{\text{abundant}}}{k_{\text{rare}}} \quad (3.27)$$

where k_{abundant} is the reaction rate coefficient for a reaction using the abundant isotopic form ($^{12}\text{CO}_2$) as reactant, and k_{rare} is the reaction rate coefficient for use of the rare isotopic form ($^{13}\text{CO}_2$) as reactant. Thus, we can trace lines of conceptual and mathematical continuity from an observed isotope effect, molar abundance ratios of common and rare isotopes in a system, and the rates of chemical and physical processes that distribute the isotopes differentially within the system.

Kinetic isotope effects will only occur if unused reactant molecules – i.e., reactant molecules left behind by discrimination – are allowed to leave the reaction. If a reactant is completely used in a reaction to form product, then there is no potential for isotopic discrimination. Discrimination will occur when the unused reactant molecules either leak from the reaction site or are used in a connected, but branched reaction. Thus, we can write:

$$\Delta_e = \Delta_{\text{max}} \phi \quad (3.28)$$

where Δ_e is the 'expressed' discrimination, Δ_{max} is the maximum potential discrimination, and ϕ is the *branching factor* or '*leakage*' which facilitates the removal of unused reactant from the site of a reaction.

Appendix 3.1. Derivation of the Q_{10} equation

The temperature dependence of chemical reactions is generally evaluated with the Arrhenius equation (see Section 3.A). Manipulation of the Arrhenius relation allows us to derive a parameter called Q_{10} . The Q_{10} is a ‘metric of convenience’, allowing biochemists to evaluate the temperature sensitivity of a reaction across a standardized range of temperature (10°C). To develop an expression for the Q_{10} , we begin with the ratio of reaction constants at two temperatures (k_1 and k_2) defined according to the Arrhenius relation at each temperature:

$$\frac{k_2}{k_1} = \frac{\exp\left(-\frac{E_a}{RT_2}\right)}{\exp\left(-\frac{E_a}{RT_1}\right)} = \exp\left(\frac{E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)\right) = \exp\left(\frac{E_a}{R}\left(\frac{T_2 - T_1}{T_2 T_1}\right)\right) \quad (3.29)$$

We can manipulate Equation 3.29 to provide an expression defined across a temperature range limited to 10°C :

$$Q_{10} = \frac{k_2 : f\{T+10\}}{k_1 : f\{T\}} = \exp\left(\frac{10 E_a}{R(T+10)}\right) \quad (3.30)$$

Relying on the fact that the reaction is characterized by the same E_a at both temperatures and that R is constant, Equation 3.30 can be expressed as:

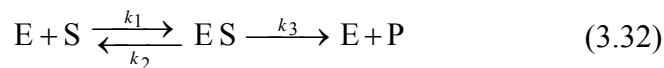
$$Q_{10} = (k_2/k_1)^{[10/(T_2 - T_1)]} \quad (3.31)$$

We caution readers in the use of Q_{10} . In the past, Q_{10} has been misused with the assumption that it is a constant property of a reaction. Many biochemical treatments have relied on the assumption of $Q_{10} = 2.0$ within the temperature range experienced by most organisms. This assumption is probably valid in homeothermic animals, where the temperature doesn’t vary significantly. However, the Q_{10} itself is sensitive to temperature because of changes in the rate by which the fraction of molecules that exceed E_a increases or decreases as a function of temperature (see discussion of the Maxwell-Boltzmann law in Section 3.A). The Maxwell-

Boltzmann effect causes the Q_{10} to decrease as temperature increases; even across those temperatures typically experienced by organisms. The temperature dependence of Q_{10} will be discussed further when we take up topic of cellular respiration and its response to temperature (Section 4.D.2).

Appendix 3.2 Derivation of the Michaelis-Menten model of enzyme kinetics

Consider a simple enzyme-catalyzed reaction in which a single substrate molecule is converted to a single product molecule. The reaction can be represented as:



where ES represents the enzyme-substrate complex which is formed according to rate constant k_1 , and can either dissociate to form product and free enzyme (according to rate constant k_3) or decompose back into the original substrate and free enzyme (according to rate constant k_2). The velocity (v) of Reaction 3.32 can be defined as:

$$v = k_3 [ES] \quad (3.33)$$

The total concentration of enzyme (E_t) can be defined as:

$$[E_t] = [E] + [ES] \quad (3.34)$$

Using these relationships, we can derive an expression for the velocity of the reaction per unit of total enzyme, which should reflect the catalytic efficiency of the enzyme:

$$\frac{v}{[E_t]} = \frac{k_3 [ES]}{[E] + [ES]} \quad (3.35)$$

An equilibrium enzyme-substrate dissociation constant (K_m) can be defined in terms of the concentrations of E, S and ES and the rate constants k_1 and k_2 as follows:

$$K_m = \frac{[E][S]}{[ES]} = \frac{k_2}{k_1} \quad (3.36)$$

Rearranging Equation 3.36 and substituting for [ES] in Equation 3.35, we obtain:

$$\frac{v}{[E_t]} = \frac{k_3 [E] \frac{[S]}{K_m}}{[E] + [E] \frac{[S]}{K_m}} \quad (3.37)$$

At rapid equilibrium, the rate-limiting step in Reaction 3.32 will be k_3 . Here, we define a term, V_{\max} , as the velocity of the reaction when $[S] \rightarrow \infty$ and $[E_t] \rightarrow [ES]$. Within this assumption, $V_{\max} = k_3 [E_t]$. Using these assumptions, and algebraically rearranging Equation 3.37, the final form of the Michaelis-Menten model is obtained as:

$$v = \frac{V_{\max} [S]}{K_m + [S]} \quad (3.38)$$

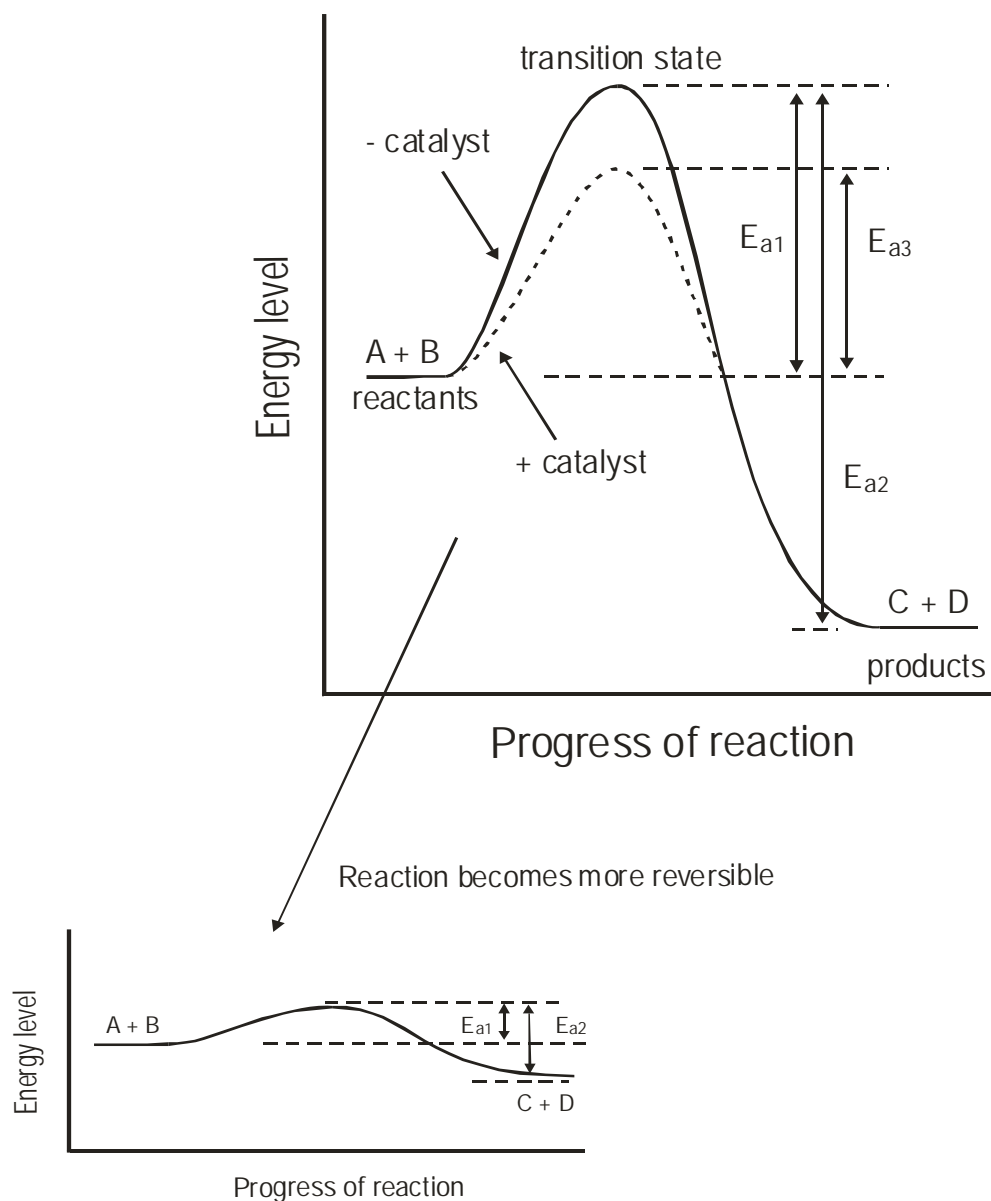


Figure 3.1. Upper figure. Energetics of a hypothetical chemical reaction in which reactants A + B react to form products C + D in the absence or presence of a catalyst. The energy of activation for the reverse reaction is represented as E_{a2} . The presence of a catalyst will reduce the energy of activation (represented as E_{a3}), allowing a greater fraction of reactions between A and B to form the transition state and proceed to products. **Lower figure.** A reaction in which the difference between E_{a1} and E_{a2} is small, and the overall difference in free energy between the reactants and products is small. These conditions favor reversibility.

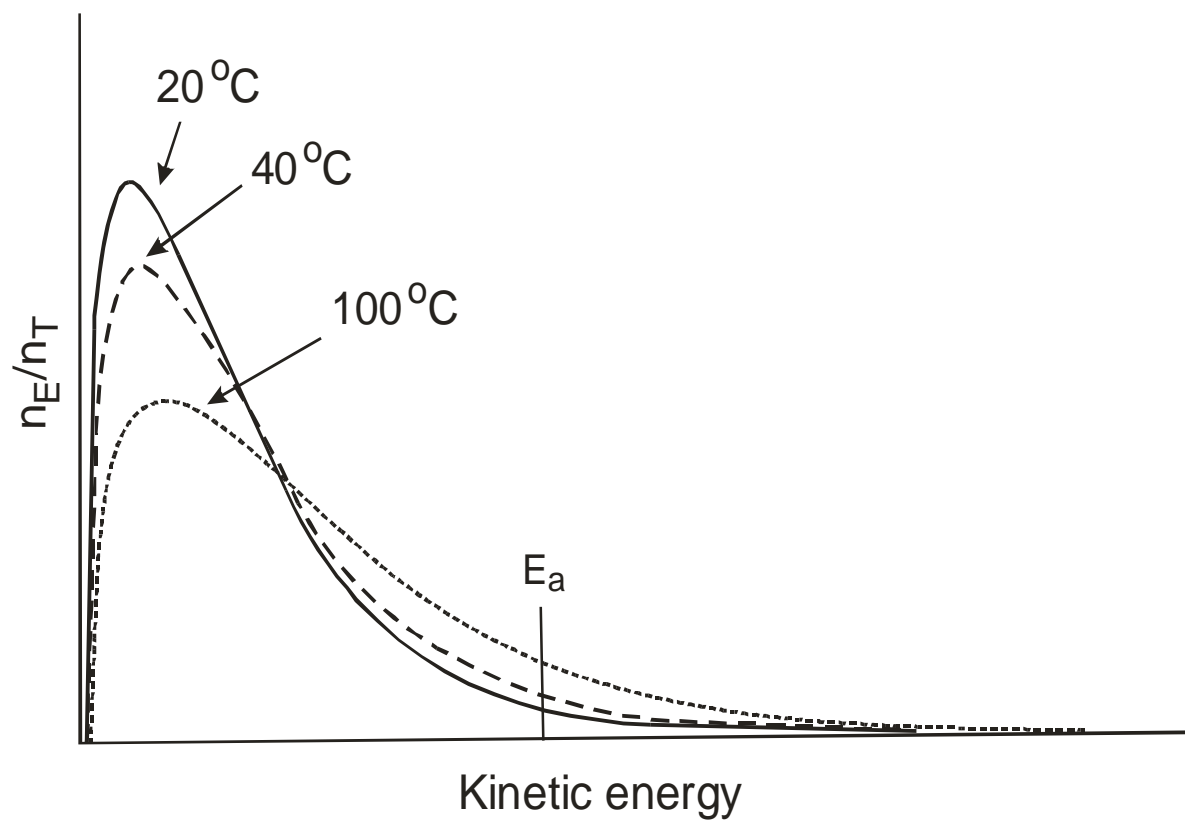


Figure 3.2. The Maxwell-Boltzmann energy distribution as a function of temperature. The molar fraction (n_E/n_T) at the higher end of the energy distribution increases with an increase in temperature, causing more molecules to exist at kinetic energies above the energy of activation threshold (E_a).

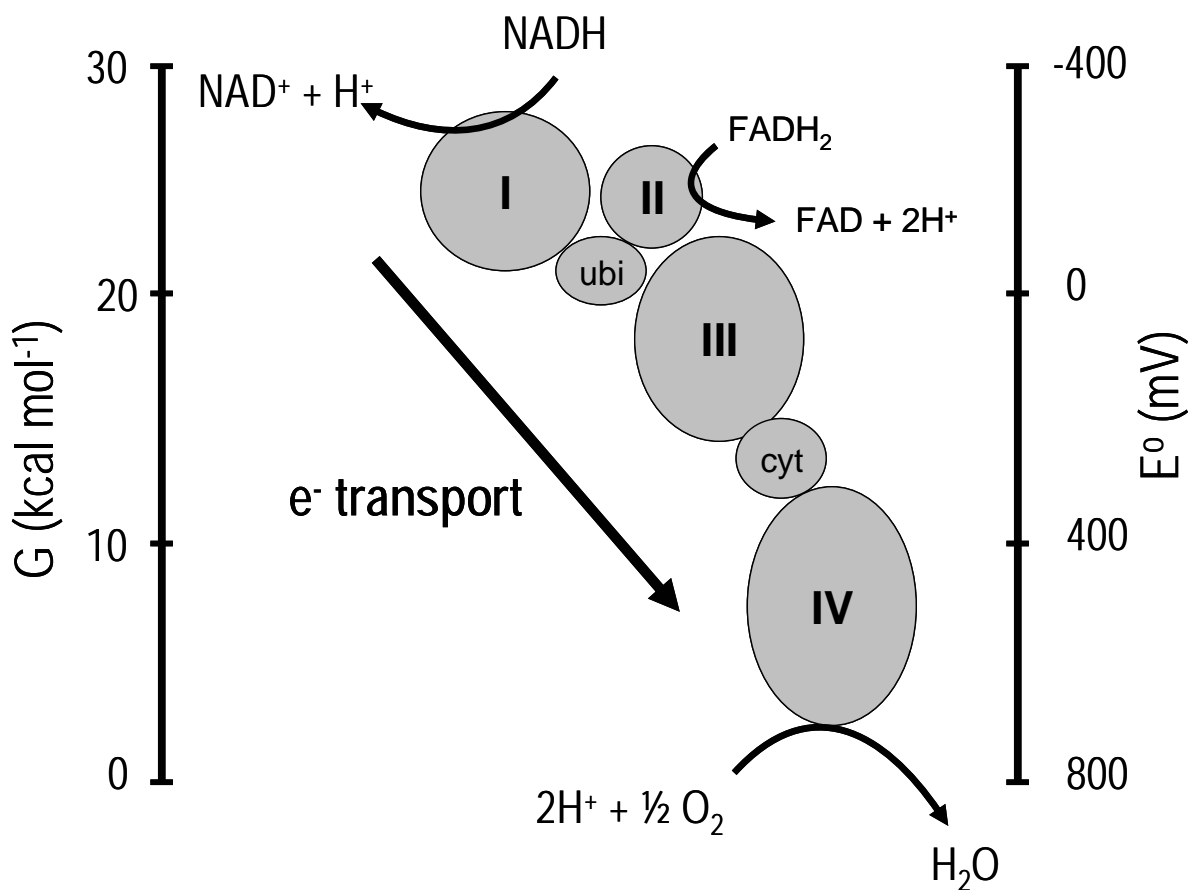


Figure 3.3. Electron transport in oxidative respiration illustrating successive redox reactions as electrons are passed along a chain of four primary protein complexes. The electrons are passed down a gradient in free energy (expressed here as free energy per electron, G) and reduction potential (E°), where the most positive E° indicates the greatest potential for oxidation. Oxygen is the ultimate electron acceptor with an E° value of ~ 820 mV. The free energy of electron transport is eventually transferred to energy rich organic phosphate compounds, in which form it is used to drive the reactions of metabolism.

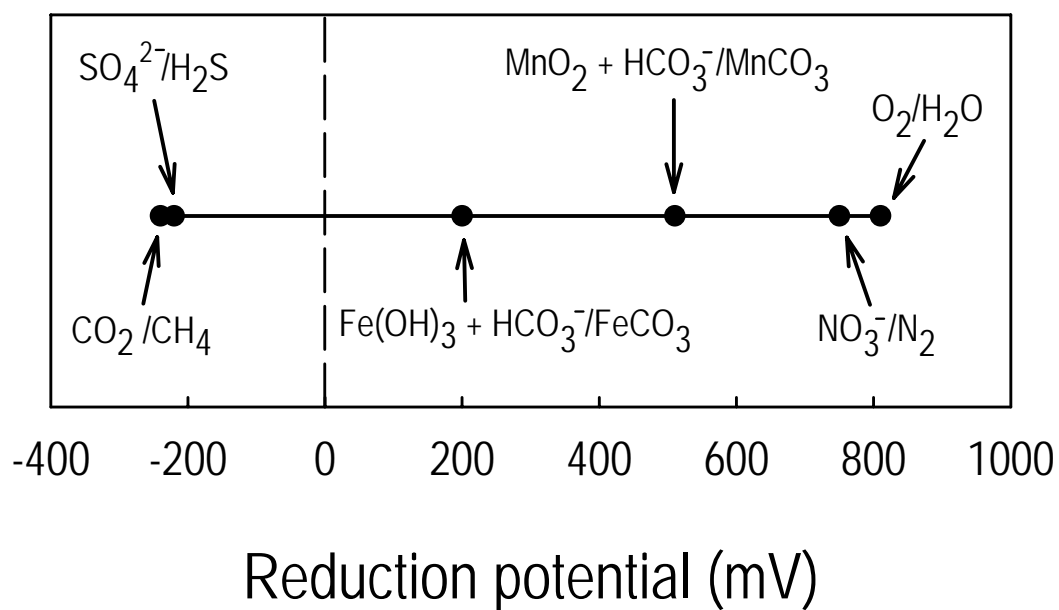


Figure 3.4. Reduction potential for significant redox couplings in soils. The left member of each pair is the oxidized member and the right member is the reduced member. Redox values taken from Liesack et al. (2000).

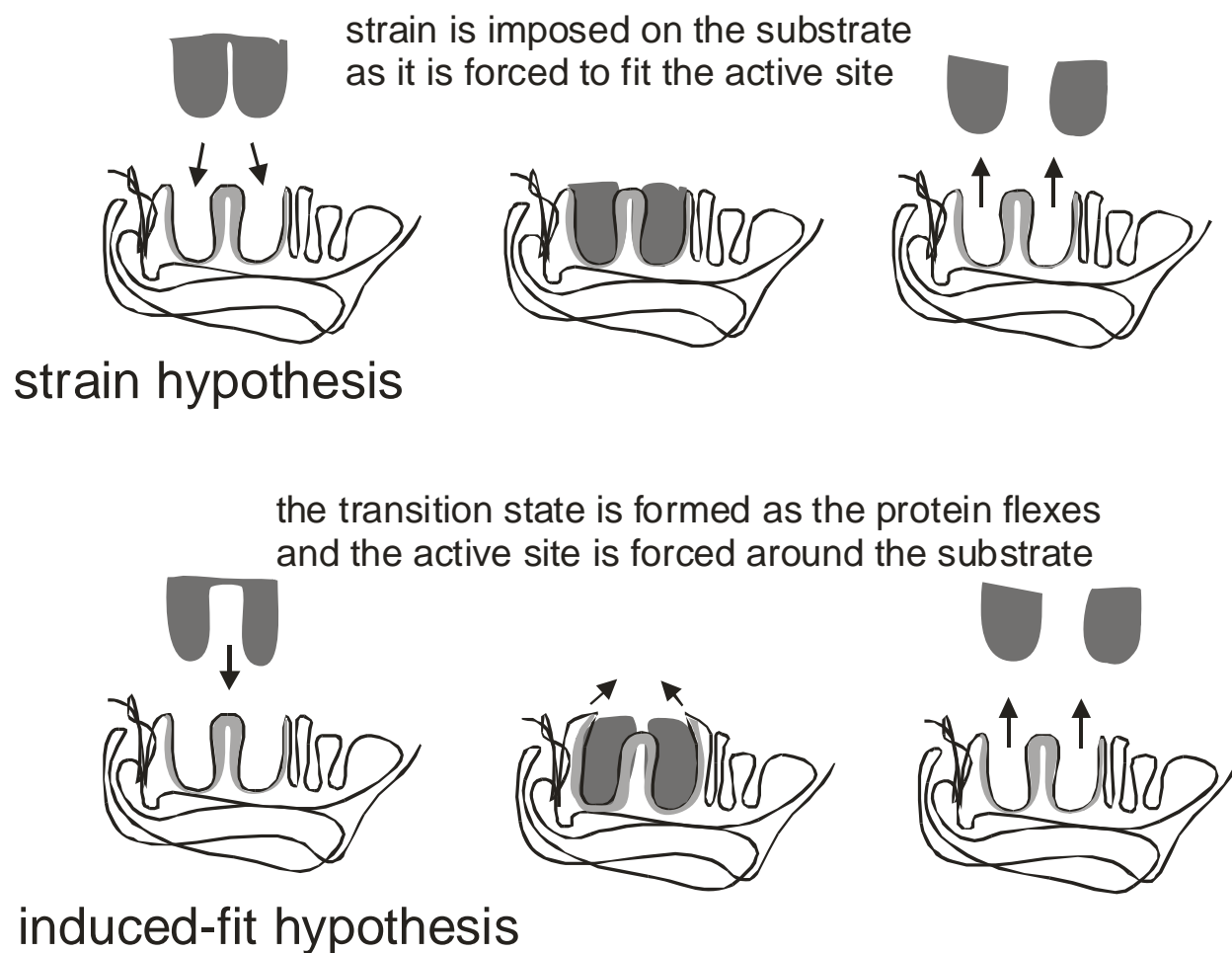


Figure 3.5. Two hypotheses to explain catalysis by enzymes as they promote the conversion of substrate through the transition state to product.

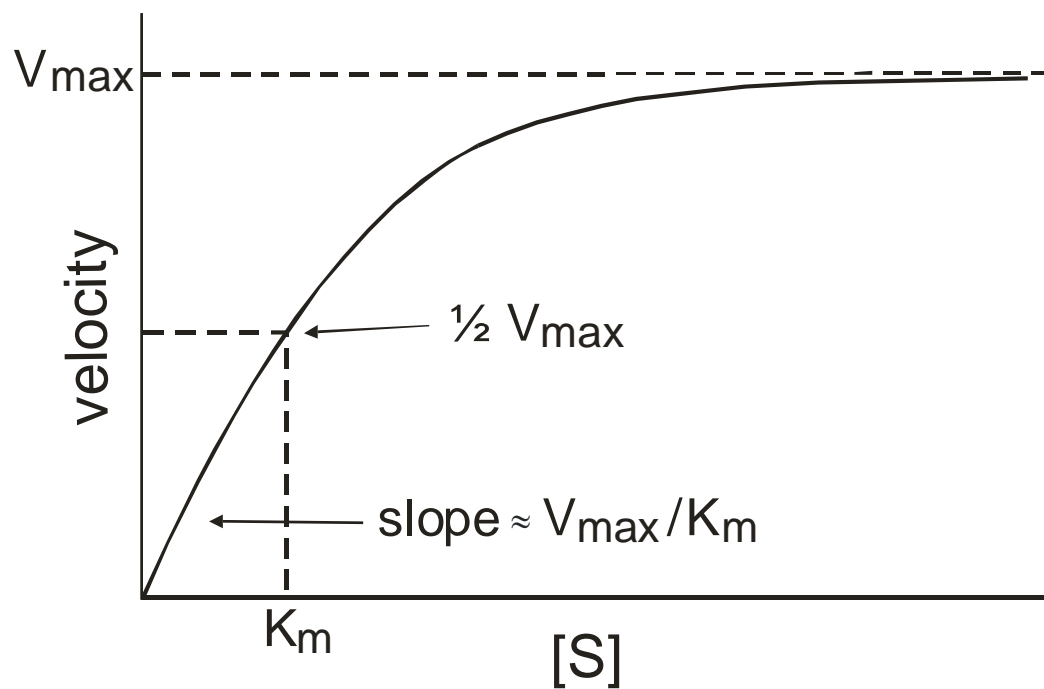


Figure 3.6. Graphical representation of the Michaelis-Menten model describing the dependence of reaction velocity on substrate concentration $[S]$.

Footnotes (Chapter 3)

¹ In this form of the Maxwell-Boltzmann distribution, a rather complex function $[2\pi(1/\pi RT)^{3/2} \sqrt{E}]$ has been condensed to provide the synthetic coefficient, A . In this case, we are using E to represent any chosen energy level used to evaluate the fraction of reactants above or below that energy level. The chosen energy level could be the energy of activation, in which case we would designate $E = E_a$.

² Ribonucleic acids (RNAs) can also function as biological catalysts, although they are typically referred to as ribozymes, rather than enzymes. Ribozymes probably served a central role in the earliest evolutionary origins of nucleic acid replication and perhaps cellular metabolism. In this book, we will focus solely on protein catalysts as these are the catalysts most involved in the biochemical reactions of ecosystem-atmosphere exchange.