CATALYSIS AND ENZYME ACTION

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The Chemistry-Biology Interface Series

Several years ago, a few dozen biologists, chemists, physicists, and other scientists spent several days on the campus of the University of Washington under the joint sponsorship of the Commission on Undergraduate Education in Biology, the Advisory Council on College Chemistry, and the Commission on College Physics. The purpose was to study ways to improve teaching in areas of mutual concern to two or more of the disciplines involved. The group considering the area between chemistry and biology agreed that a series of paperback books, prepared for elementary college level students in either biology or chemistry could serve a useful purpose toward this end.

Prepared by authorities in their fields, these books could, for the chemists, indicate the biologically significant reactions useful to illustrate chemical principals and, for the biologist, summarize up-to-date information on molecular phenomena of significance to a modern understanding of biological systems.

To implement this proposal, CUEBS and AC_3 appointed an editorial committee of:

Professor Robert H. Burris,
Department of Biochemistry, University of Wisconsin
Professor L. Carroll King,
Department of Chemistry, Northwestern University
Professor Leonard K. Nash,
Department of Chemistry, Harvard University
Professor Aubrey W. Naylor,
Department of Botany, Duke University
Professor Charles C. Price,
Department of Chemistry, University of Pennsylvania
to organize the undertaking.

As of this writing, the following volumes have been published:

O. T. Benfey, "INTRODUCTION TO ORGANIC REACTION MECHANISMS"

Roderick K. Clayton, "LIGHT AND LIVING MATTER," Vols. I and II

Charles C. Price, "GEOMETRY OF MOLECULES"

The following volumes are planned:

Melvin Calvin, "CHEMICAL EVOLUTION"

Paul M. Doty, "MACROMOLECULES"

David E. Greene, "SURFACE, FILMS AND MEMBRANES"

It is our hope that the material in these volumes will prove of sufficient interest to teachers and students in elementary college chemistry and biology courses that much of it will ultimately be incorporated in regular textbooks.

CHARLES C. PRICE

Philadelphia, Pennsylvania

PREFACE

It is the aim of this book to introduce college students in chemistry and biology to the concepts of catalysis, that is, how a reaction rate is accelerated. The catalysts vary from the very simple proton to the very complex enzymes and heterogeneous catalysts, both containing thousands of atoms.

The enzymes are biocatalysts; that is, they catalyze most of the body's functions. Heterogeneous catalysts are usually solid materials that are commercially very important. These catalysts are responsible for about 100 billion dollars worth of products annually in our economy.

In between these two extremes are catalysis by bases, such as hydroxide ions, general (undissociated) acids, general bases (usually organic compounds), metal ions, organic nucleophiles and electrophiles, as well as more complicated forms of catalysis, such as multiple catalysis, intramolecular catalysis, and intracomplex catalysis. Metal ions can act as catalysts by serving as superacids (protons of magnified charge in neutral solution), by acting as electron transfer agents, or by acting as templates on which reactions occur. Catalysis by nucleophiles and electrophiles includes a discussion of two important vitamins, thiamine pyrophosphate (B_1) and pyridoxal phosphate (B_6). Multiple catalysis, as the name implies, propounds that if a little is good, a lot is better. Intramolecular catalysis has demonstrated some important models of complex systems in which the catalyst is tied down in the same molecule as the bond to be broken. Intracomplex catalysis is closer to enzyme catalysis because in this instance, a noncovalent complexing is a necessary prerequisite for the subsequent catalytic steps.

Enzymic catalysis, including the structure, kinetics, and inhibition of enzymes, is discussed. Like intracomplex catalysis, an enzyme forms a noncovalent complex with the molecule it is acting on, called the substrate. Enzymes are usually protein in nature, being polymeric

materials composed of amino acid monomers. But sometimes small cofactors, either organic or inorganic, are needed for enzyme action. These substances, called coenzymes, can range from complex metal ions such as vitamin B_{12} to the vitamins mentioned above (B_1 and B_6). Catalysis by two enzymes, chymotrypsin and lysozyme, is described in some detail, including how the fit between the enzyme and the substrate is important. The factors by which enzymes achieve their catalytic efficiency and specificity are also emphasized.

Other books in this series emphasize the geometry of molecules, organic reaction mechanisms, macromolecules, etc. This book attempts to build on the other members of this series.

MYRON L. BENDER
LEWIS J. BRUBACHER

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ONE
INTRODUCTION

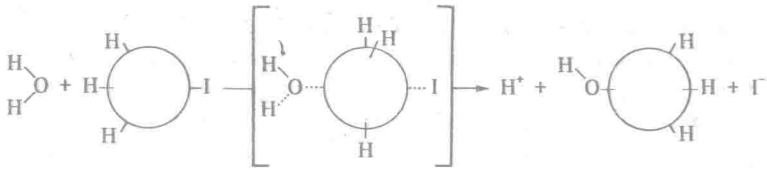
In order to understand how catalysts function, we must first understand what happens to the atoms in a molecule during a typical chemical reaction.

1-1 THE ANATOMY OF A CHEMICAL REACTION

As an example of a typical chemical reaction we shall consider the hydrolysis of methyl iodide in water solution to give methyl alcohol. The equation for this reaction, as written in Eq. (1-1), tells us nothing about what happens to the individual atoms during the course of a reaction between one molecule of methyl iodide and one molecule of water.

$$\underset{\text{Methyl iodide}}{CH_3I} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Methyl alcohol}}{CH_3OH} + \underset{\text{Hydrogen iodide}}{HI} \qquad (1\text{-}1)$$

Equation (1-2) illustrates what happens more clearly. In general a chemical reaction involves the breaking of old bonds and the making of new bonds, which may take place in several steps. The reaction in Eq. (1-1) is relatively simple. It occurs in a single step, as shown in Eq. (1-2). The intermediate stage in which two bonds are breaking while another is forming is called the *transition state*. We shall define this term more precisely later in this chapter.



(1-2)

Let us center our attention on the methyl iodide molecule in Eq. (1-2). The carbon atom is represented by a circle, and four atoms are bonded to it in a tetrahedral arrangement, as indicated by the drawing in the initial state. When a water molecule approaches the methyl iodide molecule from its backside (relative to the iodine atom substituent) with sufficient energy, a new bond begins to form as indicated in the drawing labeled "transition state." Simultaneously, the bond between the iodine atom and the carbon atom weakens. In the transition state the carbon atom of methyl iodide has three more or less normal bonds (to the three hydrogen atoms) and two partial bonds, the bond to the iodine atom which is breaking, and the bond to the oxygen atom which is forming. Although the total system is electrically neutral, the iodine atom is beginning to acquire a partial negative charge and the hydrogen atom of the water molecule a partial positive charge. When the oxygen-carbon bond is completely formed and the carbon-iodine bond is completely broken, the final state is reached and the reaction is complete.

In one aspect Eq. (1-2) is incomplete. The H^+ ion is not a free species but is covalently bonded to a water molecule as an hydronium ion, H_3O^+. That is, the proton is transferred from the forming methyl alcohol molecule to a neighboring H_2O molecule. Such a proton transfer reaction occurs very readily and may be viewed as occurring almost simultaneously with the breaking and forming of the two bonds involving the central carbon atom in Eq. (1-2).

The way Eq. (1-2) is written establishes a convention that will be used in this book. The transition-state representation is shown in large brackets within the arrow. This is to emphasize that the transition state is not an intermediate compound, but is a structure through which the reactant(s) pass in the 10^{-13} s or so that it takes to go from the initial state to the final state.

The description in this section of the course of events during a chemical reaction is called the *mechanism* of the reaction (see O. T. Benfey in this series). A study of the *kinetics* of a reaction is a study of the rate at which the reaction occurs, i.e., the number of molecules of methyl iodide that react per second under various conditions of temperature, solvent, etc.

1-2 THE ENERGY REQUIREMENTS OF A CHEMICAL REACTION

It takes energy to break a chemical bond such as the carbon-iodine bond in Eq. (1-2). Conversely, energy is released when a bond, such as the carbon-oxygen bond in Eq. (1-2), is formed. In the initial stages of the reaction in Eq. (1-2), the energy being released by the forming bond is *not* sufficient to begin to break the carbon-iodine bond. Additional energy is required, and it comes from the kinetic energy of the two molecules. Thus, if a reaction is to occur, the water and methyl iodide molecules must collide with sufficient force to provide the additional energy. In this particular reaction, the additional energy requirement, or *energy barrier*, is rather large. Consequently, only a very small fraction of all collisions involve sufficient kinetic energy for the reaction to occur and the reaction rate is low. At 25°C it takes 108 d for half the methyl iodide molecules to react. This is the half-life of the reaction. Raising the temperature of the solution increases the number of sufficiently energetic collisions which occur per second. At 50°C the reaction rate is 35 times faster than at 25°C; the half-life of the reaction is only 3.1 d.

The energy requirements for the reaction of a single molecule according to Eq. (1-2) may be represented graphically as in Fig. 1-1. Energy is plotted along the ordinate; the abscissa, labeled "reaction coordinate," represents the extent of reaction. Thus the curve in Fig. 1-1 shows schematically the minimum energy required by a molecule of water plus a molecule of methyl iodide as they go from the initial state to the transition state and on to the final state. The point of maximum energy on this curve corresponds to the transition state. For convenience we have assigned zero energy to the initial state; since the final state is at lower energy, a small amount of energy is actually released in the course of the reaction. Our immediate interest, however, is in the energy barrier to reaction, 4.52×10^{-20} cal per molecule of methyl iodide or 27.18 kcal mol^{-1}, which is the difference between the energy of the transition state and the energy of the initial state. This energy difference is referred to as the *free energy of activation* $\Delta G^{\ddagger}$. It is one of the few aspects of Fig. 1-1 which can be quantitatively determined. Diagrams like Fig. 1-1 are only schematic representations. The extent of reaction of a single molecule (i.e., the abscissa) is not a directly measurable quantity. It does not correspond directly to the dimensions

of either space or time; however there is a qualitative relation between the extent of reaction and the distance between the carbon and iodine atoms. We can say with simple certainty that for a given pair of reactants there is an initial state where the extent of reaction is 0 percent, and a final state where the extent is 100 percent, and that in going from the former to the latter, a molecule requires a certain minimum energy input $\Delta G^{\ddagger}$.

The free energy of activation is composed of two types of energy according to Eq. (1-3), where $\Delta H^{\ddagger}$ is the enthalpy of activation, T is the absolute temperature (in degrees Kelvin), and $\Delta S^{\ddagger}$ is the entropy of activation.

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\,\Delta S^{\ddagger} \tag{1-3}$$

Enthalpy is simply heat energy, the manifestation of molecular motion. At 25°C, $\Delta H^{\ddagger}$ is 27.37 kcal mol^{-1} for the hydrolysis of methyl iodide.

Entropy is a quantity that is difficult to visualize but may be thought of as a measure of disorder or randomness of orientation in a system. As a system becomes more disordered, its entropy increases. Conversely, as a system becomes more ordered, the entropy decreases. At 25°C, $\Delta S^{\ddagger}$ for the hydrolysis of methyl iodide is +0.62 cal deg^{-1} mol^{-1}; at 50°C it is −3.94 cal deg^{-1} mol^{-1}. A brief rationalization of

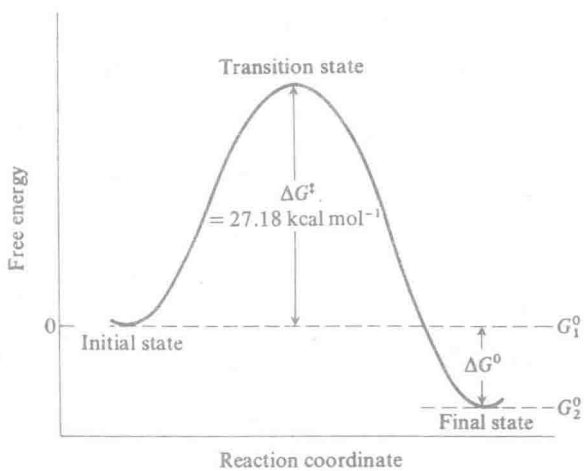


FIGURE 1-1 Free-energy diagram for the hydrolysis of methyl iodide at 25°C.

why $\Delta S^{\ddagger}$ decreases as temperature increases will give the reader some feeling for the concept of entropy as a measure of disorder.

The methyl iodide reactant molecule is always surrounded by a changing layer of water molecules, one of which will eventually become a reactant. This layer is the solvent shell. In the process of going from the initial state to the transition state we must ask what happens to this solvent shell as well as what happens to the reactants themselves. In other words, $\Delta S^{\ddagger}$ (and $\Delta H^{\ddagger}$, too, for that matter) has two components, $\Delta S^{\ddagger}_{\text{solvent shell}}$ and $\Delta S^{\ddagger}_{\text{reactants}}$ [Eqs. (1-4) to (1-6)].

$$\Delta S^{\ddagger} = \Delta S^{\ddagger}_{\text{solvent shell}} + \Delta S^{\ddagger}_{\text{reactants}} \tag{1-4}$$

$$\Delta S^{\ddagger}_{\text{solvent shell}} = (\text{entropy of solvent shell in transition state}) - (\text{entropy of solvent shell in initial state}) \tag{1-5}$$

$$\Delta S^{\ddagger}_{\text{reactants}} = (\text{entropy of reactants in transition state}) - (\text{entropy of reactants in initial state}) \tag{1-6}$$

According to one theory now in favor, the solvent shell has a certain degree of order in the initial state, perhaps similar to the ordering of water molecules in ice; in the transition state, this order breaks down somewhat (entropy increases). Therefore, $\Delta S^{\ddagger}_{\text{solvent shell}}$ is always positive. On the other hand, the two reactants must be lined up in just the right way in the transition state (high order, low entropy) but may have any orientation in the initial state (high disorder, high entropy). Therefore, $\Delta S^{\ddagger}_{\text{reactants}}$ is always negative. Since $\Delta S^{\ddagger}$ is slightly positive experimentally at 25°C, $\Delta S^{\ddagger}_{\text{solvent shell}}$ must have just a slightly greater magnitude than $\Delta S^{\ddagger}_{\text{reactants}}$.

Now increasing the molecular motion in the system (by raising the temperature) will increase the disorder (entropy) of the solvent shell in the initial state more than in the transition state, which has less order to start with. Thus $\Delta S^{\ddagger}_{\text{solvent shell}}$ will decrease in magnitude as the temperature is raised. On the other hand, $\Delta S^{\ddagger}_{\text{reactants}}$ should, if anything, also become more negative as the temperature is raised. Thus $\Delta S^{\ddagger}$ should become more negative as the temperature is increased. This is indeed what is observed. Consequently, the idea that entropy is a measure of disorder is a reasonable one.

In summary, we have described some aspects of the mechanism of

the hydrolysis of methyl iodide. The course of events in bond-making and bond-breaking is illustrated by Eq. (1-2). Quantitatively 27.18 kcal of energy is required on the average to take 1 mol of methyl iodide molecules from the initial state to the transition state at 25°C. A little more than this amount of energy is released, however, in going from the transition state to the final state, so that the net result of this reaction is the release of some free energy. The rate of a reaction at a given temperature is determined by $\Delta G^{\ddagger}$. The larger $\Delta G^{\ddagger}$ is, the fewer collisions per second there are that will have sufficient energy to attain the transition state, and the slower the reaction.

1-3 THE BASIS OF CATALYSIS

In 1902 the German chemist Ostwald gave the first adequate definition of a catalyst: "a substance which alters the velocity (rate) of a chemical reaction without appearing in the end product." According to this definition a catalyst may either increase or decrease the velocity of a chemical reaction. However, in current usage, a catalyst is a substance which increases the reaction velocity; a substance which decreases the rate of a reaction is called an inhibitor. Ostwald's definition also implies that a catalyst is not consumed during the course of the reaction it catalyzes, but serves repeatedly to assist molecules to react. There are other substances which may also properly be called catalysts, but which are consumed in the reactions they catalyze. In biochemistry this is true of many coenzymes. However, these coenzymes are often restored to their original form by a subsequent reaction, so that in the larger context the coenzyme is unchanged. A typical example is the coenzyme nicotinamide adenine dinucleotide which is chemically reduced during reactions it helps to catalyze. The reduced form is oxidized back to its initial form in a subsequent reaction. In later chapters we shall meet many other coenzymes.

A catalyst increases the velocity of a reaction by increasing the number of conversions of reactant molecules to product molecules that occur each second. In order to do this the catalyst must in some way reduce $\Delta G^{\ddagger}$ for the reaction it catalyzes.

Most commonly the catalyst performs this function by providing a different pathway for the reaction, which will usually have more steps than the uncatalyzed pathway. An essential feature of the catalyzed pathway is that all $\Delta G^{\ddagger}$ values are smaller than the largest $\Delta G^{\ddagger}$ value of the uncatalyzed pathway.