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The Molecular Anvil Model of an Enzyme Taking into Consideration the Flexibility of Enzyme Molecules

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Abstract. The concept of a molecular anvil model of an enzyme, assuming a rigid enzyme molecule, is introduced. Two distinct features of enzymes, high catalytic power and high specificity, are reasonably and consistently explained. The dynamic nature of molecular anvil action is stressed. The origin of the high catalytic power is the spontaneous creation of a high energy state at the anvil site. The origin of the high specificity is a high sensitivity of the maximum accessible potential energy to the relatively extruded distance of the molecular anvil. The flexible model is developed by assuming a flexible enzyme molecule. It is deduced from this flexible model that enzyme activity shows a maximum with a wide range of monotonous change of the configuration of the enzyme molecule. This is the origin of the general property of enzymes that enzyme activity shows a maximum with monotonous variation of environmental parameters such as pH, temperature, pressure or some times concentration of chemical substances. The induced fit theory of Koshland is reasonably explained. The relation and differences between individual theories of enzymes are discussed. The enzymological basis of the complex regulation of biological organisms is discussed. The inversion of the sign of control of effectors is predicted when environmental parameters are varied. This concept may be useful in designing artificial enzymes or high specificity catalysts.

Key words: Molecular anvil, high catalytic power, high specificity, spontaneous creation of a high energy state, relatively extruded distance, semi-inclusion phenomenon, slightly imperfect fitting, antibody, hapten, enzyme, enzyme activity, regulation of biological system, Boltzman factor.

1. Introduction

The concept of the molecular anvil has been proposed by us in a previous paper [1] in order to explain the high specificity of enzymes for discriminating optical isomers. In this paper we will describe this molecular anvil in a more general way and make clear its features and the importance of its dynamic nature. Then the flexibility of the enzyme molecule is introduced and we will discuss how the efficiency of the molecular anvil will vary with a change of configuration of the enzyme molecule. By assuming a linear relationship between the configuration of the enzyme molecule and the environmental parameters, the general properties of enzyme, *viz.* that enzyme activity shows a maximum with a monotonous change of environmental parameters such as pH, temperature etc. is derived. The importance of this general property of enzymes in the regulation of biological organisms is discussed.

2. The Rigid Molecular Anvil Model

2.1. THE MOLECULAR ANVIL AND THE CONDITIONS OF ITS FORMATION

An anvil is a mechanical instrument to produce very high pressures from low

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pressure sources using pistons. Such a mechanism can be realized at the molecular level if the following two conditions are satisfied. The first is that two molecules can contact simultaneously at multiple points. This may be called the simultaneous multi-point contact. The first condition is satisfied for a pair of substrate and enzyme molecules because it is confirmed by X-ray experiments that an enzyme molecule has a cleft over its surface into which the substrate molecule can fit. It is generally realized that when the concave surface of one molecule comes into contact with the convex surface of the other molecule. This is called the semi-inclusion phenomenon. The second condition may be satisfied because it is not probable that different molecules can always fit strictly exactly. Various types of molecular anvils are schematically shown in Fig. 1. In these figures the number of simultaneous contacts, n, is chosen as 5. The relatively extruded point is called an anvil site and the $n-1$ contact points in the enzyme molecule distinct from the anvil site are called the contact sites. We do not exclude a case where a small molecule like water is sandwiched between the two molecules as the molecular anvil as shown in Fig. 1 (e).

Let us explain how the molecular anvil operates. When the two molecules are separated at distances greater than the equilibrium distance, an attractive force exists between every pair of molecular contacts. However when they come closer to each other, a repulsive force begins to exert only at the anvil site and the other contact sites remain to be attractive. In such a range of distances attractive forces at the contact sites are focused at the anvil site and high pressure is produced at this point and a high energy state is spontaneously created at this anvil point. This is molecular anvil action.

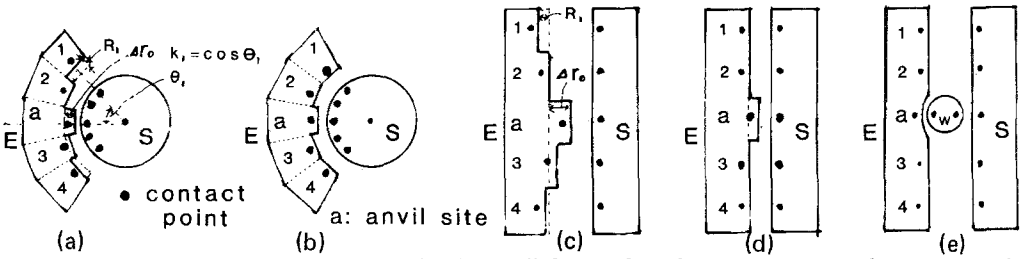

Fig. 1. Illustrations of various types of molecular anvil for $n=5$. a: $k_i s \neq 1$, $R_i s \neq 0$. b: $k_i s \neq 1$, $R_i s = 0$. c: $k_i s = 1$, $R_i s \neq 0$. d: $k_i s = 1$, $R_i s = 0$. e: a small molecule like water is sandwiched. S – substrate; E – enzyme; w – water.

2.2. POTENTIAL ENERGY AT THE ANVIL SITE

Let us discuss molecular anvil action more quantitatively. The total potential energy of the molecular anvil $E_t(r)$ is expressed by assuming pair approximation, that is the total energy is the sum of the energy of each molecular contact pair,

$$E_t(r) = f_a(r - \Delta r_0) + \sum_{i=1}^{i=n-1} f_{ci}(k_i r + R_i) \tag{1}$$

where r is the intermolecular distance, r_0 is the relatively extruded distance, $f_a(x)$ is a potential energy function of the anvil site pair, $f_{ci}(x)$ is the one for the i-th contact pair in the $n-1$ contact pairs, k_i is a ratio of the intermolecular distance change at the i-th contact site to the one at the anvil site, and R_i is a constant relating to the

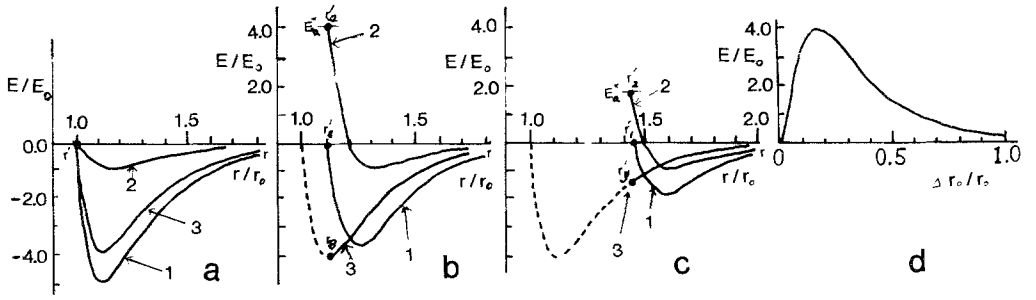

Fig. 2. Potential energy curves of the molecular anvil for $n = 5$ with different values of the relatively extruded distance Δr_0 and its relationship between maximum peak potential energy at the anvil site, E_a^*. Figures 2(a)–(c) are curves for values of $r_0/r_0 = 0$, 0.2 and 0.5 respectively. Curves 1, 2 and 3 in each figure indicate the anvil site, the contact sites and the potential energy respectively. Figure 2(d) is the dependence of E_a^* on Δr_0.

intermolecular surface distance of the i-th contact pair in the contact sites. For simplicity, we can assume without loss of intrinsicity of the molecular anvil that all $k_i s = 1$ and all $R_i s = 0$ and further $f_a(x) = f_{c1}(x) = f_{c2} = \cdots = f(x)$, then Equation (1) is reduced to

$$E_t(r) = f(r - \Delta r_0) + (n-1) \cdot f(r) \tag{2}$$

If we assume a proper function for $f(x)$ we can calculate the numerical value of each term of Equation (2). By assuming a Lenard-Jones 12-6 type potential function

$$f(r) = 4E_0\{(r_0/r)^{12} - (r_0/r)^6\} \tag{3}$$

where E_0 is the minimum value of the potential energy and r_0 is the distance at which the potential energy crossed zero. For $f(r)$, we calculated each term of Equation (2) for the molecular anvil $n = 5$ with $\Delta r_0/r_0 = 0$, 0.2 and 0.5 as functions of r and are shown in Figure 2. The potential energy of the state when the two molecules are separated at infinite distance at rest is taken to be zero. When the two molecules begin to approach, they are accelerated by their attractive force and finally they stop at the distance where the total potential energy $E_t(r) = 0$, due to the principle of conservation of energy, and then reverse again. During these processes $E_t(r)$, $f(r - \Delta r_0)$, and $(n-1) \cdot f(r)$ values change along curves 1, 2 and 3 respectively and each molecular contact pair moves from r_1 to $r_{1'}$, r_2 to $r_{2'}$, and r_3 to $r_{3'}$ respectively. Positive values of potential energy appear only at the anvil site except when $\Delta r_0 = 0$.

The peak positive potential energy values at the anvil site for a particular value of the relatively extruded distance Δr_0 varies very sharply with Δr_0 and is shown in Figure 2(d) for $n = 5$. The maximum value in the curve of this figure E_m^* is a function of n and is equal to $(n-1) \cdot E_0$. Values of $\Delta r_0/r_0$ corresponding to E_m^* vary from 0.153 for $n = 2$, 0.200 for $n = 5$ and 0.237 for $n = 10$ and are nearly near 0.2. Since r_0 is an order of a few Å, the molecular anvil has its maximum efficiency for Δr_0 of about 1 Å. This means that the efficiency of the molecular anvil is very sensitive to the relative size and shape of the substrate and enzyme molecules. This is the origin of the high specificity of enzymes.

2.3. TIME VARIATION OF POTENTIAL ENERGY AT THE ANVIL SITE

In an ideal case when the two molecules are isolated from the surroundings only one collision may occur if they had kinetic energy initially. In an actual case, each molecule has kinetic energy and is confined to a small space by collisions with surrounding molecules. In such case we can reasonably assume that the two molecules forming the molecular anvil complex will continue to oscillate in the intermolecular potential well. The range of distance of oscillation may be approximated between $r_{\min}$ at which $E_t(r)=0$ and $r_{\max}$ at which $E_t(r)=-kT$, the mean kinetic energy. Then the potential energy at the anvil site may vary periodically with time as shown in Figure 3. A high energy state is spontaneously and periodically created at the anvil site. Since chemical reactions are triggered by instantaneously created high energy states, the peak values govern the enhancement factor of an enzymatic reaction. The peak and average values of potential energy at the anvil site of the molecular anvil of maximum efficiency are calculated for various values of n and are shown in Figure 4. The average value is approximated to the one when the enzyme-substrate complex is at rest at equilibrium distance where $E_t(r)$ is the minimum. It is seen from this figure that the peak values are significantly higher than the average values. The peak potential energy is produced by the dynamic nature of molecular anvil action. It is stressed that the high catalytic power of an enzyme is produced by its dynamic nature. During repeated appearance of a high energy state at the anvil site, enzymatic reaction may be triggered.

Preliminary calculations of the peak pressures at the anvil site give values of several hundred thousand Mpa. for $n=5$ assuming E_0 corresponding to a vaporization energy of 10 Kcal/mol and r_0 corresponding to a molar volume of 100 ml.

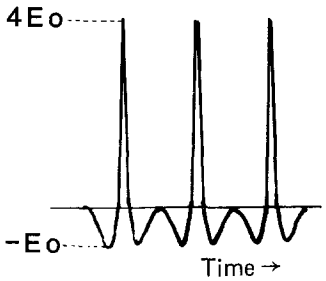

Fig. 3. Schematic illustration of periodic variation of potential energy values at the anvil site.

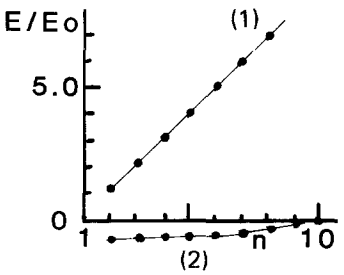

Fig. 4. Peak (1) and average (2) values of potential energy at the anvil site of the molecular anvil for various values of n with maximum efficiency.

2.4. THE ORIGIN OF HIGH CATALYTIC POWER AND HIGH SPECIFITY OF ENZYMES

The total reactivity of an enzymatic reaction is governed by two factors. The first one is the probability of formation of an enzyme-substrate complex. The second one is the enhancement factor of lowering the activation energy in a chemical process. The complexation energy E_c relates to the probability of formation of the complex by the factor $\exp(E_c/kT)$. The value of E_c is equal to the minimum value of the total potential energy of the molecular anvil $E_t(r)$. We can calculate E_c as a function of Δr_0 of the extruded distance for a particular value of n. The relationship between E_c and $\Delta r_0/r_0$ for $n = 5$ is shown by curve 1 in Figure 5. It is seen that the complexation energy decreases with increasing Δr_0 due to the increase of the repulsive force at the anvil site. We can assume that the local high energy state at the anvil site is effectively utilized to enhance enzymatic reaction. High pressure at the anvil site may help chemically bonded enzyme-substrate intermediate formation. The enhancement factor is proportional to $\exp(E^*/kT)$, where E^* is the peak positive potential energy value at the anvil site. E^* is reproduced as curve 2 in Figure 5 for $n = 5$. The total reactivity is the product of the above mentioned two factors and is shown as curve 3 in Figure 5.

For the molecular anvil of maximum efficiency the ratio of the peak potential energy at the anvil site $(n-1)\cdot E_0$ to E_c is nearly 1 ranging from 0.810, 0.946, 1.036 to 1.243 for $n = 3, 4, 5$ and 10 respectively. It may be said that in enzymatic reactions specificity is greatly elevated by utilization of the complexation energy twice, while catalytic power is enhanced by the factor of $\exp(E_c/kT)$, corresponding to utilization of E_c once. This is the origin of the high catalytic power and high specificity of enzymes.

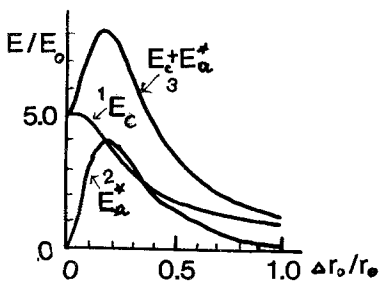

Fig. 5. Dependence of complexation energy, E_c, peak energy at the anvil site, E_a^*, and their sum on the relatively extruded distance, Δr_0, for the molecular anvil with $n = 5$.

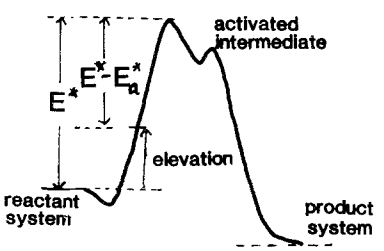

Fig. 6. Energy diagram of an enzymatic reaction showing elevation of starting level of a reactant system by molecular anvil action.

It may be noted that enhancement of a chemical reaction by the molecular anvil action is due to elevation of the starting level of the reactant system but not the lowering of activation energy at the top of the reaction intermediate state as shown in Figure 6. It also may be noted that molecular anvil action is useful in discriminating a substrate smaller than the size of the cleft of the enzyme.

3. The Flexible Molecular Anvil Model

3.1. ENZYME ACTIVITY AND CONFIGURATION OF ENZYME MOLECULE

We assume that a flexible molecular anvil is composed of at least one rigid molecular anvil with one anvil site and $n_a - 1$ contact sites and n_c pieces of other rigid contact sites with n_j contact points and each rigid body is connected by a flexible chain and the relative positions are changeable. Various types of flexible molecular anvil are shown in Figure 7. We do not exclude the case of having two rigid molecular anvils by which the substrate molecule is sandwiched as shown in Figure 7-c. The relative position of each rigid body is supposed to be changed by various environmental parameters such as temperature, pressure, pH and also concentration of chemical substances in the medium in which the enzyme molecule exists.

The total potential energy of the flexible molecular anvil is generally expressed as a function of various environmental parameters such as

$$E_t(r) = f_a(r - \Delta r_0) + (n_a - 1) f_{ac}(r) + \sum_{i=1}^{i=n_c} n_j f_c k_i(T, P, C_i) r + R_i(T, P, C_i) \qquad (4)$$

where $f(x)$s are intermolecular potential functions and the suffixes a and c indicate anvil site and contact site respectively and ac indicates contact site in the anvil, k_i and R_i are the same as Equation (1) but now they are not constant but functions of environmental parameters such as temperature T, pressure P, and concentration of chemical substances c.

For simplicity we can assume without loss of intrinsicity that all k_is are 1 and only R_is are varied with environmental parameters. The change of R_is is essentially equivalent to the change of the relatively extruded distance Δr_0 in the rigid model since the change of R_is relative to the anvil site surface is important in expressing molecular anvil action. Then this problem is reduced to the dependence of enzyme activity on the relatively extruded distance except that it is caused environmentally but not structurally. Thus it is derived that enzyme activity varies with values of environmental parameters. If one of the environmental parameters is varied over a wide range, enzyme activity should show a maximum at some optimum value. This conclusion is in accordance with the general properties of enzymes that each enzyme has its optimum pH or optimum temperature. When the major environmental

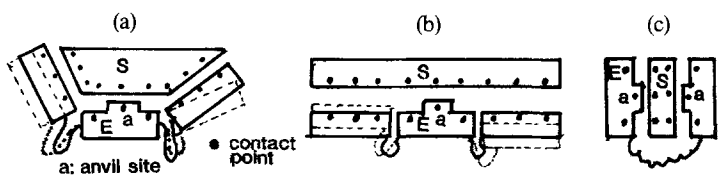

Fig. 7. Illustration of various types of flexible molecular anvil for $n_c = 3$. a: $k_i \neq 1$ and variable. b: $k_i s = 1$ and constant. c: Two anvil site. Solid line indicates original position and dotted one after shifting.

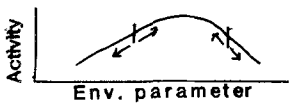

Fig. 8. Enzyme activity vs. environmental parameter curve showing maximum. Arrows indicate direction of change of environment to both sides.

Fig. 9. Schematic illustration of gradual change of peak energy at the anvil site with time during a period of induced fitting.

parameters are fixed and only a minor environmental parameter is changed monotonously, the change of enzyme activity is either a monotonous decrease or a slight increase followed by a decrease, since the original point is not always under conditions showing maximum activity. This is illustrated in Figure 8. The induced fit proposed by Koshland is a case where the configuration of an enzyme molecule is induced by approaching the substrate molecule so as to increase its activity. A gradual increase of peak potential energy at the anvil site may occur and is illustrated in Figure 9.

It is predicted theoretically that some special chemical substances may cause a bell shaped change of enzyme activity with a maximum even over a very small concentration range. This seems important in that if this substance is an effector controlling a certain enzyme in a biological organism, inversion of sign of control will occur when its concentration exceeds some threshold value or other environmental parameters are changed. It seems closely related to the enzymological basis of inversion from an unstable positive feedback state to a stable negative feedback one or vice versa in a biological regulation system.

In actual biological organisms many enzymes exist not in free solution but attached to cell membranes or the skeleton. In such cases changes of configuration of the enzyme molecule caused by a morphological change of the cell are much greater than in free enzymes in solutions. This model offers the principle of activity change of enzyme in cells playing an important role in the regulation of biological systems. This concept seems to be useful in understanding complex biological phenomena from an enzymological point of view.

4. Discussions

The most distinct feature of the molecular anvil is the spontaneous creation of a local high energy spot at the anvil site by accumulating energy from the surroundings even though instantaneously and periodically. The probability of appearance of a high energy state at the anvil site is higher than that expected from the Boltzman factor. The usual thermodynamic laws seem to be invalidated even though instantaneously and locally. The high catalytic power produced by this molecular mechanism of pumping energy from the surroundings by molecular anvil action may be the thermodynamical basis of producing the most far-from equilibrium state such as a biological organism.

The famous key and lock or template theory proposed by E. Fischer [2] implicitly assumes a perfect fitting without an extruded part in the template and in such cases a high energy state is never created and cannot explain the important properties of enzymes of high catalytic power. A slightly imperfect fitting of the molecular anvil is essential to produce high catalytic power.

Recently it was found that an antibody for a bapten works like an enzyme, though weak, for a substrate molecule which has a structure similar to the bapten molecule [3, 4]. This fact indicates that the bapten molecule fits perfectly with the antibody molecule and does not form a molecular anvil, but a certain molecule slightly different from the bapten may form a molecular anvil with the antibody molecule since these molecules fit slightly imperfectly. This seems strong support for the concept of molecular anvil and encourages artificial enzyme design.

According to the molecular anvil model it is predicted that if a certain enzyme has several substrate molecules of different size, relative enzyme activities for these substrates may vary with their molecular volume showing a maximum value for the proper size. Preliminary experiments on urease for substrates such as urea, formamide, methylurea, ethylurea, urethane, show that relative enzyme activities, vary with molecular volume of the substrate molecules, as shown in Figure 10.

There are many individual theories explaining the high catalytic power of enzymes based on particular experimental results on a particular enzyme such as a charge relay system [5], or facilitated proton transfer [6], polyfunctional catalysis [7] etc. Our model does not contradict with these theories because these models explain the lowering of activation energy at the metastable intermediate state while our model is to explain the elevation of the starting level of the reactant system resulting in equivalent lowering of the activation energy. We do not deny that the ease of an enzymatic

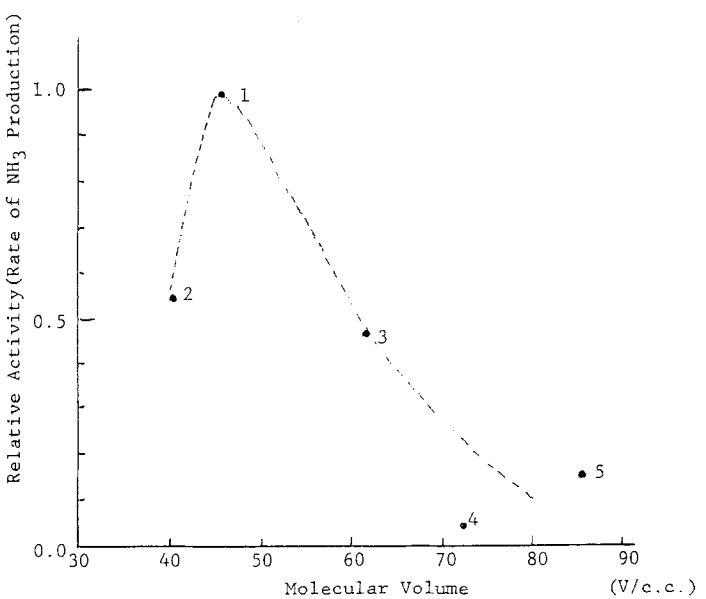

Fig. 10. Relationship between relative activity of urease and the molecular volume of various substrates.

reaction depends on the kind of group in contact at the anvil site, that is the enzymatic reaction is not wholly determined by the potential energy value at the anvil site but there exist many factors to affect it.

Of all enzymes, about 30% are metal containing ones and the metal atoms play important roles in enzymatic reactions. The electronic properties of these metal atoms may contribute greatly to enhance related enzymatic reactions. But it may be pointed out that molecular anvil action may to some extent contribute to enhancing these enzymatic reactions, because metal atoms are usually greater in size than atoms such as H, C, N, and O which constitute an organic compound surrounding metal atoms and the metal part may be relatively extruded. In some metal porphyrin containing enzymes, the position of the metal atoms are affected by environmental parameters such as temperature, or concentration of chemical substances or substrates and metal atoms come in and out of the plane of the porphyrin molecule. Unusual properties of this group of enzymes may be interesting if examined from the stand point of molecular anvil. The peculiar properties of hemoglobin is also related to this subject, as mentioned by Perutz [8].

According to the flexible molecular anvil model it is predicted that enzyme activity may show a maximum with change of concentration of some effectors on chemicals.

Such a prediction is actually observed, in the L-Lactate-Debydrogenase system [9].

A monotonous decrease of enzyme activity was observed by the addition of 10 to 40 p.p.m. of soap to the amylase-starch system. Whereas in the case of dodecyl benzene sulfonate a slight increase followed by a decrease was observed for the some concentration range [10].

The conclusion of the flexible molecular anvil model that enzyme activity is varied by the change of configuration of the enzyme molecule offers an enzymological basis of regulation of complex biological systems. The inversion of sign of control of effectors may be related to the enzymological basis of cytodifferentiation as well as carcinogenesis [11].

The concept of the molecular anvil may relate to some extent to catalytic power and also to the specificity of non-enzymatic catalysis. This concept may be useful in designing high specific catalysts and artificial enzymes.

5. Conclusions

Two distinct features of enzymes, high catalytic power and high specificity are reasonably and consistently explained by the rigid molecular anvil model. The flexible model similarly explains some general properties of enzymes such as showing maximum activity at the optimum values of environmental parameters such as pH, temperature, concentration of chemical substances. The relation between these fundamental properties of enzymes and biological phenomena is discussed.

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