THE EXPLANATION OF HIGH SPECIFICITY OF DISCRIMINATING OPTICAL ISOMERS IN ENZYMATIC REACTIONS BY THE MOLECULAR ANVIL MODEL OF ENZYME

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ABSTRACT. The molecular anvil model of enzyme is proposed and applied to explain high specificity of discriminating optical isomers in enzymatic reactions. The molecular anvil is a mechanism which can accumulate energy from two interacting molecules and produce locally a high energy spot called anvil site. Two conditions neccessary for formation of the molecular anvil are described. For a pair of enzyme and substrate molecules these two conditions are considered to be satisfied. Assuming proper shapes and sizes for molecules of optical isomers and a hole on the surface of the enzyme molecule into which the optical isomers can fit and also assuming Lenard-Jones 12-6 type potential for each pair ofinteracting molecular sites. The amount of energy accumulated at the anvil site is calculated. Following the assumption that the total reactivity is determined by binding process and chemical process in which the accumulated energy at the anvil site is utilized to enhance the reaction, total reactivities for L- and D-isomers are calculated and the values of specificity of discriminating L-isomer from D-isomer are derived for various values of interaction energy. It is shown that the molecular anvil plays an important role in elevating specificity as well as producing high catalytic power of enzyme.

1. INTRODUCTION

It is known that enzymes catalyse mainly only one of the two optical isomers. For example, in a case of tryptophanpyrolase it catalyses only L-tryptophan almost exclusively. This corresponds to a discrimination ratio of infinity. Such a high discrimination ratio cannot be expected if one considers physical process such as binding only. For example the heats of mixing of concentrated aqueous solutions of L-isomers and D-isomers of some compounds are nearly zero[1] and the differences of lattice energies of some crystalline optically active compounds and their corresponding racemic compounds are several kilo Joules per mole at highest[2] and this corresponds to the discrimination ratio of about several tens at most. This suggests that there must exist some discriminating mechanism relating to some chemical reaction proccess other than physical proccess.

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The author proposed a concept of the molecular anvil in 1979[3] and in this paper high specificity of enzyme of discriminating optical isomers will be explained by employing the molecular anvil model of enzyme.

2. THE MOLECULAR ANVIL MODEL OF ENZYME

Let us first explain the meaning of'anvil'. A mechanical anvil is an apparatus which generates very high pressure from lower pressure. It consists of two counter positioned pistons each of which has a flat plane with large cross section area at one end and a cone shaped head with a plane of very small cross section area at the other end as shown in Fig.1. By applying low pressure to each of the large flat plane of two pistons,

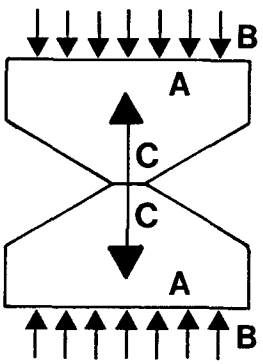

Fig.1 Illustration of a mechanical anvil, A. Piston. B. Flat plane with large cross section area. C. Cone shaped head with plane of very small cross section area. The arrows indicate direction and magnitude of forces.

very high pressure is produced between the two contacting cone shaped heads.

A similar mechanism may be realised in molecular scale if certain conditions are satisfied. Two conditions are neccessary. The first is that simultaneous multi-site contact occurs between two interacting molecules. In the case of two molecules of enzyme and its substrate, this first condition may be satisfied, because it is experimentally confirmed by X-ray diffraction analysis that there is a cleft on the surface of the enzyme molecule into which the substrate molecule can fit. This semi-inclusion phenomenon ensures contact of concave surface of one molecule with convex surface of another molecule and makes it possible for the two molecules to contact at many sites. This is called simultaneous multi-site contact. Such phenomena can never happen between ordinary molecules with convex surface only. This is the distinct feature of enzyme systems.

The second condition is that there exist one or a few relatively extruded sites in the above mentioned simultaneous multi site contact. This site is called the anvil site. This condition may be considered to be realized with some probability, because it is unlikely that two molecular surfaces can always fit strictly exactly together. These two cond-

itions are schematically illustrated in Fig.2.

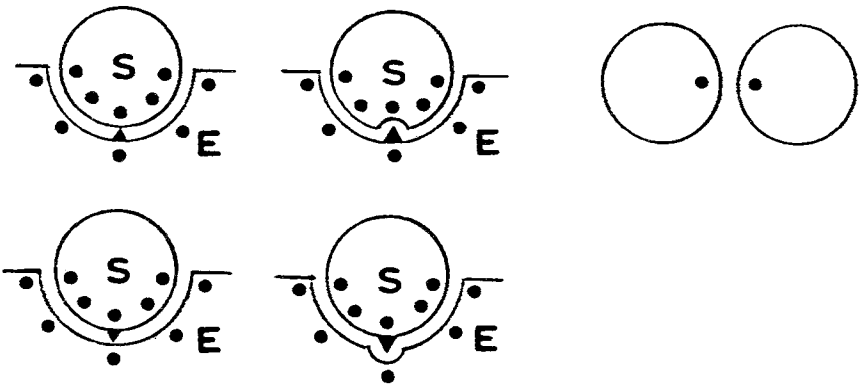

Fig.2 Schematic illustration of four different types of the molecular anvil with one anvil site and number of sites n=5 in simultaneous multi-site contact. The dots indicate sites of contact. E. Enzyme. S. Substrate. In comparison single site contact for ordinary molecules is shown together.

Let us next explain how the molecular anvil works. The four different types of molecular anvil shown in Fig.2 can be reduced in principle without loss of intrinsicity to the one shown in Fig.3.

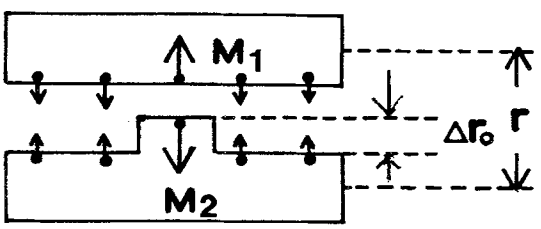

Fig.3 Schematic illustration of the molecular anvil with n=5 and one extruded site. M_1, M_2. The two interacting molecules. r indicates intermolecular distance and Δr_0 indicates extruded distance. The arrows indicate direction of force and their length indicates its magnitude.

Let us consider the molecular anvil in which the number of sites in simultaneous multi-site contact is n and the number of anvil sites is one and its extruded distance is Δr_0. When the two molecules are separated at infinite distance, there is no intermolecular force to exert and the potential energy at this distance is taken to be zero. Then if the two molecules come nearer attractive forces begin to exert between all n site pairs. The two molecules move closer to each other by their attractive forces by themselves. If the two molecules come much closer to each other repulsive force begins to exert between the anvil site pair while between the rest of n-1 site pairs forces remain to be attractive, the molecular anvil begins to work. The closest distance of the anvil site pair is the

distance at which the total potential energy of the multi-site contact reaches zero. At this point the positive value of potential energy of the anvil site balances the negative value of the rest of n-1 sites and high energy state is created at the anvil site. Such spontaneous creation of high energy state by accumulating energy from the neighbours does not occur in the ordinary systems. This is the distinct feature of the molecular anvil. The accumulated energy at the anvil site may be utilized to enhance the reaction and this is the origin of high catalytic power of enzyme. The amount of energy accumulated at the anvilsite E^* and hence the value of $\exp\cdot(E^*/kT)$, the enhancement factor of reaction is very sensitive to relative shape for fitting and this leads to elevation of discrimination ratio through chemical proccess.

Let us explain this situation more quantitatively. Since the total potential energy of the molecular anvil is the sum of potential energies of the anvil site pair and the rest of the n-1 site pairs, the total potential energy of the molecular anvil is expressed generally as

$$E(r) = E_0\{f(r-\Delta r_0) + (n-1)\cdot f(r)\} \qquad (1)$$

where f(r) is the potential function assumed, r is the inter-molecular distance, Δr_0 is the extruded distance of the anvil site, and n is the number of contacting site pairs in the molecular anvil, and E_0 is the binding energy of each site pair. Here we assumed that all potential functions for each of site pairs are equal. Three curves corresponding to each term of eq.(1) are shown in Fig.4 for n=3 and $\Delta r_0=0.173r_0$, where r_0 is the inter-molecular distance at which f(r) crosses zero. At this value of Δr_0 the molecular anvil of n=3 has the maximum efficiency. The Lenard-Jones 12-6 type potential function is assumed for f(r).

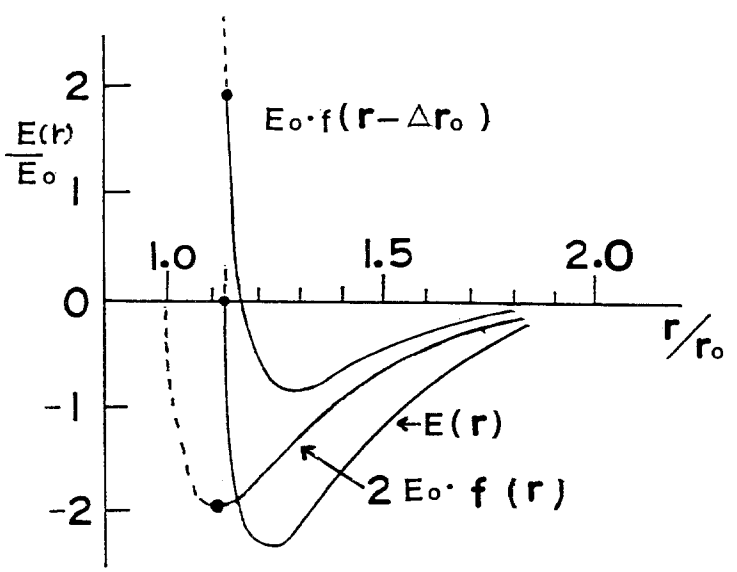

Fig.4 Potential curves for the molecular anvil with n=3 and $\Delta r_0=0.173r_0$ assuming Lenard-Jones 12-6 type potential function.

It is easily seen that when the two molecules come closer to each other high energy state is created at the anvil site.

The amount of energy accumulated at the anvil site when E(r) crosses zero, E , depends on the values of Δr_o and also on that of n, and are shown in Fig.5.

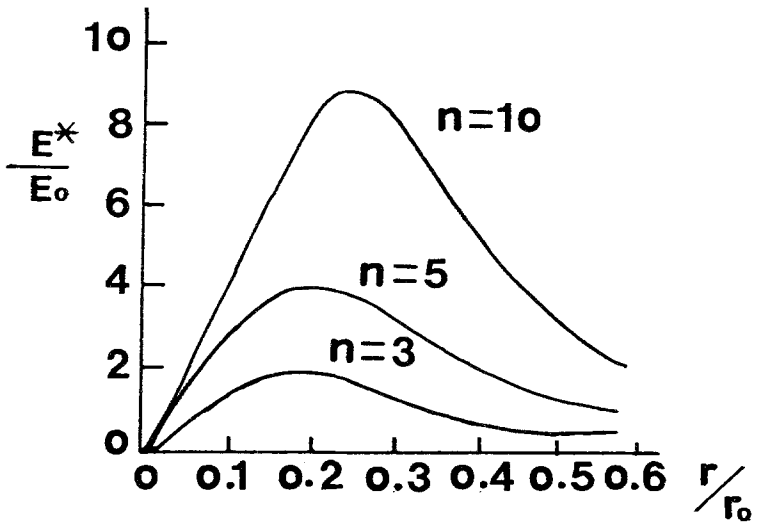

Fig.5 Dependence of E^*, the amount of enrgy accumulated at the anvil site on the values of Δr_o for various values of n.

It is seen from the figure that the value of E^* changes sharply with the value of Δr_o. The value of Δr_o at which E^* is maximum is around $0.2r_o$ and varies from $0.153r_o$ for n=2 to $0.243r_o$ for n=10. Since the value of r_o is an order of several angstroms, $0.2r_o$ is an order of one angstrom. It is reasonable to assume that the energy accumulated at the anvil site is utilized to enhance the reaction and this enhancement factor may be proportional to $\exp(E^*/kT)$. A slight change in molecular size of an order of 1 Å may change the value of E from zero to several kilo Joules per mole or more and this means that the change of enhancement factor through enzymatic reaction is about ten or more. This may be the origin of high specificity of enzyme as mentioned before.

Strictly speaking, the total reactivity is determined by binding factor and chemical one. In this case the binding factor without molecular anvil is proportional to $\exp(n \cdot E_o/kT)$ and if the molecular anvil is formed it decreases slightly by repulsive force at the anvil site to the value of $\exp(n \cdot x \cdot E_o/kT)$. The value of x is around 0.7 varying from 0.753 for n=2 to 0.693 for n=10. However this decrease of binding factor is compensated by chemical enhancement factor which has the value $\exp(n-1) \cdot E_o/kT$, so that the net gain of enhancement through formation of the molecular anvil is $\exp(1.7 \cdot n-1)/n \cdot E_o/kT$ instead of $\exp(n-1) \cdot E_o/kT$. This does not affect the intrinsic feature of the molecular anvil.

3. CALCULATION OF DISCRIMINATION FACTOR OF OPTICAL ISOMERS

Employing the molecular anvil model described above and making some assumptions for shapes of optical isomers and enzyme molecule, discrimination factor for optical isomers were calculated.

3.1. Assumptions

For a shape of molecules of optical isomers we assumed that the molecule consists of three pieces of one third of cylinders of different length cut symmetrically to the axis and all of these are bundled together to form a defective cylinder the one end of which is flat but the other end is not flat and has a three screwed steps. It is further assumed that the order of arrangement of three pieces of one third of the cylinders is 1, 2 and 3 clockwise viewed from flat side for L-isomer and 1,2 and 3 in counter-clockwise for D-isomer, in which 1,2 and 3 denote the longest, the middle and the shortest pieces respectively as is shown in Fig.6.

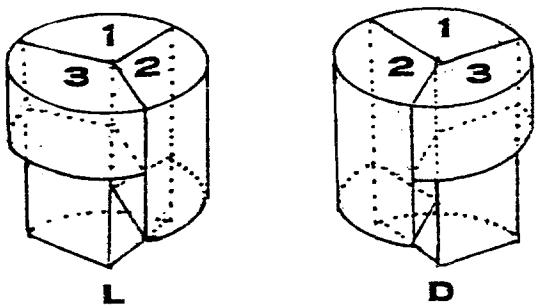

Fig.6. Illustration of assumed molecular shape for optical isomers.

For enzyme molecule it is assumed that there is a hole on the surface of the enzyme molecule and its size and shape is so chosen that it satisfies the two conditions of formation of the molecular anvil. It is also assumed that enzyme side has the extruded site at the deepest part of the bottom surface as is indicated in Fig.7 by the black dots.

It is also assumed that the optical isomer molecules in the hole of enzyme can take three different angular positions and for axial direction take the deepest positions. Three configurations of each isomer in the hole of enzyme molecule viewed from periphery and extended to a plane are shown in Fig.7.

To make calculation easy the value of Δr_o is chosen to be of maximum anvil efficiency and the length of three pieces of one third of thecylinders l_1,l_2 and l_3 respectively for 1,2 and 3 are chosen to be $l_1-l_2=l_2-l_3$ and its value is an order of r_o. By the last assumption interaction energies of site pairs except the closest one can be neglected. As for the potential function, Lenard-Jones 12-6 type potential of eq.(2) is used.

$$E(r) = 4E_o\{(r_o/r)^{12}-(r_o/r)^6\} \qquad (2)$$

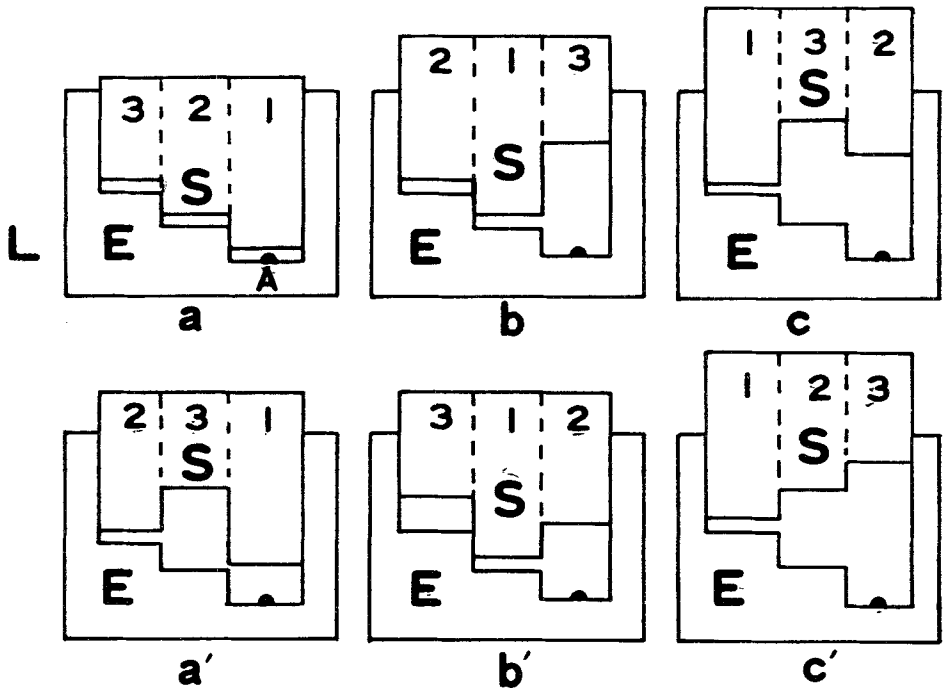

Fig.7 Illustration of three different configurations of fitting between enzyme and each of optical isomers. E. Enzyme S. Substrate A. Anvil site a, b, c, for L-isomer a', b', c', for D-isomer

3.2. Calculation and result.

Calculations were made assuming that the total reactivity is the product of the binding factor and the chemical factor which comes from enhancement action of the molecular anvil. The total reactivities are calculated for all configurations and summed for each isomer and their ratios for various values of binding energy and the results are shown in TABLE I.

TABLE I. DISCRIMINATION RATIOS FOR OPTICAL ISOMERS

E_o/kT	Discrimination ratio (A) with molecular anvil	(B) without molecular anvil	A/B
0.0	1.000	1.000	1.000
0.5	2.773	1.789	1.550
1.0	11.95	3.702	3.228
1.5	62.55	8.522	7.340
2.0	347.1	20.99	16.53
2.5	1956.	53.87	36.31
3.0	11070.	141.5	78.23

4. DISCUSSIONS

It is clear from the Table that discrimination ratio with molecular anvil is very high. To make clear the effect of the molecular anvil, discrimination ratios without molecular anvil are shown together for comparison. It is clear that improvement of discrimination ratio by molecular anvil goes up as the value of binding energy increases. As for the values of E_o it can be derived from heats of vaporization. E_o is nearly equal to $\Delta H \cdot 2/z$ where ΔH is the value of heat of vaporization and z is the number of nearest neighbours and is between 6 and 12. For heat of vaporization of about 10 K cal. the value of E_o/kT is about 3. For amino acid there is no data of heat of vaporization, but their lattice energy values are of similar order or more, an order of discrimination factor of L-and D-isomer may be explained by this model.

5. CONCLUSIONS

The molecular anvil model of enzyme is proposed and it can explain consistently the two distinct features of enzyme, high catalytic power and high specificity. By employing this model discrimination ratios for optical isomer were calculated for various values of binding energy. It is shown that the molecular anvil plays an important role in elevating discrimination factor.

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