CONCEPT OF ENZYME CATALYSIS

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Summary

A typical enzyme reaction runs through several steps. The structure of the enzyme-substrate intermediate in each step is reorganized to an optimal state to pass the next transition state. A very limited number of basic chemical catalytic principles are used in each transition state, and this is accomplished by a limited number of functional groups like amino acid side chains. Various methods of kinetic and structural studies are used to understand the function of the intermediates and transition states.

1. Background

According to an old definition “an enzyme is a protein with catalytic properties due to its power of specific activation”. The definition includes three aspects: the protein nature, catalytic efficiency, and specificity. Many of the general features of enzymes can be applied also for some other biocatalysts like ribozymes. They speed up the reactions or catalyze, and they show specificity towards their substrates, but since they are RNA-based, they are not classified as enzymes in a strict sense. Another obscure limit is towards to the binding proteins, which are proteins and specific, but sometimes it is not clear whether they catalyze a reaction or not. For instance antibodies have been shown to have catalytic properties.

Term "structure-function analysis" is used to express how structural studies and kinetic and specificity studies are connected in order to understand the enzyme function. The number of solved X-ray structures of proteins (including enzymes, enzyme-substrate and enzyme-inhibitor complexes and other protein-ligand complexes etc.) has risen over to 10 000. High-resolution models of the active sites from hundreds of different enzymes are available. The function of the enzymes is studied mainly by kinetic means.

The basic kinetic methods have been known for decades. The basic Michaelis-Menten theory was developed in 1913. The information from the kinetic studies can be used to interpret the roles of the functional groups in the structural models, and inversely, the detailed structure suggests how new kinetic studies should be carried out. The roles of exact ionic forms of the functional groups, substrates and products, the rates of formation and degradation of enzyme intermediates and the estimation of proton transfer reactions (e.g. by isotope effects) can be experimentally tested.

What is the number of different enzyme mechanisms? The Enzyme Nomenclature of the Enzyme Commission lists and classifies the enzymes according to the catalyzed reactions. The list from year 1992 includes about 3200 different enzyme reactions, which are divided into six main groups. All enzymes are somehow different from each other. However, the number of different reaction mechanisms does not rise to thousands, i.e. different enzymes use similar mechanisms. Partial chemical events in the total reaction follow the same order, and the same kind of functional groups are involved. For instance the number of enzymes using the serine protease type catalytic center rises to hundreds. Especially the reactions using the same coenzyme can closely resemble each other. In the Enzyme Nomenclature the number of sub-sub groups is 200. This can be regarded as a rough estimate for the lower limit of different mechanisms.

A minimal enzyme reaction contains three steps: first the substrate is bound, then the catalytic reaction occurs, and in the third step the product is dissociated. The ES complex and EP complex are the real intermediates of the reaction. There are only very few examples of such a simple reaction. Another extreme is the reaction of the aminoacyl-tRNA synthetases (which make the correct aminoacyl-tRNA:s at the start of protein synthesis). There are three substrates and three products, magnesium ions and polyamines are activators, and the reaction runs in two separate phases: activation of the amino acid and transfer of the aminoacyl group to tRNA. The number of intermediates in such a reaction increases to more than one hundred, and even the number of "important" steps is more than ten. An average enzyme reaction may have two substrates and two products. Such reactions take place in 5 to 9 important steps.

The main reason for the several successive reaction steps is to make the total reaction possible, but there are also some other physiological consequences. Such a reaction can follow the Michaelis-Menten kinetics, but the K_m values tend to be lower than the corresponding substrate dissociation constants K_s , and their difference depends on the mechanistic features of the enzyme reaction. It means that there are possibilities to adjust the efficient functional enzyme level to the metabolite levels in the cells not only by changing the substrate binding but also by other mechanistic changes.

2. Enzyme Specificity

More than 100 years ago Emil Fischer developed the idea of analogy of lock and key to describe the steric relationship between enzyme and substrate. The descriptive value of the analogy has not changed, although it tells little about the real structures leading to the specificity. The initial step in the formation of specificity is binding where the bond-forming structures in the enzyme and substrate must fit together (like key to lock). In many enzymes this already gives sufficient specificity, but normally additional steps are

necessary.

The term “induced fit” (an expression about 40 years old) is used to describe the next step: the substrate binding is followed by a conformational change which is (sterically or energetically) possible only with the correct substrates. In fact a normal enzymic reaction includes a series of conformational changes where the intermediates are structurally reorganized for the next steps, and these changes can affect both the catalytic efficiency and specificity.

The specificity is still improved by proofreading steps. In the “kinetic proofreading” a wrong substrate is dissociated from an enzyme-substrate intermediate. The wrong substrate has weakened bonds to the enzyme compared to the correct substrate. The kinetic proofreading is most efficient in multi-intermediate enzyme reactions, where a relatively slow step exists before the proofreading step. In some enzymes requiring very high specificity a mechanism called “hydrolytic proofreading” is used. The enzyme shifts the product to a hydrolytic site, separate from the active site, where a wrong product is hydrolyzed but not the correct product. The hydrolytic proofreading has been detected for instance in DNA polymerase and some aminoacyl-tRNA synthetases, which are responsible for the correct transfer of the genetic information to the proteins. In the isoleucyl-tRNA synthetase the hydrolytic site is 34 Å apart from the active site while within an active site the distances are only a few ångstroms.

The stepwise increase in specificity in the discrimination of valine by the isoleucyl-tRNA synthetase occurs approximately as follows: After the initial binding the relation valine : isoleucine is 1:3.6. The next conformational change lowers the error frequency to 1:250. After the first proofreading step the error frequency is about 1:12000 and after the second, hydrolytic, proofreading 1:38000. The term "double sieve" has also been used for a system like this where the primary binding preferentially eliminates the amino acids bigger than isoleucine, and the hydrolytic editing eliminates the smaller ones.

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Biographical Sketch

Kalervo Airas was born in 1942. He obtainerd his Ph.D. in 1976 at the University of Turku. He works as a teacher, docent and research scientist at the University of Turku, Finland. He has conducted research at the University of New Mexico, Albuquerque, USA and the Max-Planck-Institut für experimentelle Medizin, Göttingen, Germany. His research has dealt with the kinetics and mechanisms of enzymes like pantothenase and aminoacyl-tRNA synthetases.