VETERINARY TODAY INTERNATIONAL
ISSN 2583-8288

Three-Dimensional Structure, Flexibility and Conformational Mobility of Enzymes

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doi.org/10.5281/VeterinaryToday.21489741

Enzymes

Enzymes are biological catalysts that increase the rate of reaction without affecting the reaction equilibrium. They work by lowering the activation energy (E_a) for a reaction, which leads to an increase in reaction rate and faster product formation.

Enzymatic reactions are also characterized by high substrate and reaction specificity and fewer side reactions.

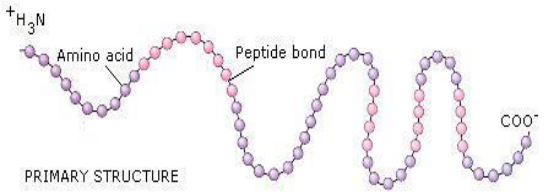
Enzyme Three Dimensional structure

Enzymes have **four levels of structures**. These are **Primary, Secondary, Tertiary and Quaternary structures**

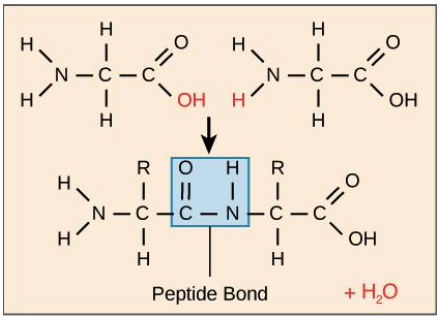
The enzyme structure ranges from a basic amino acid sequence to a three dimensional (3D) structure in a folded protein. The amino acid sequence in polypeptide chains in each enzyme is distinct and determines the three-dimensional shape. Further, it is the 3D structure of an enzyme that determines the enzyme activities.

Primary structure

The sequence of amino acids in an enzyme is the **primary structure**. In the primary structure, the constituent amino acids are linked by peptide bonds (-CONH-) bonds.



The peptide bond is formed between the amino group (-NH_2) of one amino acid and the carboxyl group (-COOH) of another, along with the release of water molecule. The primary structure dictates three dimensional structures of the proteins. The different ways in which amino acids will be arranged in the chain will influence proper protein folding for the enzyme to be functionally active.



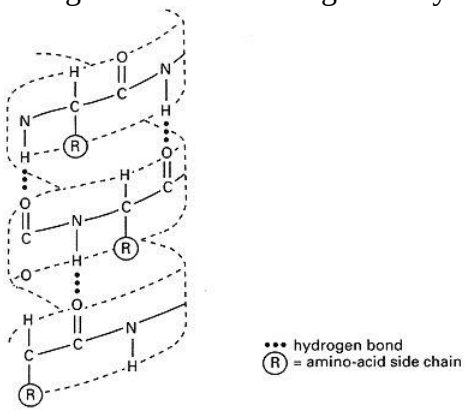
Secondary structure

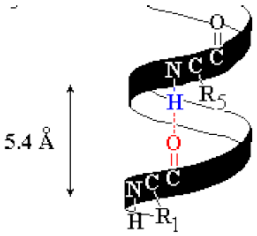
The secondary structure in enzymes refers to the interaction of amino acids in a chain (primary structure) which are closely located. There are two types of secondary structures: helical (called **α helices**) and pleated sheets (called **β pleated sheets**).

Alpha helix

The alpha helix is a helical structure, coiled around an axis. The helix is right-handed in nature and is characterized by intramolecular hydrogen bonds between the O atom of the C=O of each peptide bond in the strand and the N-H group of the peptide bond. The side-chain substituents of the amino acids extend to the outside from the helix, which has about 3.6 amino acids per turn on an average, meaning that it will have 36 amino acids in 10 turns. The pitch is 5.4 Å. Alpha helices form more readily in enzymes than any other possible

conformations owing to the optimal use of internal hydrogen bonds is made in these arrangements for attaining stability.





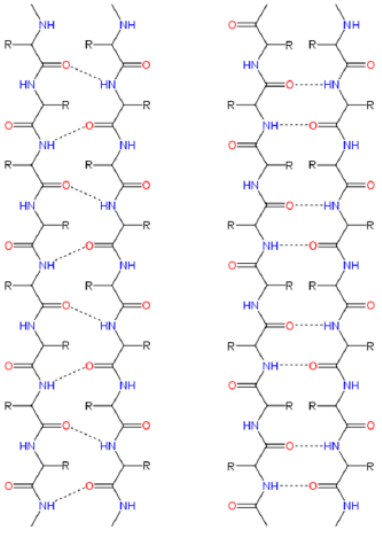
Beta pleated sheet

The second form of secondary structure in enzymes is the beta pleated sheet. This structure is formed by intermolecular hydrogen bonding between two or more straight chains. The O atom of the C=O of peptide bond in one strand hydrogen bonds with the N-H group of the peptide bond in an adjacent strand.

Again, the two strands involved in the formation of beta pleated sheets can run either parallel to each other or anti-parallel to each other. If the amino groups of both chains are on the same side, the sheet are said to be *parallel* to each other. On the other hand, if the amino groups of both chains are on the opposite side, the chains are said to run in the opposite direction. In this case, the sheet is termed *anti-parallel*. The anti-parallel ß-sheet is more stable than parallel sheet owing greater alignment in the hydrogen bonds.

Difference between alpha helix and beta pleated sheet structure

Properties	Alpha helix	Beta pleated sheet
Structure	Right handed, helical	Sheet like
Formation	Formed by H bonds within the polypeptide chain	Formed by H bonds between two adjacent polypeptide chains
Side chains	Side Chains (R groups) orient out of the helix	Side chains (R groups) are directed both inside and outside the pleated structure
Types	Only one type	Can be of two types: parallel and antiparallel
Preferred amino acids	Ala, Leu, Met, Phe, Glu, Gln, His, Lys, Arg	Tyr, Trp, Phe, Met, Ile, Val, Thr, Cys



Tertiary structure

The arrangement of amino acids in the three dimensional space defines the tertiary structure of enzymes. The protein molecule arranges itself three dimensionally in such a way as to achieve low energy and maximum stability. The various interactions involved in the formation/stabilization of a tertiary structure are Hydrogen bonds, polar-polar interaction, hydrophobic interaction, ionic interaction, formation of disulfide bonds, Van der Waals forces.

Under physiological conditions, the side chains of amino acids which are hydrophobic in nature such as phenylalanine or isoleucine, tend to remain buried within the protein/enzyme core, owing to their minimal affinity for the aqueous medium. The alkyl groups of Ala, Val, Leu, Ile often form hydrophobic interactions between one-another. Acidic or basic amino acid side-chains are polar in nature, and therefore remain exposed on the enzyme surface, to allow for greater water solubility.

Quaternary structure

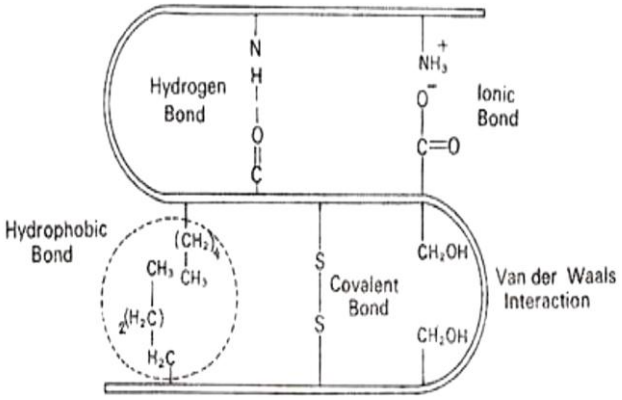
Sometimes, proteins or functional enzymes can be made up of more than one polypeptide chains, which are known as subunits. The interaction between these subunits is called the quaternary structure. Various interactions, including H-bonding, disulfide-bridges and salt bridges are also involved in stabilizing the overall complex.

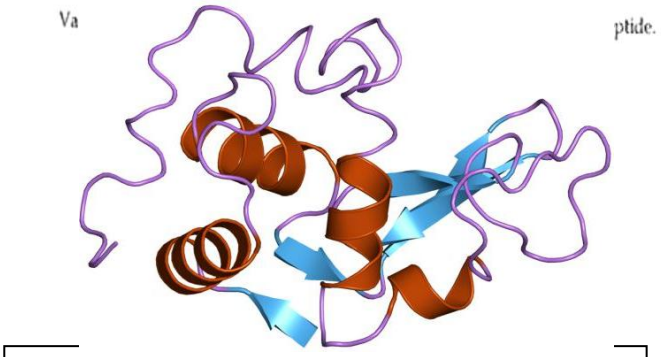
Enzyme Structure Analysis

Analysis of enzyme/protein structure can be done by the following analytical techniques/equipments:

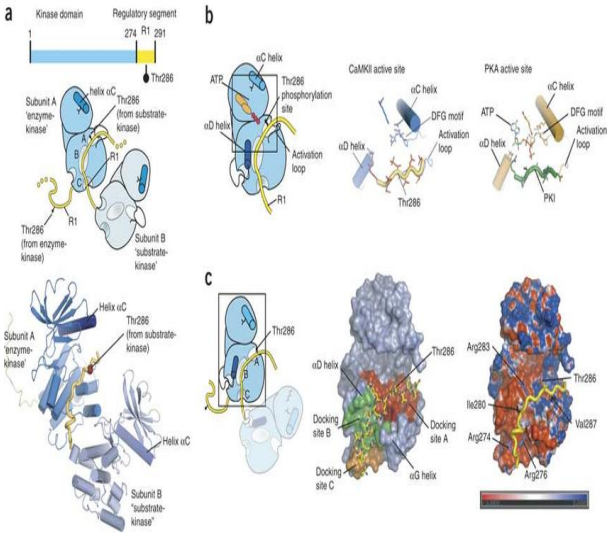
- Determination of amino acids which are present in an enzyme and the molar ratios of each can be analyzed by an **amino acid analyzer**.
- The sequence of amino acids in the enzyme can be analyzed by **peptide mapping, Edman degradation** or **mass spectroscopy**.
- Secondary structure of an enzyme can be determined by **circular dichroism spectroscopy** (CD).
- The tertiary structure of an enzyme can be determined by **fluorescence spectroscopy**.
- **X-ray crystallography** or **nuclear magnetic resonance** (NMR) analysis can be used to obtain a high-resolution analysis of the 3D structure of an enzyme.

Crystal structure of the CaMKII enzyme–substrate complex.(a) Schematic diagram of the crystallized CaMKII enzyme–substrate complex. The construct contains the kinase





Lysozyme. The red helices are α-helices, blue arrows the β-sheets.



domain of CaMKII and the R1 portion of the regulatory segment. The structure shown (at right) is that of crystal form A. (b) The CaMKII enzyme–substrate-complex active site is in the active conformation. Comparison of the major active-site components of the CaMKII enzyme–substrate complex with that of protein kinase A in the active state32 (PDB 1ATP). (c) Docking sites used by the R1 element are indicated as docking sites A, B and C and are colored red, green and gold, respectively, on a surface representation of the kinase domain. The R1 element is shown in a sticks representation. At right, an electrostatic surface potential representation (produced with Adaptive Poisson-Boltzmann Solver tools39) of the CaMKII kinase domain in the enzyme–substrate complex illustrates a negatively charged region (red) encompassing docking sites B and C that is used by residues in the R1 element (basic residues shown in blue, hydrophobic residues shown in black).

Flexibility and Conformational Mobility of Enzyme

Active Site of an Enzyme

All enzymes have an **active site**, where the reaction is catalysed. This part of the enzyme has the specific shape and functional groups to bind to the *substrate*. Hence the active site contains a small number of catalytic amino acids, which are essential in catalysing the reaction. The substrate molecule can bind to the active site via non-covalent interactions:

- **Electrostatic interactions**: Arg, His, Lys (Positively charged); Asp, Glu (Negatively chrged)
- **Hydrogen bonding**: amide and carboxyl groups of the amino acid can form hydrogen bonds with the substrate.
- **Van der Waals interactions**: Ser, Thr, Gln, Asn
- **Hydrophobic interactions**: Ala, Val, Ile, Leu, Met, Phe, Tyr, Trp

Enzyme and evidence of its flexibility:

Earlier it was believed that enzymes are highly specific catalysts, with one structure correlating to one function, as ‘‘key-lock’’ theory of Fischer(1894). Later this view was challenged, by Koshland (1958) as “induced-fit model” which introduced the concept of an enzyme active site which undergoes structural changes induced by the binding of the substrate. This theory is based on the idea that protein structures or at least their active sites possess a flexibility which enables them to adopt more than one conformations.

Protein–protein and protein–small ligand interactions, signal transduction and assembly of macromolecular machines, allosteric regulation and thermal enzymatic adaptation are processes which require structural flexibility. Flexibility of the active site is considered as a requirement for reduction of free energy barrier and acceleration of the enzymatic reaction and there may be connection between flexibility and substrate turnover rate. Moreover, the role of conformational flexibility has been well established in connection with the accessibility of the active site, the binding of substrates and ligands, and release of products, stabilization and trapping of intermediates, orientation of the substrate into the binding cleft, adjustment of the reaction environment, etc.

Techniques including *X-ray crystallography* and *Nuclear Magnetic Resonance* (NMR) have been widely used to obtain information about the protein structure that provides insights into the mechanism of function. It revealed that the functioning protein is present in slightly different but related conformations, with some areas of the protein being more flexible than others. Evidence came from the analysis of crystallographic temperature factors, which had lower values for buried/semi-buried areas and systematically higher for solvent exposed residues. In some cases, loops and side chains exposed to the solvent have very high values indicating high mobility. Moreover, relatively high temperature factors also characterize regions of the active site of enzymes.

It has been proposed that the protein function may involve multiple conformations, and that the observed deviations are not just inconsequential random thermodynamic fluctuations; rather, flexibility may be closely linked to protein function, including the catalytic efficiency of enzymes. Protein flexibility plays a promoting role in the

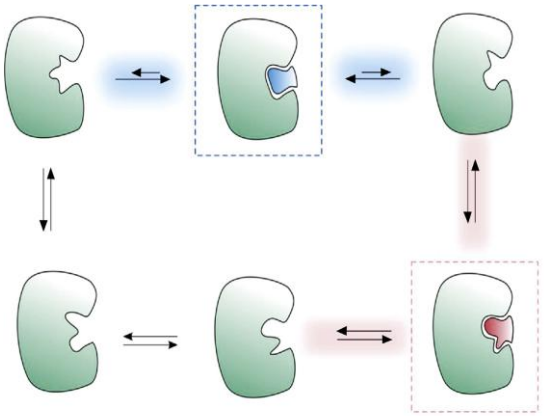
biophysical mechanism of enzymes. Conformational flexibility of enzymes has been associated with substrate (and cofactor) binding and product release.

Methods used to study flexibility of proteins (and thus enzymes):

A. Crystallography and Time-Resolved X-ray Methods
B. X-ray free electron laser (XFEL)
C. Spectroscopies, NMR, NSE, and Hydrogen–Deuterium Exchange
D. Biomolecular simulations (computational based)

Enzymes are dynamical entities that can change their conformation in many different ways, from local fluctuations of side chains, through to large scale loop and even domain motions. These changes can be intimately linked to an enzyme's function: for example, many enzymes undergo conformational changes to attain catalytically active conformations, allosteric regulation is critical to the function of many enzymes, and several proteins undergo order-disorder transitions to facilitate chemistry.

These conformational transitions also facilitate catalytic promiscuity, allowing enzymes to adapt to bind substrates at the same (or



sometimes even multiple) active site(s)

In this model, proteins can interchange between multiple conformations, with the dominant conformation being considered to be the native state, which interacts with the native ligand (blue). Conformational fluctuations such as, for example, side chain or loop dynamics, can then lead to multiple alternative conformations which can either interact with the native ligand, or with promiscuous ligands (red).

Determinants of Active Site Flexibility:

Enzyme active sites are formed by relatively weak molecular interactions and hence conformationally more flexible than the intact enzymes. The activity of enzymes is strongly dependent on their conformational integrity.

In principle, upon catalysis, residues in and around the active site must undergo conformational changes associated with the binding and release of substrates and cofactors, the protection of the reaction space from the aqueous environment, stabilization of reaction intermediates, and interactions between catalytic residues and binding subsides with various groups of reactants upon completion of catalysis.

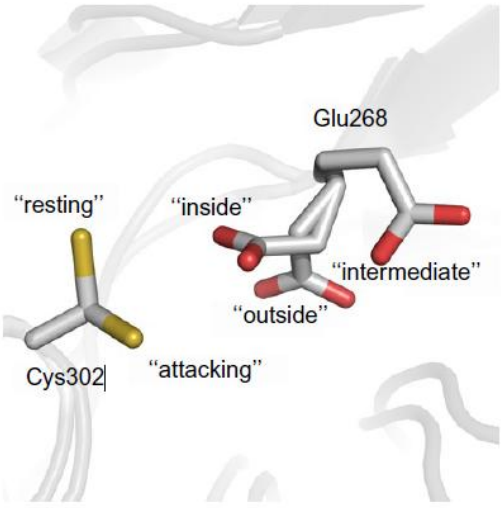
- In some cases, the structural requirements for conformational flexibility are limited to individual amino acids especially to side chains capable of adopting different rotamers.
- In other cases, the interactions of the macromolecule with substrate and cofactors require conformational changes mainly involving the external loops located in the periphery of the active site.
- On the other hand, several times the required structural reorganization associated with catalysis involves large global movements and rearrangements of whole protein domains.

Active Site flexibility:

A. *Flexible Active Site Loops:* extremely flexible, mobile loops in the proximity of active sites frequently play various roles in catalysis and it is widely accepted that their pronounced conformational flexibility may contribute to their functions
B. *Active Sites Located at Domain Interfaces:* A significant portion of oligomeric enzymes form their catalytic and binding sites at the interface

between subunits while, in the case of monomeric enzymes, active and binding sites often lie between domains, called as "lobes", ligand is first bound to one lobe; subsequent movement of the other lobe brings it near to the ligand permitting additional interactions which stabilize the structure

C. *Flexibility of Active Site Residues:* Active site residues are involved in catalysis and substrate binding, stabilize the intermediates of the reaction or the structure of the binding cleft. They provide suitable for the catalysis microenvironments and enable substrates to form enough contact points for strong binding. Some degree of flexibility is inevitable for the active site residues to achieve their functions and accommodate the conformational changes which are necessary during the catalytic cycle. This flexibility facilitates active site rearrangements during the numerous intermediate steps of the reaction.

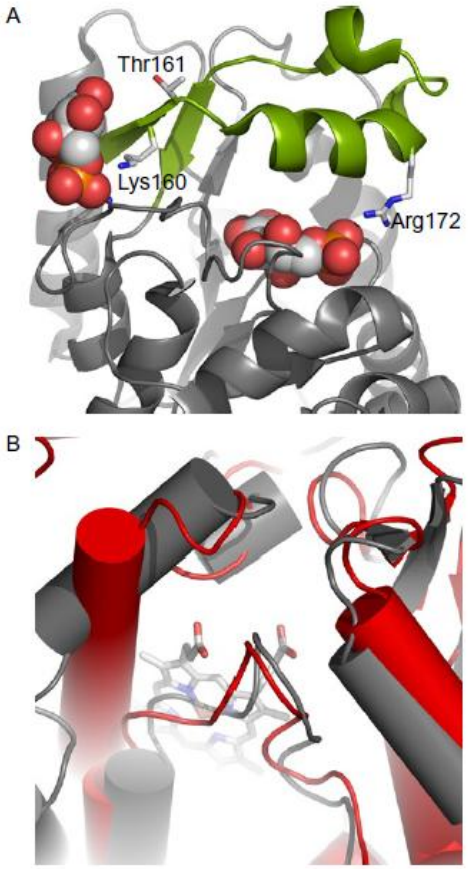


Local flexibility of the active site residues of aldehyde dehydrogenases is demonstrated by the multiple conformations of Cys302 and Glu268. As it is shown, Cys302 can adopt two conformations called ''resting'' and ''attacking'' while Glu268 adopts three, the ''inside'' ''intermediate'' and ''outside'' conformations. Combinations of these conformations determine different stages of the catalytic cycle (PDB entries 1UZB and 1O02).

Flexibility and Special Aspects of Enzyme Properties:

- Flexibility is important for Thermal Enzymatic Adaptation in different organisms which are residing in extreme temperatures.

- Flexibility is an Essential Component of Enzymatic Allostery, the process by which conformational changes at one site of a protein (called regulatory or allosteric site) are coupled with changes to a different and usually distant functional site (active site). Studies mainly by X-ray crystallography showed that the functional states of an allosteric enzyme are well represented by two, structurally different forms: (i) the ''R'' form (relaxed), which has an optimal affinity for the substrate and (ii) the ''T'' form (tensed), which has a minimal affinity.



(A) Cartoon representation of the glucosamine-6-phosphate deaminase. The enzyme possesses a complex active site lid (shown in green) which is directly connected with both, active and allosteric sites. To illustrate the location of these sites, a glucosamine 6-phosphate (space-filling model) is shown bound to each of them (PDB entry 1FS5).

(B) Structural comparison of the P450 cytochromes CYP101 (P450cam, PDB entry 2L8M) in gray from Pseudomonas putida and CYP108 (P450terp, PDB entry 1CPT) in red, a bacterial enzyme from Pseudomonas. The enzymes adopt a conserved fold with a substantial variability around the substrate-binding region. The molecule of heme is shown as stick representation. Small displacements of structural elements which abuts the active/binding toward the heme together with the segmentation of one of the helices (shown on the left of the figure) decreases the size of the active/binding site of CYP101 relative to that of the CYP108.

- Flexibility also plays a role in Ligand Specificity. The broad substrate specificity which characterizes some enzymes has been explained under the view of a flexible active site cavity capable of accommodating stereochemically diverse substrates. The different substrates may either induce conformational rearrangements to the broader area of the flexible active site or, if the protein structure is seen as a dynamic ensemble of conformations, each substrate is bound to different conformations with different affinity. In some cases, the local flexibility around the active site and the possible reposition and repack of only few individual side chains could cause sufficient space and shape alterations inside the active site to switch from one substrate to another without substantial structural changes. However, in other cases, global flexibility seems to be a requirement for promiscuity.