PAPER TITLE: ENZYMOLOGY AND ENZYME TECHNOLOGY

ENZYME CATALYSIS

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References

1. Lehninger Principles Of Biochemistry Fourth Edition: David L. Nelson And Michael M. Cox
2. Biochemistry; Donald Voet And Judith G. Voet

How Enzymes Work

- An enzyme provides a specific environment within which a given reaction can occur more rapidly.
- An enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site**
- The molecule that is bound in the active site and acted upon by the enzyme is called the **substrate**.
- The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation.
- The enzyme-substrate complex is central to the action of enzymes , {ES } was first proposed by Charles -Adolphe Wurtz in 1880,
- A simple enzymatic reaction might be written as:

$$E+S \longleftrightarrow ES \longleftrightarrow EP \longleftrightarrow E+P$$

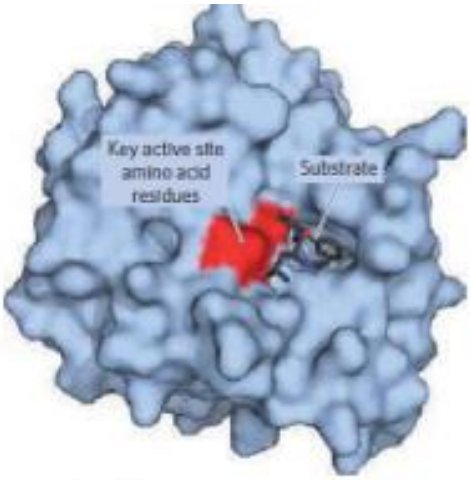

FIG. : Binding of a substrate to an enzyme at the active site. The enzyme chymotrypsin with bound substrate. Some key active -site amino acid residues appear as a red splotch on the enzyme surface.

Catalysts do not affect reaction *equilibria*.

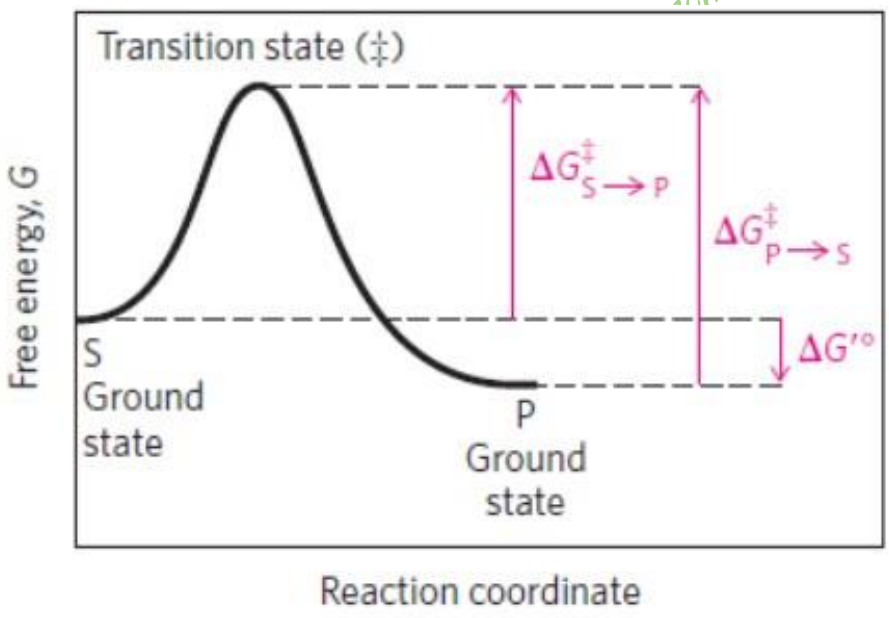

FIGURE: Reaction coordinate diagram. The free energy of the system is plotted against the progress of the reaction.

- Reaction *equilibria* are inextricably linked to the standard free-energy change for the reaction, *G*, and reaction *rates* are linked to the activation energy, *G*‡.
- An equilibrium such as $S \rightleftharpoons P$ is described by an **equilibrium constant**, ***K*eq**,
- Under the standard conditions equilibrium constant is denoted *K*eq (or *K*):

$$K'_{eq} = \frac{[P]}{[S]}$$

- From thermodynamics, the relationship between *K*eq and *G* can be described by the expression $\Delta G'^{\circ} = -RT \ln K'_{eq}$
- where *R* is the gas constant, 8.315 J/mol

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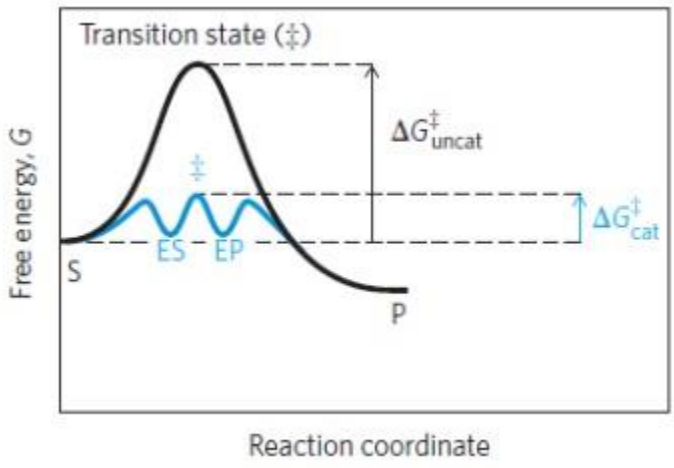
There is an energy barrier between S and P: the energy required for alignment of reacting groups, formation of transient unstable charges, bond rearrangements, and other transformations required for the reaction to proceed in either direction.



ENZYME CATALYSIS

Prominent physical and thermodynamic factors contributing to $G^{\ddagger}$, the barrier to reaction, might include:

(1) the entropy (freedom of motion) of molecules in solution, which reduces the possibility that they will react together,

(2) the solvation shell of hydrogen bonded water that surrounds and helps to stabilize most biomolecules in aqueous solution,

(3) the distortion of substrates that must occur in many reactions, and

(4) the need for proper alignment of catalytic functional groups on the enzyme.

Binding energy can be used to overcome all these barriers.

- **First,** a large restriction in the relative motions of two substrates that are to react, or **entropy reduction**, is one obvious benefit of binding them to an enzyme.
- **Second,** formation of weak bonds between substrate and enzyme results in **desolvation** of the substrate.
- **Third,** binding energy involving weak interactions formed only in the reaction transition state helps to compensate thermodynamically for any distortion, primarily electron redistribution, that the substrate must undergo to react.

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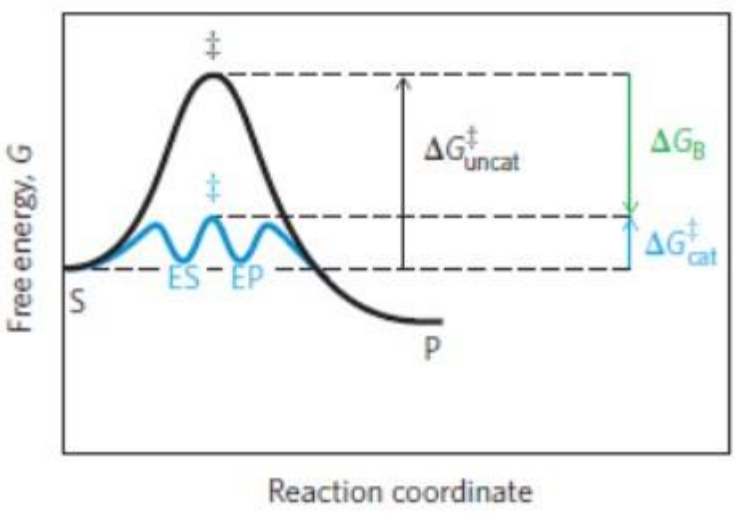
ENZYME CATALYSIS

Role of binding energy in catalysis.



To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which $G^{\ddagger}$ is lowered.
Much of this energy comes from binding energy (G_B) contributed by formation of weak noncovalent interactions between substrate and enzyme in the transition state.

ENZYME CATALYSIS

- Some weak interactions are formed in the ES complex, but the full complement of such interactions between substrate and enzyme is formed only when the substrate reaches the transition state. These weak interactions make the primary contribution to catalysis.
- An enzyme must provide functional groups for ionic, hydrogen -bond, and other interactions, and also must precisely position these groups so that binding energy is optimized in the transition state.
- The groups on the substrate that are involved in these weak interactions can be at some distance from the bonds that are broken or changed.
- ***The weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis .***
- The free energy (binding energy) released by the formation of these interactions partially offsets the energy required to reach the top of the energy hill.
- The summation of the unfavorable (positive) activation energy G‡ and the favorable (negative) binding energy GB results in a lower *net* activation energy .

LOCK AND KEY MODEL

- **Lock and Key** model was first postulated in 1894 by Emil Fischer.
- The specific action of an enzyme with a single substrate can be explained using this hypothesis.
- Any lock, which is analogous to an enzyme, can have only one suitable key of appropriate shape and size to open it.
- The various available keys, which are analogous to the thousands of substrates available, can attempt to open the lock but only one will be the perfect fit that is capable of opening the lock.
- Similarly only one particular substrate will fit into the active site of the enzyme and the enzymatic reaction can occur.
- According to the Fischer's hypothesis, enzymes and their substrates possess specific complementary geometric shapes that fit exactly into each other.
- This model accounts for the specificity of enzymes but fails to account for stabilization of the transition state.

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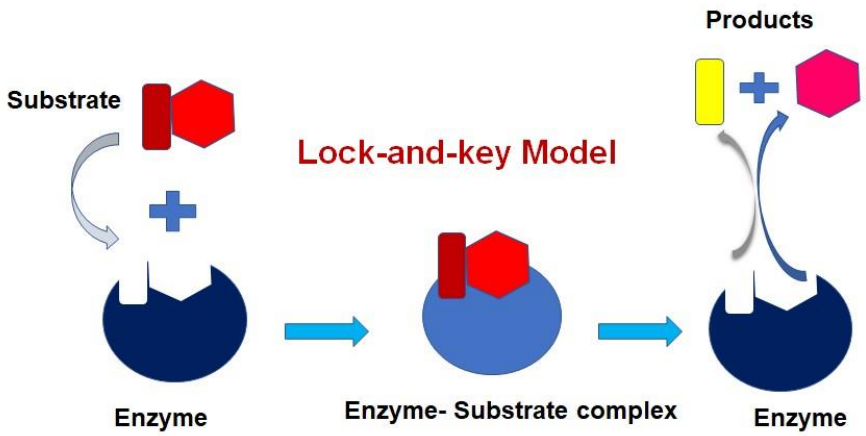
LOCK AND KEY MODEL

Weak Interactions between Enzyme and Substrate Are Optimized in the Transition State



- John (J. B. S.) Haldane, in 1930, pointed out that if the **binding energy** was used to distort the substrate in such a way as to facilitate the subsequent reaction, then less energy would be required for the reaction to take place.
- This concept was developed further by Linus Pauling, in 1948.
- If the structure of the active site is rigid, the substrate must be distorted slightly in order to bind to the enzyme. This distortion might result in the stretching, and thus weakening of a bond which is subsequently to be cleaved, thus assisting the forward reaction

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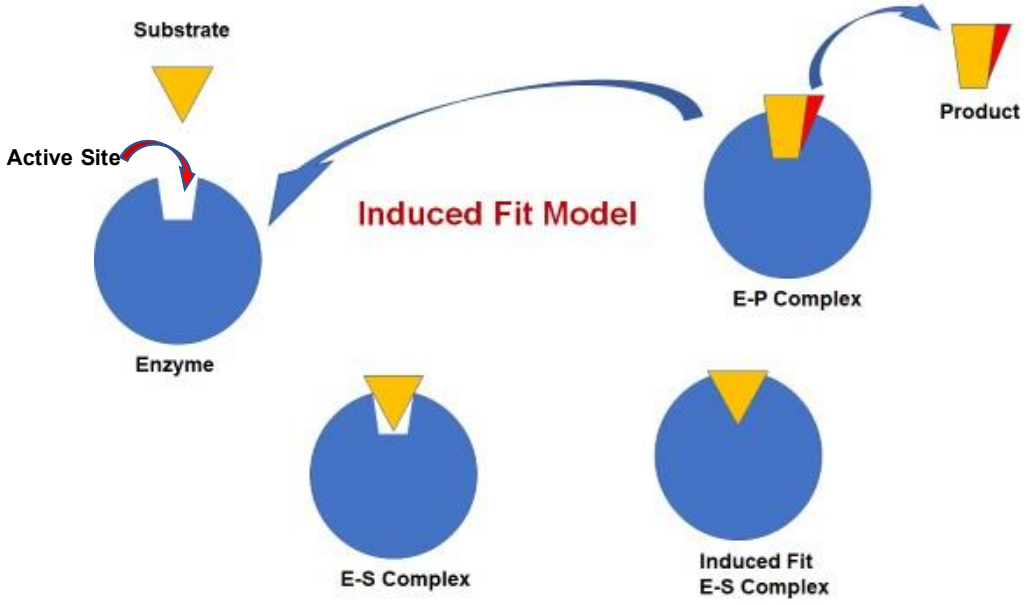
INDUCED FIT MODEL



INDUCED FIT MODEL

- Daniel Koshland in 1958 has proposed a modified hypothesis and suggested that the active site of an enzyme gets continually reformed based on the interactions that it establishes with the substrate molecule.
- The model is known as "Induced Fit model".
- The enzyme initially has a conformation that attracts its substrate. Enzyme surface is flexible and only the correct catalyst can induce interaction leading to catalysis.
- Conformational changes may then occur as the substrate is bound.
- Finally the reaction products will move away from the enzyme and the active site returns to its initial shape.
- This hypothesis is supported by the observation that the entire protein domain could move several nanometers during catalysis.
- This movement of protein surface can create microenvironments that favor the catalysis.

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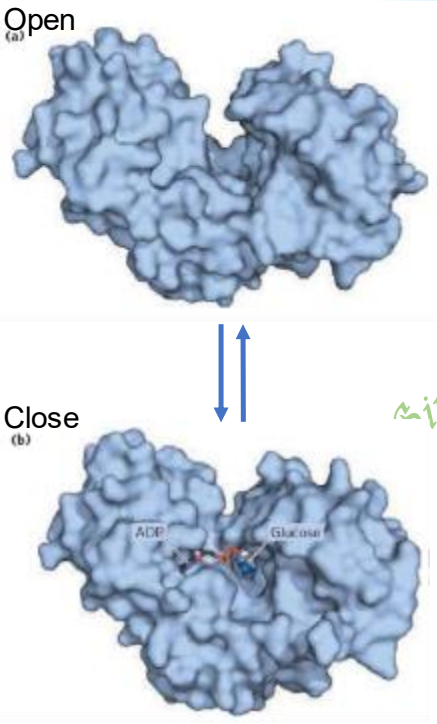
HEXOKINASE INDUCED FIT MODEL



- The active site residues for Hexokinase are Asp205, Lys169, Asn204, Glu256,and Thr168.
- These residues are located in the deep cleft at the interface between the two lobes.
- Hexokinase undergoes an induced fit conformational change when glucose binds.
- This conformational change prevents the hydrolysis of ATP, and is allosterically inhibited by physiological concentrations of glucose-6-phosphate the product.
- Hexokinase has two conformational states. The open state occurs prior to glucose binding. ATP is bound to the large lobe, but is far away from the glucose binding site, and in a different position than it assumes in the active site.
- When the glucose binds to Hexokinase a large conformational change occurs. This change closes the two lobes around the glucose substrate.
- This conformational state is referred to as the closed state.

TRANSITION STATE MODEL

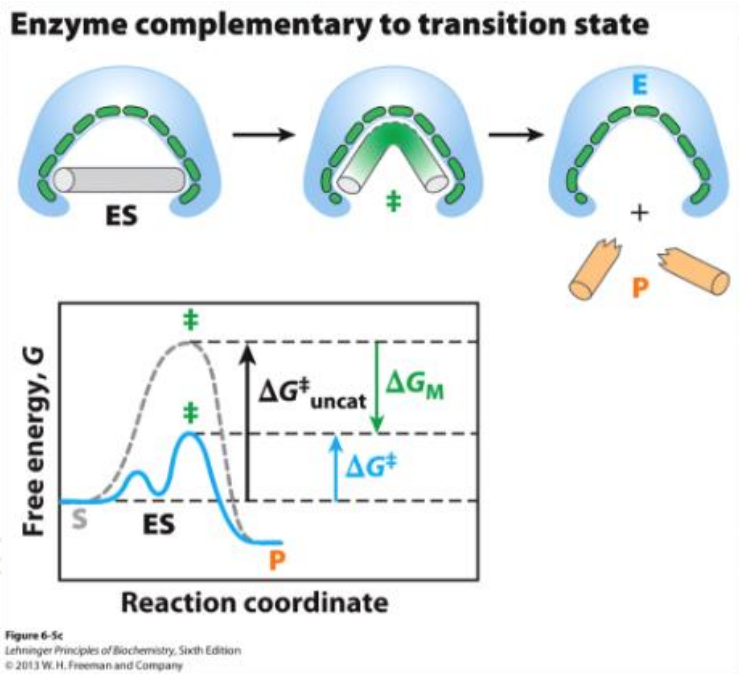


Figure 6-5c
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TRANSITION STATE MODEL

- An alternative, and possibly more likely, mechanism for driving the reaction forward is **transition-state stabilization**.
- This assumes that the substrate is bound in an undistorted form, but the enzyme-substrate complex possesses various unfavourable interactions.
- These tend to distort the substrate in such a way as to favour the following reaction sequence:

  enzyme-substrate complex → transition-state → products
- Thus, the overall effects of **strain** and **transition-state stabilization favors the reaction in forward direction**.
- An example of an enzyme-catalysed reaction proceeding via a transition-state stabilization mechanism is the hydrolysis of peptides by Chymotrypsin.
- Lysozyme is also an example of an enzyme which operates transition-state stabilization.

References

1. Lehninger PRINCIPLES OF BIOCHEMISTRY Fourth Edition: David L. Nelson and Michael M. Cox
2. BIOCHEMISTRY; DONALD VOET and JUDITH G. VOET