Chapters 5-6 Enzymes

Catalyst: A substance that speeds up the rate of a chemical reaction but is not itself consumed.

Most biological catalysts are **proteins** but some **RNA** are catalysts too.

e.g. Peptide bonds are made by the catalytic activity of the RNA in ribosomes.

Some enzymes require organic coenzymes and / or metal ions.

Apoenzyme / Apoprotein = Protein

Holoenzyme = Protein + Coenzyme

Classification of Enzymes Add "*ase*" to the activity to obtain the name.

1. **Oxidoreductases**: transfer e^{-1} as H or H^-.
2. **Transferases:** group transfer.
3. **Hydrolases:** bond breakage through addition of water.
4. **Lyases:** addition to or formation of double bonds.
5. **Isomerases:** group transfer yielding isomers.
6. **Ligases:** formation of C-C, C-S, C-O, C-N coupled to ATP cleavage.

Why are enzymes necessary?

1. Most biological molecules are **stable** at pH 7, 37^{o} C in H_2O.

Enzymes accelerate bond formation and breakage by $10^{2} – 10^{17}$.

2. Enzymes are **specific**; usually there are no side-reactions.

3. Enzymes can be **regulated**.

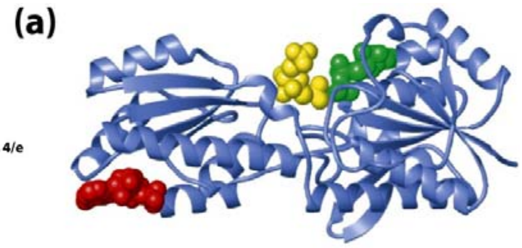
But, enzymes can be easily **denatured**.

Enzymes function *via* **changes in conformation**. They are dynamic, not static. Their atoms are constantly in motion.

How do Enzymes work?

1. **Specificity**: Specific reacting molecules called **substrates (S)** bind to the enzyme **(E) active site** and are converted into a **product (P)**. The active site fits the substrate like a hand in a glove (but see below).

e.g. Phosphofructokinase transfers a phosphate group from ATP to Fructose-6 phosphate forming **Fructose-1,6 bisphosphate** and **ADP**. The reaction is controlled by the binding of **ADP** in the **allosteric** binding site.



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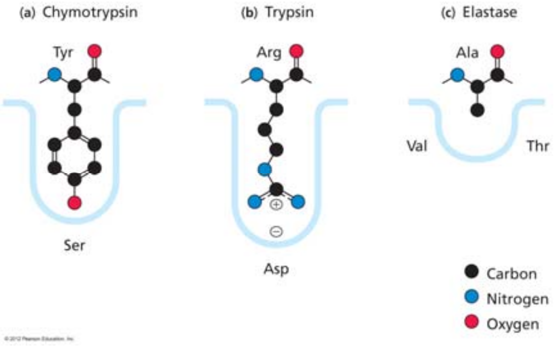
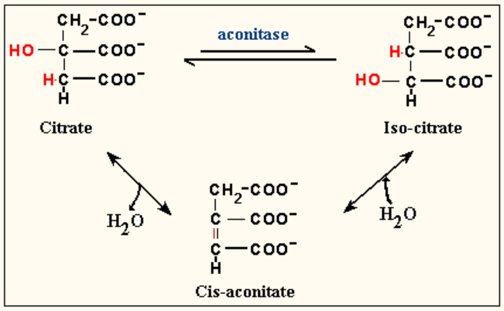
e.g. *Trypsin*, *chymotrypsin*, and *elastase* recognize different classes of AA *via* the size and shape of their binding sites:



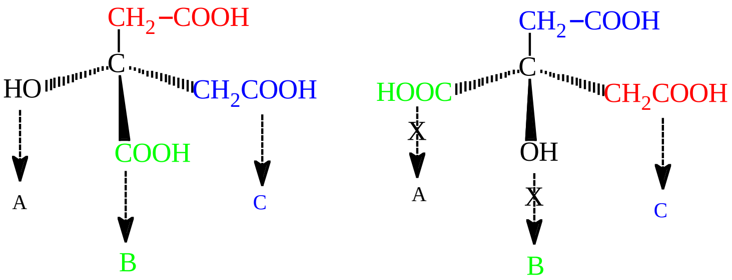
e.g. *Aconitase* can distinguish between the two ends of citrate even though there is no chiral C.



Citrate is converted into iso-citrate by firstly a dehydratation (cleavage of water) so that cis-aconitate is formed. Then cis-aconitate is hydrated (water added) and iso-citrate is formed. This reaction is catalysed by aconitase.

http://www.natuurlijkerwijs.com/english/Citrate_cycle.htm

It can, because the enzyme is a 3-dimensional molecule with 3 sites of interaction:



On the other hand, enzymes are less specific and can sometimes bind several different substrates.
e.g. *Hexokinase* phosphorylates glucose, fructose, and mannose.

So the enzymes provide a binding site that is **complementary** to the **steric** and **electronic** features of the substrate: “*hand-in-glove*”.

How do Enzymes work?

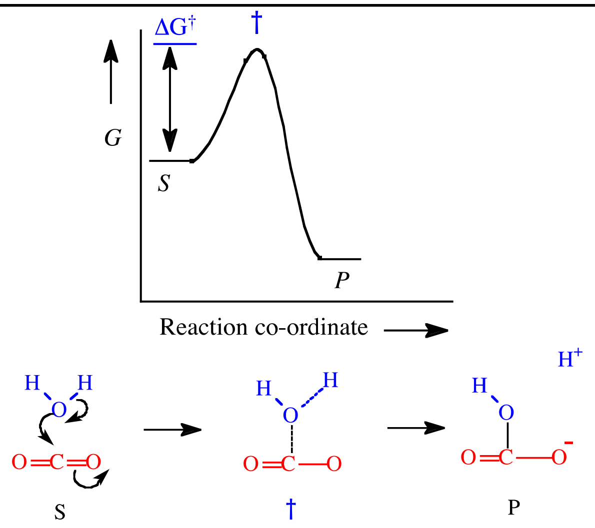
2. Enzymes provide a *special environment* in which bond formation / breakage is easier.

$$H_2O + CO_2 \rightleftharpoons HCO_3^- + H^+$$

is slow because energy is required to break the O-H bond of water and stretch one of the C=O bonds of CO_2.

We can keep track of the G changes by a “**Reaction co-ordinate diagram**”.

The Reaction co-ordinate indicates the free energy changes as the reaction progresses from substrate, *S*, to product, *P*.

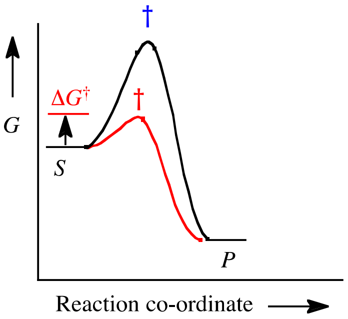


G must be added to **stretch the bonds** to the point of breaking.

The **Transition State †** is the point at which there is an equal probability of the O–H and C=O bonds re-forming and new HCO_3^- bonds forming. $\Delta G^\dagger$ = **Activation Energy**.

$\Delta G^{\dagger}_{S \rightarrow P} = G^{\dagger} - G_S$

Enzyme catalysts work by lowering $\Delta G^{\dagger}$, so that in the presence of an enzyme $\Delta G^{\dagger}_{cat} << \Delta G^{\dagger}_{uncat}$.



And the reaction catalyzed by *Carbonic anhydrase*:

$$H_2O{-}E{-}CO_2 \rightleftharpoons E{-}HCO_3^- + H^+ \rightleftharpoons E + HCO_3^-$$

is 10^7 times faster than by itself in water.

Notice that K_{eq} is **not** affected by a catalyst. If the enzyme increases the forward rate, then the backward rate will also be increased because $\Delta G^{\dagger}_{P \rightarrow S} = G^{\dagger} - G_p$ is lowered.

K_{eq} depends on the difference in G between product and substrate: $\Delta G^o = G^o_P - G^o_S$ as follows:

$$\Delta G^o = -RT \bullet \ln_e(K_{eq})$$

and is **path independent**.

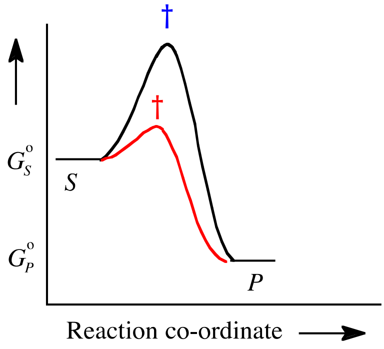
R = Gas Constant = 8.31 J / mol · K;

T = Temp in Kelvin

ln is the natural logarithm to base *e*;

e is 2.71828….

The rate constants depend on $\Delta G^{\dagger}$.



$$\Delta G^{\dagger} = -RT \bullet \ln_e\left(\frac{k \bullet h}{k_B \bullet T}\right)$$

k_B = Boltzmann constant = 1.38 x 10^{-23} J / K

h = Planck's constant = 6.6 x 10^{-34} J · s

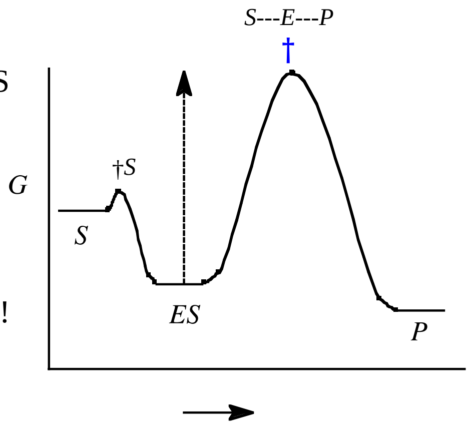
The smaller is $\Delta G^{\dagger}$ the bigger is k.

$+6.9 = \ln(0.001) \qquad 0 = \ln(1) \qquad -6.9 = \ln(1000)$

How do enzymes lower $\Delta G^{\dagger}$?

Strong binding between E and S would lower the G of ES (ES would be more stable) and increases $\Delta G^{\dagger}$.

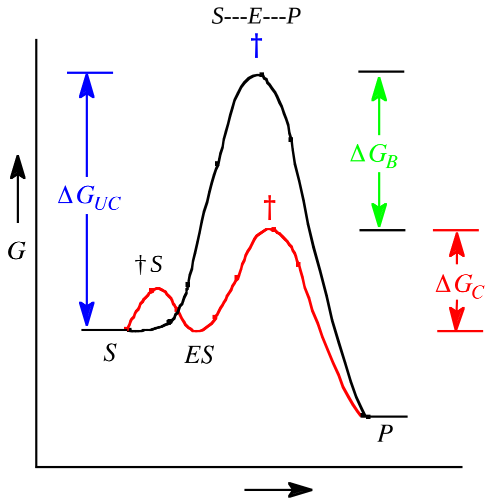
So this would slow the reaction!

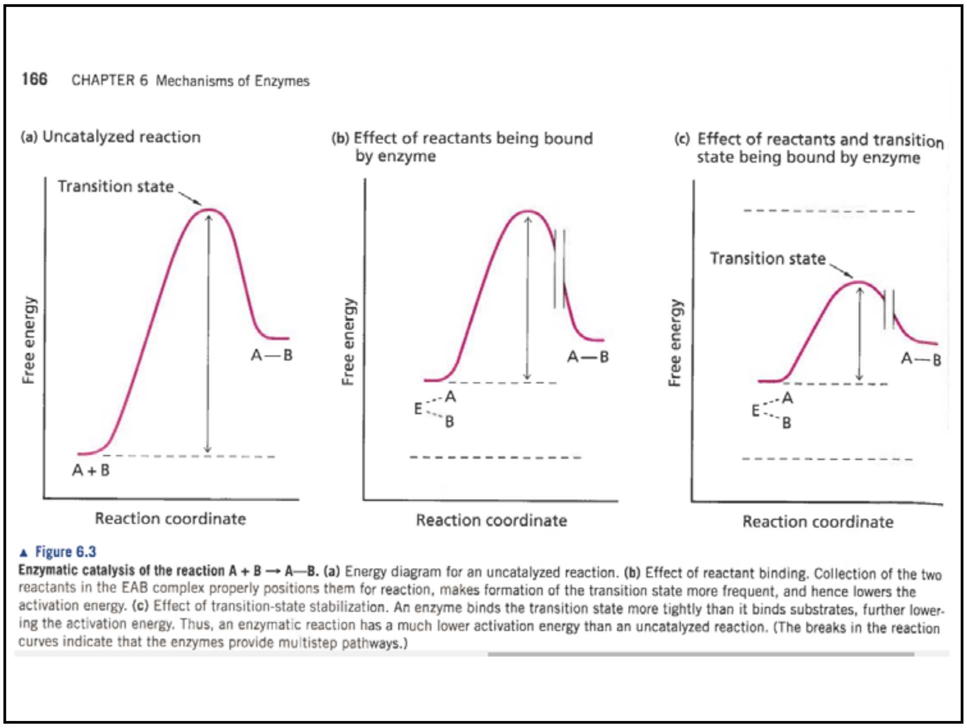


Instead, the E binds the **transition state of the substrate** tightly.

Formation of bonds between E and † releases **Binding Energy**, $\Delta G_B = G^{\dagger}_{uncat} - G^{\dagger}_{cat}$, which can be used to reduce $G^{\dagger}$.

$$\Delta G^{\dagger}_{cat} = \Delta G^{\dagger}_{uncat} - \Delta G_B$$

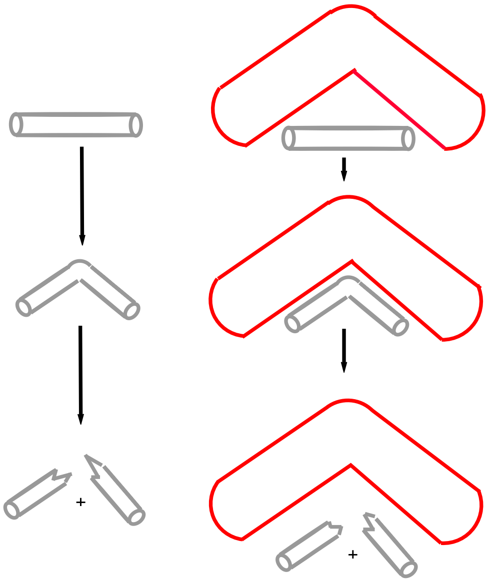




▲ **Figure 6.3**

Enzymatic catalysis of the reaction A + B → A—B. (a) Energy diagram for an uncatalyzed reaction. **(b)** Effect of reactant binding. Collection of the two reactants in the EAB complex properly positions them for reaction, makes formation of the transition state more frequent, and hence lowers the activation energy. **(c)** Effect of transition-state stabilization. An enzyme binds the transition state more tightly than it binds substrates, further lowering the activation energy. Thus, an enzymatic reaction has a much lower activation energy than an uncatalyzed reaction. (The breaks in the reaction curves indicate that the enzymes provide multistep pathways.)

Here is a mechanical analogy: The enzyme is a magnet.



The substrate is an iron rod.

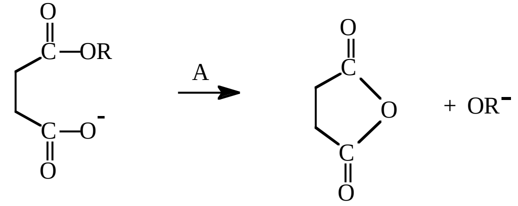
$^{\dagger}S$ = bent rod

Products are the rod fragments.

Binding Energy is also used for:

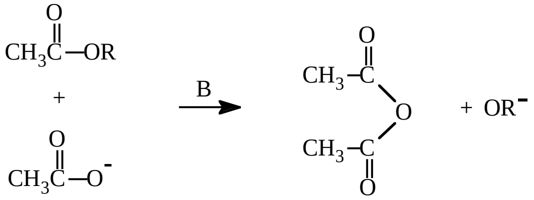
1. **Entropy Reduction**: enzymes hold the substrates close together in the proper orientation for reaction.

Low S



For *e.g.* reaction A is 10^5 times faster than reaction B.

High S



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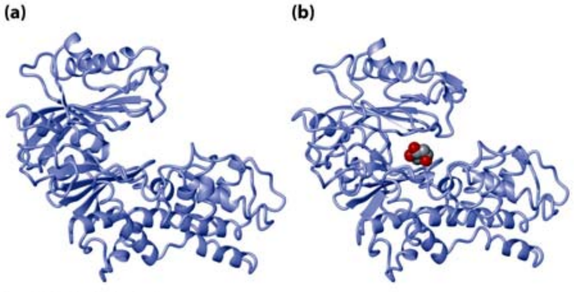
2. **Desolvation:** Substrate molecules are surrounded by a water hydration shell that usually must be removed for reactions to occur.

3. **Strain Reduction:** Substrate steric and/or electronic strain must be overcome.

4. **Induced Fit:** Change the conformation of the enzyme to obtain the proper orientation of the active site AA.

e.g. glucose changes the conformation of *hexokinase*.



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Enzymes usually also **participate** in the chemical transformation by their AA side-chains. Here is a classic example:

Chymotrypsin: General Acid-Base Catalysis

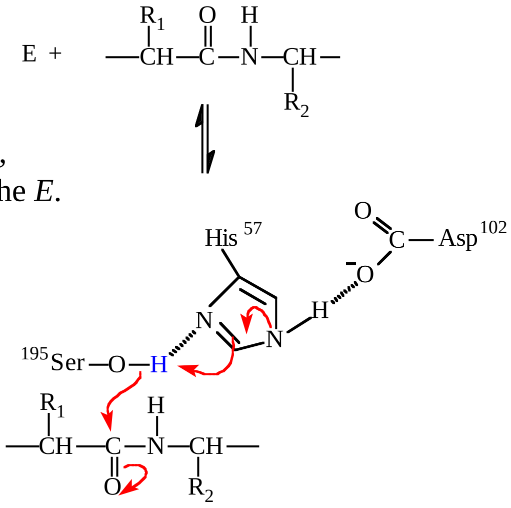
Acids: donate H^+ & accept electron pairs (Lewis).

Bases: accept H^+ & donate electron pairs (Lewis).

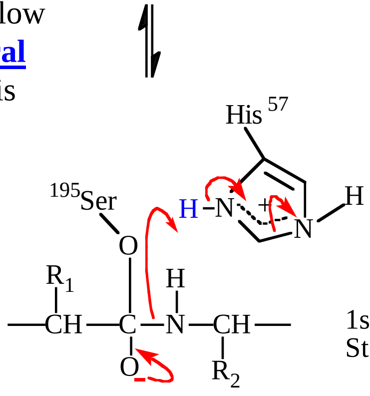
Recall that *chymotrypsin* is a proteolytic enzyme that severs peptide bonds at Trp, Tyr, Phe.

The side chains of His, Asp, Ser in the chymotrypsin active site form a **Catalytic Triad**.

In the first step,
the *S* binds to the *E*.



The 2nd step involves electron flow (arrows above) from the **General Base** Catalytic Triad into S. This creates the 1st transition state below:



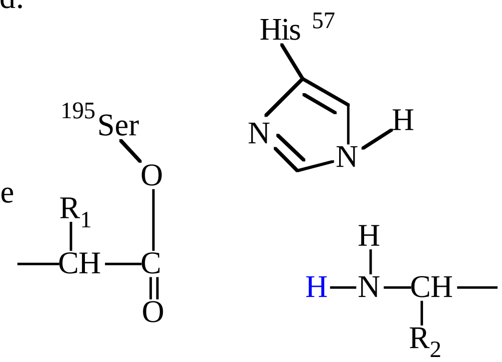
1st Transition State

The **oxyanion** (O^-) is stabilized by H-bonding to groups in the protein (not shown). Notice that a covalent bond has formed between the E and S.

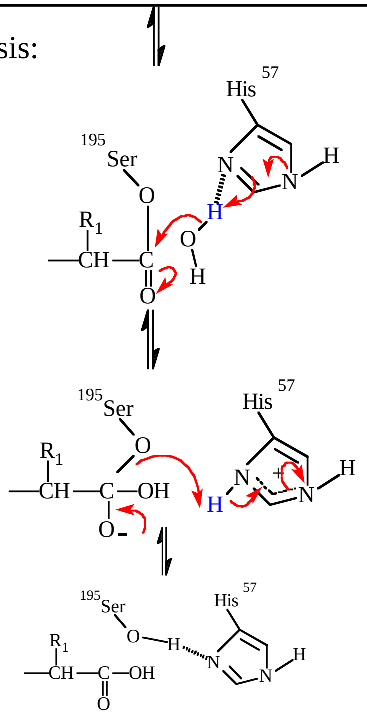
In the next step, electrons flow from the substrate to the **General Acid** Catalytic Triad (arrows above).

The C-terminal peptide is released:

Covalent Intermediate



The N-terminus is released by hydrolysis:



The mechanism is the same as in the first half of the reaction – general base catalysis followed by general acid catalysis.

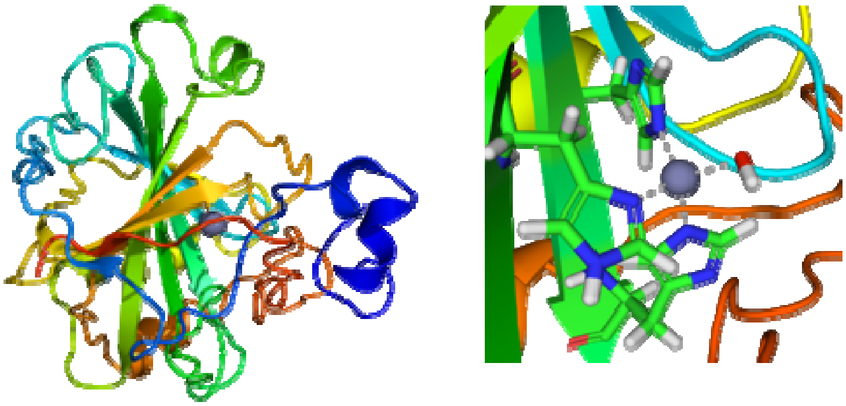
Go here to view an animation: http://www.angelo.edu/faculty/nflynn/Biochemistry/CT%20Catalytic%20Mechanism.htm

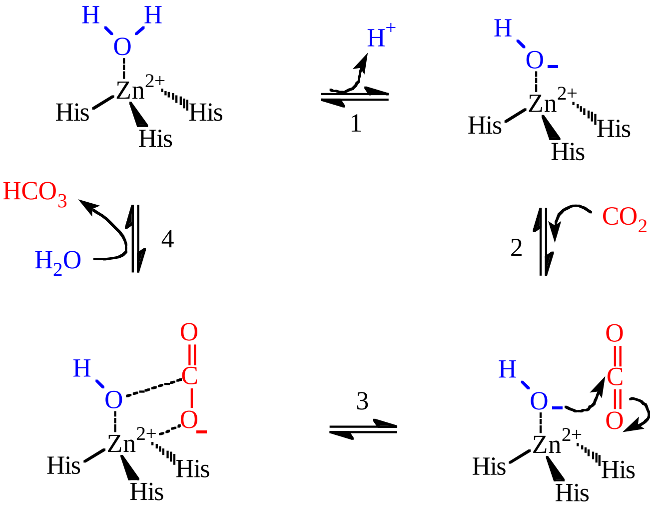
About 1/3 of all enzymes use metal cofactors.

1. Weak interactions between metals and the substrate help stabilize the charged † and may help orient and bind the substrate.

e.g. zinc in carbonic anhydrase

PDB 1CA2





2. Metals accept & donate electrons in Redox reactions:

$$Mn^{+2} + e^{-1} \rightleftharpoons Mn^{+1}$$