Chapter 11

Web Text Box 1

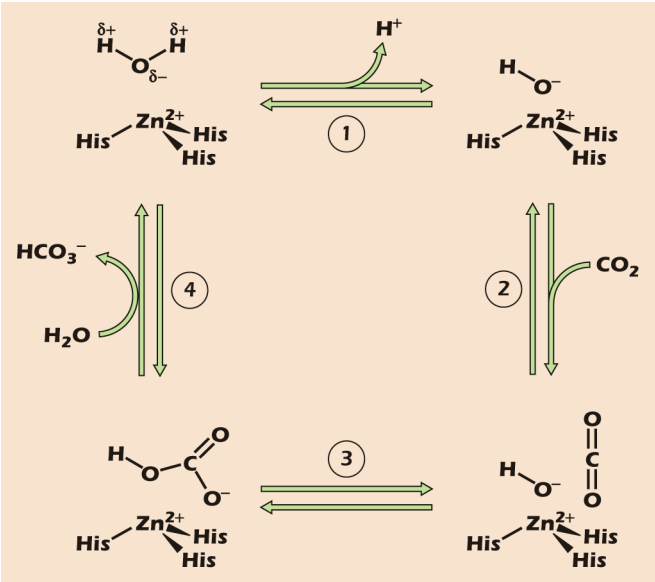
Enzyme catalytic strategies

We can get an idea of the catalytic effectiveness of an enzyme by comparing the rate of the reaction in the absence of the enzyme with the rate in its presence. One of the largest enhancements is achieved by the enzyme orotidylate decarboxylase. The reaction it catalyzes, part of nucleotide synthesis, would in the absence of the enzyme be half complete 78 million years after mixing the substrates. The enzyme enhances the rate by a factor of 1.4×10^{17}, so that it is half complete in a fraction of a second! Clearly it is important to understand just how enzymes achieve such remarkable enhancements and the problem has been approached from many different directions including structural studies, computer modeling, kinetics, inhibition, and site-directed mutagenesis (the last is a technique in which proteins are made with specific amino acid changes designed in, such as the position 222 methionine to alanine mutation in subtilisin described on book page 114). Each type of enzyme has its own mechanism but all utilize the binding of the substrate so that appropriate side chains and (if there is one) the cofactor are in the correct positions to carry out the reaction. The free energy of binding may drive changes in the protein structure that may even distort the shape of the substrate towards that of the product and hence make the transition state between substrate and product easier to achieve. Many enzymes have two substrates and in these cases they are bound in exactly the correct orientation for the reaction.

One strategy that enzymes employ is to allow the reactions to take place at the surface of a metal ion. Carbonic anhydrase carries out this comparatively simple reaction in which carbon dioxide is hydrated:

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

This reaction is vital in the body as part of the blood buffering system. The unanalyzed reaction is fast (half over in 5 seconds) but not fast enough. Carbonic anhydrase gives a rate enhancement of almost 8 million, which allows the blood to pick up CO_2 in body tissues in the short time available as it flows through. Carbonic anhydrase holds a zinc ion in a tetrahedral structure through bonds to three histidine residues in the polypeptide chain. The fourth side of the Zn^{2+} is bare and attracts a water molecule. This is the structure shown at the top left of the diagram.



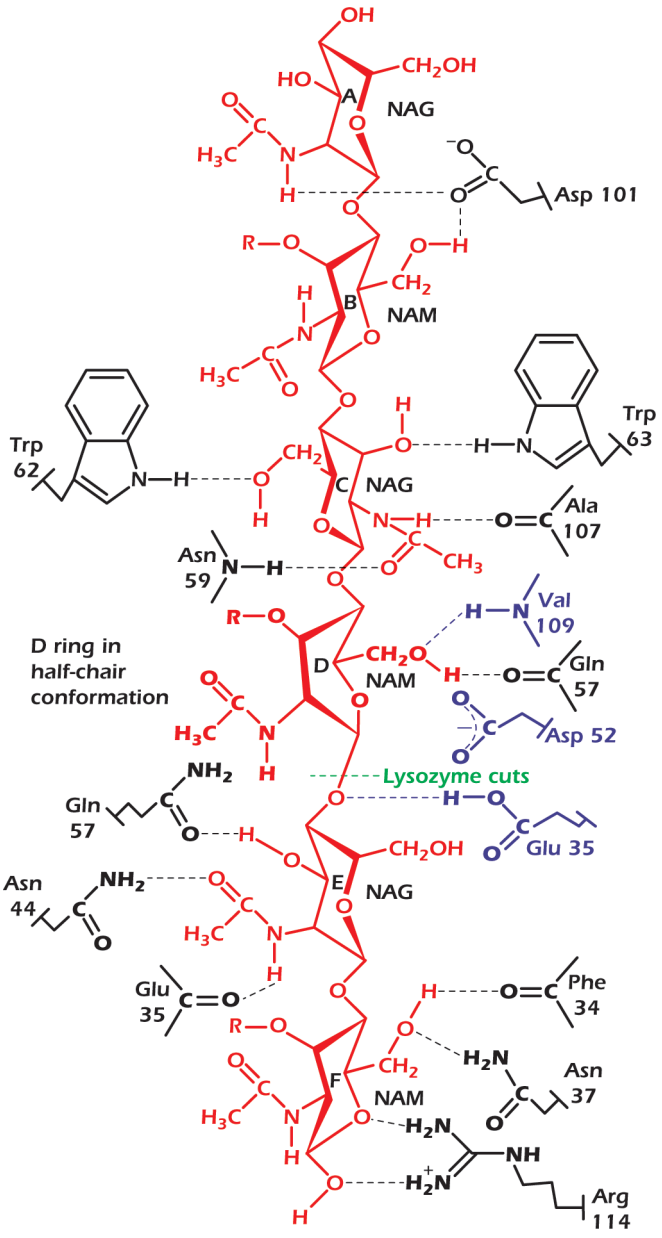
The positively charged zinc ion attracts the electrons in the water molecule, and there is a tendency for H^+ to leave: as usual, this statement is shorthand for saying that the H^+ is transferred to a water molecule (step 1 in the diagram). When a carbon dioxide molecule approaches it picks up the OH^- to form HCO_3^- (steps 2 and 3 in the diagram), which then leaves as another water molecule slips in to replace the lost OH^- (step 4). All the steps are easily reversible and run in the opposite direction when blood flowing through the lungs rapidly dumps its bicarbonate as CO_2 into the alveolar air.

The trick of using a zinc ion to persuade H_2O to become OH^- that then transfers to CO_2 has arisen independently at least three times in evolution. Metal ions are used as cofactors in many enzymes including alcohol dehydrogenase (book page 211) and nitrogenase (book page 222). This use of a metal ion in facilitating a reaction should be distinguished from the simpler use of an ion in forming bonds and therefore stabilizing a protein structure or location, as seen in zinc finger proteins (book page 149) and the association of protein kinase C with membrane phospholipid (see Web Text Box 7.2, A GFP chimera used to study protein translocation).

Lysozyme (book page 144) is an example of other strategies that are often employed by enzymes. First, it uses the free energy of the binding of the substrate to distort one of the substrate sugar rings and this helps to stabilize the reaction transition state, thus lowering the activation energy, and to precisely position amino acid residues in the correct environment to carry out the reaction. Second, the polypeptide chain itself participates in the reaction so that an intermediate, in which the protein is covalently bound to the substrate, is transiently formed.

The cell wall of some types of bacteria is made of long, strong covalent chains of polysaccharide and peptide. The sugars in these chains are two modified glucoses: N acetyl glucosamine (Figure 2.11 on book page 27) and N acetyl muramic acid. Lysozyme hydrolyses the β glycosidic bond between the two glucose residues. A six sugar length of the chain slots into a cleft in the enzyme. This can be seen in three dimensions on the protein data bank site, which shows the complex immediately after the bond has been broken, with the four-sugar and two-sugar fragments still held in the cleft (see http://www.rcsb.org/pdb/explore/explore.do?structureId=1LZS). The binding involves many hydrogen bonds as well as hydrophobic interactions and is therefore thermodynamically favored, even though it distorts one of the sugar rings. The bonds are shown in the diagram below. Six residues of the polysaccharide are bound; the bond to be cleaved lies between residues D and E. Note the extensive interactions the substrate makes with the protein. Sugar rings A, B, C, E and F are shown in the “chair” conformation; this is a more accurate representation of their favored structure than the

simple hexagons we use in the book figures. The ring of the D residue is distorted, making what is normally a very stable bond to residue E liable to attack. The β glycosidic bond is hydrolyzed and the two separate lengths of chain released.



The chemical reactions involved are known in some detail. The active site has an aspartic acid side chain and a glutamic acid side chain positioned near the bond to be cleaved. The aspartic acid carboxyl group is in a polar region and so will have lost its hydrogen ion. The glutamate, however, is buried in a hydrophobic area and will hold onto its hydrogen ion. The binding of the polypeptide chain causes the cleft to close slightly and distorts the N acetyl muramic acid (NAM) ring. The glutamic acid then transfers its hydrogen ion to the oxygen of the bond to be cleaved making a positively charged transition state (an oxonium ion) that is stabilized by the negative charge on the aspartic acid and by the distortion of the NAM sugar ring. The aspartic acid carboxyl then transiently forms an ester link with the NAM sugar (which relaxes to its undistorted shape) as water adds and part of the product (the chain ending in N acetyl glucosamine) is released. An enzyme: sugar intermediate is left that very rapidly breaks down with the addition of water, thus releasing the other part of the product (the chain ending in NAM) and regenerating the enzyme.

For more detail see Voet & Voet. 2010. Biochemistry 4th edition. John Wiley & Sons. pp 517-525.