

Highlights Enzymes IV

1. Chemicals, such as DIPF and iodoacetate, covalently (and irreversibly) bind to the side chains of specific amino acids (serine and cysteine, respectively) and if these side chains are essential for the catalytic action of the enzyme, the enzyme will not catalyze reactions after being treated with these chemicals.

2. Penicillin is a substance that resembles the substrate of an enzyme in bacteria that helps make the bacterial cell wall. When it binds to the enzyme, it inactivates the enzyme by covalently bonding to the active site, thus destroying the enzyme (and killing the bacterium containing it). An inhibitor of this type is known as a suicide inhibitor.

Highlights Catalytic Mechanisms

1. Proteases catalyze the hydrolysis of peptide bonds in polypeptides. They are usually fairly specific for certain amino acids and cut at or near those amino acids.

2. Chymotrypsin is a protease whose activity has been closely studied. Conveniently, the activity of chymotrypsin can be studied using an artificial substrate which, when cleaved by the enzyme, releases a yellow product.

3. When the release of the colored substrate by the enzyme is studied, it appears to occur in two different rates. First there is a VERY rapid release of the colored substrate. After that initial burst of activity, the remaining yellow color is released slowly.

4. The reason appears to be that the reaction catalyzed occurs in two steps. The first step cleaves the bond to produce the yellow product, which is rapidly released. The other product of this reaction is the remainder of the substrate that is covalently linked to the enzyme. In order for the enzyme to bind another substrate molecule and release more yellow color, it must first release the covalently bound molecule. This step occurs slowly and explains why subsequent yellow molecules are released slowly - after the initial one is released, the enzyme must remove the covalently bound molecule, bind a new substrate, and cut the substrate and the continue the process repeatedly.

5. Chymotrypsin is an example of a protease that employs reactive serine in its active site. Such an enzyme is called a serine protease. Treatment of chymotrypsin with DIPF, which covalently links to serines, inactivates the enzyme.

6. Serine proteases form covalent intermediates with their polypeptide substrates. The first step involves nucleophilic attack of an alkoxide ion on the polypeptide substrate to form an acyl-enzyme intermediate. Formation of this intermediate results in cleavage of the peptide bond and release of the first polypeptide fragment. The acyl-enzyme intermediate is resolved by addition of water to release the other portion of the original polypeptide along with regeneration of the original enzyme active site. This last step occurs relatively slowly.

7. In the active site of chymotrypsin (and other serine proteases) is a so-called catalytic triad of amino acids that includes a serine hydrogen bonded to a histidine. The histidine is, in turn, hydrogen bonded to an aspartic acid residue in the active site. Each of the hydrogen bonds of the catalytic triad is important in the catalytic

mechanism. The nucleophilic alkoxide ion on serine is made possible ultimately by interactions in the catalytic triad and these hydrogen bonds.

8. The catalytic triad is not unique to chymotrypsin. The serine protease known as subtilisin also has the same catalytic triad and employs a similar mechanism.

9. Besides the catalytic triad, the enzyme has two other important sites to consider. The first is the oxyanion hole that stabilizes a tetrahedral intermediate that arises during the catalysis. The second, called as S1 pocket, is where the substrate binds. Both the oxyanion hole and the S1 pocket are adjacent to the active site (catalytic triad).