

## **LECTURE #5, 9/6/00**

### **ENZYME CATALYZED REACTIONS**

**Why do cells need enzymes?**

**Engineers run rxns at high T and P**

**Cells must run rxns at moderate T and P.**

**Also, many chemicals exist that the cell must manufacture or decompose.**

**Reactions must be specific!!!**

### **A LITTLE HISTORY**

**1897: Buchner extracted first active enzyme from living cells**

**Showed that they are active outside of cell**

**Initiated isolation of pure enzyme.**

**Accomplished by Sumner 1926**

**Found that enzymes can be used in industrial processes to form valuable products.**

**> 2000 enzymes known today!!!**

**Enzymes Are Used Two Ways:**

**Purified form (separated from cell)  
Free in reactor or immobilized**

**Used in microbial, animal, or plant cells  
Disadvantages:**

- **A high amt. of substrate is converted to biomass.**
- **Wasteful side reactions.**
- **Condition for growth of micro. may not be the same as for the product.**
- **Isolation and purification of product**

## **Differences Between Enzymes and Catalysts**

- 1. Synthetic catalysts aren't as specific. (i.e. will catalyze similar reactions involving different reactants)**

**Many enzymes catalyze one reaction involving one substrate**

**Due to 3D folding and formation of active sites.**

**Some enzymes are nonspecific like catalysts.**

**Specificity can be a drawback.**

**Each rxn requires a different enzyme**

**Enzymes have little development potential for other reactions.**

- 2. Enzymes frequently need cofactors**  
**Combines with inactive protein (apoenzyme) to form active complex (holoenzyme)**

**Simplest cofactors: metal ions Table 3.2**

**Complex cofactors: coenzymes  
(nucleotide derivatives)**

**Cofactors weakly bind to enzyme**

**3. Enzymes are more fragile - can lose activity easier.**

**Lose at high temp. where catalysts become more active.**

**Synthetic catalysts lose activity but not as fast.**

**4. Enzymes in general are more active - allow rxn to go faster.**

**Turnover #'s (# of substrate molecules reacted/site/second)**

$$3 \times 10^{-3} - 6 \times 10^5$$

**Synthetic catalysts - high temp.**

$$25 \text{ C: } 3 \times 10^{-8}$$

$$420 \text{ C: } 2 \times 10^4$$

**5. Enzymes are subject to cellular control. Genetic control can determine rate of synthesis and final cellular concentration.**

**Produce mutants that produce large amounts of enzymes.**

**Most industrial enzymes are extracellular**

**Intracellular enzymes are becoming more common**

**Examples:**

**Glucose oxidase - diagnostic tool for diabetes.**

**Asparaginase - Cancer therapy**

**Penicillinase - Converts penicillin to pencilloic acid.**

**A History Lesson: Sales of industrial enzymes was small until 1965 when enzymes in detergents became popular (improve washing results)**

**Massive increase in production until workers started showing allergic symptoms.**

**Taken out of detergents. But then started to take precautions and encapsulating enzymes before reaching the factory.**

**Costs are falling - 20 to 35% cheaper than in the mid 70's.**

**Specialized enzymes at high purities are still very costly.**

**Most enzyme kinetics assume pure enzymes. Not always the case in industrial processes.**

**What are enzymes used for: (Early 1990's)**

**55% used for food applications**

**40% industrial applications (detergent, paper, leather)**

**5% animal feed/agriculture**

**Now more enzymes are used for pharmaceuticals, analytical purposes, and medical research.**

## Nomenclature of Enzymes:

**Named for what it does [suffix "ase" is added to substrate name or to reaction which is catalyzed.]**

**Ex: urease: catalyzes urea decomposition  
alcohol dehydrogenase: catalyzes the  
dehydrogenation of alcohol.**

**Exceptions: trypsin (hydrolyzes peptide bonds  
of proteins)  
rennin (cheese making)  
old yellow (causes apples to brown)**

## Classification of Enzymes:

**Commission of Enzymes of the International  
Union of Biochemistry  
See Table 3.1**

**EC 1.1.4 (First # represents classification, other  
# represent subclasses which take into account  
coenzymes, type of bond etc.).**

**Some enzymes don't fit into classification:  
Trypsin. So, some have come up with other  
classifications (e.g. proteases).**

**Some enzymes have more than one name**  
**Citrate oxaloacetate lyase**  
**citrate lyase**  
**citrase**

**Another note about names of enzymes;**

**Some enzymes will have same name but come from different source: Could have different amino acid sequence, different properties, and different activity.**

**Example: Glucose isomerase: catalyze isomerization of glucose to fructose.**

**Glucose isomerase (Bacillus coagulans)**  
**Other micro. also synthesize enzymes that catalyze this rxn and are called glucose isomerases.**

**Catalytic activity is different. e.g. one above requires cofactor. Other do not.**

## Hydrolytic Enzymes

Most common type of enzyme

Catalyze hydrolysis reactions

Classified according to bond hydrolyzed:

- **Esterases:** hydrolyze ester bonds  
Common ester bond - binds fatty acids to glycerol to form fats (Enzyme - Lipase)  
  
Pectinases: Decay fruit. Extraction of fruit juices.
- **Carbohydrases:** bonds between sugar molec. (polysacc. to disacc. to monosacc)

Amylase: Common carbohydrase

2 types:  $\alpha$ -amylase,  $\beta$ -amylase  
Both act on starch

Remember starch: amylose (str. chain)  
amylopectin (branched)

**Amylopectin is more soluble and responsible for high viscosity of starch soln.**

**alpha-amylase: acts on amylopectin  
Reduces viscosity (starch-liquefying enzyme) - starch is then used for other purposes**

**beta-amylase: acts on amylose**

### **Hydrolysis of Cellulose**

**Use cellulase - Obtain cellobiose (2 glucose)**

**Cellulase is a complex mixture of several different enzymes. Types depend on source of cellulase and on method of production.**

- **Proteases: Nitrogen bonds (bonds betwn amino acids to form proteins)  
(Proteins to polypeptides to amino acids)**

**Interesting: Enzyme (protein) is breaking down proteins!!!**

**Usually synthesized inactive and then transported from site of synthesis to site of**

**desired activity - Activated by cofactor or coenzyme**

**Ex: Pepsin, Trypsin, Chymotrypsin, Carboxypeptidase)**

**Most hydrolytic enzymes are extracellular, Some are intracellular**

### **Enzyme Mixtures**

**Very common: Provides better success**

**Can be of same type (all carbohydrases)**

**or of different type (proteases, esterases)**

**Example: Detergent (Made up of amylase and proteases to remove stains more effectively)**

**Medical Applications of Hydrolytic Enzymes are becoming more important.**

**Trypsin: anti-inflammatory agent**

**Lysozyme: antibacterial agent**

**Urokinase: Dissolving and preventing clots**

**Asparaginase: Anticancer agent**

**Penicillinase: Hydrolyzes penicillin. Used to treat allergic reactions to penicillin.**

**Also used for diagnosis of some diseases. Initial stages of disease give rise to elevated or depressed levels of enzyme conc. in body fluids. Can test for enzyme to determine if disease is present.**

**Nonhydrolytic Enzymes are not as common**

**Good Reference on Enzymes: Biochemical Engineering and Biotech. Handbook , Atkinson, and Mavituna, 1991, Stockton Press.**

## **PROCESS FOR OBTAINING ENZYMES**

### **3 general methods**

- 1. Leave enzyme in cell - put reactants in with cell and take out products. Cell does all the work.**
- 2. Take enzyme out of cell**
- 3. Synthetic enzymes**

**Altering existing enzymes  
Building from scratch**

## **Enzymes From Cells**

- 1. Grow microorganisms - details later**
- 2. Obtain enzyme from cell**
  - Intracellular - break cell**
  - Extracellular - filtration or centrifugation (enzyme extract)**
- 3. If intracellular, end up with cell fragments and soluble component (enzyme extract). - Separate by Filtration.**
- 4. Enzyme extract : other enzymes + biochemicals**
  - Must purify enzyme**
    - I. Ionic separation:**
      - a. Fractional separation (decr. solubility and cause precipitation).**
        - pH to isoelectric pH (net charge is zero)**
        - proteins have small solubility at isoelectric pH**
      - Change T to denature protein (this will decrease solubility)**

**Salting out: Salts form ions with ionized groups on proteins and decr. solubility  
Solvents also used that raise chemical potential of protein and hence, reduce solubility**

**b. Electrophoresis**

**Electric field and protein will separate  
(Can run at isoelectric pH of protein)  
Prob.: soln will heat up due to energy**

**c. Ion Exchange**

**+ charged proteins will stick if packing is negative. visa versa**

**To remove: change pH or ionic strength.  
Weakly bound proteins will detach first.**

**II. Separation by Adsorption**

**a. Physical adsorption:**

**Proteins have varying tendencies to stick to various materials such as starch, polyacrylamide gel**

**Weak forces (e.g. Van der Waal)**

**Affinity depends on equil. partition coef. of enzyme for particular adsorbent - have been measured.**

- b. Affinity chromatography: Similar Chemically bind species to material that has high affinity for enzyme.**

**e.g. Coat polyacrylamide beads with inhibitor of enzyme. E & I will want to bind. Then separate complex.**

### **III Separation By Size**

- a. Gel permeation chromatography:  
Pack column with gel particles with specific diameter.  
Molec. with diameters larger than this size can't diffuse into gel and will rapidly pass thru column.**

**Smaller molec. will permeate gel and move more slowly.**

- b. Ultrafiltration: Using membrane to separate different size species.**

**c. Ultracentrifugation: Centrifuge at a certain speed to separate particles. Can determine speed needed for certain size particles.**

**Actual separation usually involves using many of these procedures.**

**Your process so far:**

**Microbe or animal cell involved**

**Enzyme involved (intracellular, extracellular)**

**Classification of enzyme**

**Reaction mechanism**

**Separation and purification procedure**

**Company selling enzyme (Cost??)**

## **HOW DO ENZYMES WORK???**

**Two theories:**

### **1. Lock and Key Analogy:**

**Enzyme structure gives a specific site that is complementary to the substrate.**

**Site is called the active site.**

**Explains specificity of enzyme but does not explain transition-state stabilization.**

### **2. Induced Fit Theory**

**Takes into account flexibility of proteins (bonds are weak so proteins have flexibility).**

**Binding causes conformation of the enzyme: close fit obtained and hence, leads to stability.**

**Active site has 2 parts:**

- 1. Site that recognizes and binds substrate**
- 2. Site that catalyzes the reaction once substrate binds.**

**These two sites (amino acids) don't have to be adjacent in linear sequence but are brought in close proximity during folding.**

**Substrate binds by weak forces.**

**Amount of enzyme used in a reaction is given in "units".**

**Unit: Unit of activity: amount of enzyme which gives a certain amount of activity under a prescribed set of conditions.**

**Different for different enzymes and reactions. It is important to specify what "unit" means for each enzyme and each reaction.**

