10.542 – Biochemical Engineering
Spring 2005

Applications of Enzyme Catalysis – Biocatalysis

- **Working definition** – the use of an enzyme-catalyzed reaction to convert a single starting compound to a single product
    - distinguished from the use of whole cells for multi-step synthetic pathways, which also use enzymes to catalyze each conversion

Why use enzyme catalysis over traditional (usu. solvent-based) organic synthesis?

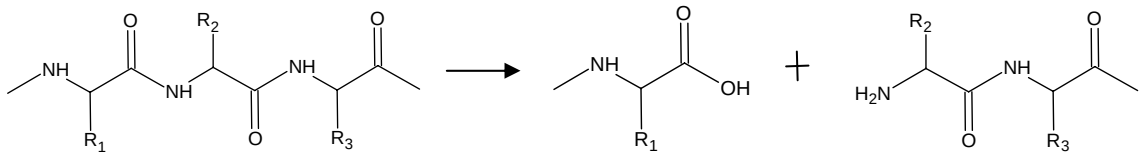
(Rozzell, J. D. "Commercial scale biocatalysis: myths and realities." *Bioorg Med Chem* 7, no. 10 (October 1999): 2253-61.)

- Catalyst selectivity/specificity
- Mild reaction conditions
- Environmentally friendly, "green chemistry"
- High catalytic efficiency
- ➢ *Greatest interest industrially is for production of chiral compounds* (see handout for examples)

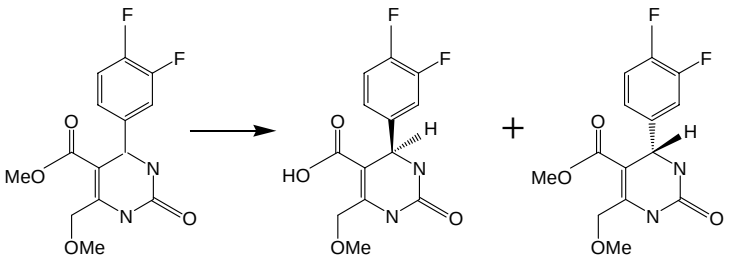
Substrate selectivity versus substrate range

- Industrial emphasis on selectivity is most often in the context of *stereo*selectivity, *i.e.*, selective conversion or production of one enantiomer, but
- The best industrial enzymes will have a broad substrate range, *i.e.*, the ability the catalyze the same type of reaction (attack the same functional group) with a variety of substrates.

Example – Subtilisin (serine protease)
Natural reaction: peptide bond hydrolysis, though activity against esters was known



Unnatural reaction: resolution of a racemic ester mixture for production of a pharmaceutical intermediate (Courtesy of Merck & Co. Used with permission.)



DHP Methyl Ester R-DHP Acid S-DHP Methyl Ester

See “Survey of Biocatalytic Reactions” handout for additional examples.

Quantifying enantioselectivity (stereoselectivity)

- The “enantiomeric excess (EE)” is determined relative to one enantiomer.

$$EE(S) = \frac{S - R}{S + R} \times 100\%$$

- Range of values are from -100% (all undesired enantiomer) to +100% (all desired enantiomer). A racemic mixture has an EE of ‘0’ for both enantiomers.
- Typical EE target is >95%, or >97.5% yield of the desired enantiomer.

Selecting an appropriate catalyst

- Typically, more than one enzyme will perform a desired conversion (species diversity)
- Derivation of rate equations is not efficient as a means of evaluating catalyst performance in a screen
- Determine the following instead, from a set of near-identical reactions (catalyst is variable), fixed volume, fixed time:

(1) $Conversion = X = \frac{S_0 - S_f}{S_0} \times 100\% = \frac{\sum P_{i(usu.R,S)}}{S_0} \times 100\%$

(2) $Yield_{P_i} = \frac{P_i}{S_0} \times 100\%$ for each product, and if the products are chiral,

(3) $EE(R) = \frac{R - S}{R + S} \times 100\%$

- Conversion is the proxy for a reaction rate.
- For a “decent” conversion, EE should be most important factor
  - Process development could improve conversion (How do you increase V_{max}?)
  - EE is more often an inherent property of the enzyme.
- Conversion vs yield = substrate consumed vs product obtained
  - distinction is important for reactions in which multiple products are made, including enantiomers and by-products