Biocatalysis

Introduction

Biotransformations is a novel trend in organic chemistry also known under the synonym of green chemistry
Together with the novel trend of microwaves in chemical synthesis

Until now you have been introduced to all kinds of different aspects of reaction and chemical engineering
Bioreactors and chemical reactors and how to model them
How do we introduce Biocatalysis into this

Bioreactors and bioreactor modelling

Takes into account all aspects of reactors
Treats the biological material as a black box.
Models microorganisms only as Biomass without any further details

Chemical reactors

Takes the reactor and the chemical reaction into account.

Biocatalysis

Biocatalysis

Uses all the reactors and reactions as in bioreactors and chemical reactors, but also takes into accounnt that the catalyst is a biomolecule, protein.

Common prejudices against enzymes in organic chemistry

- Enzymes are sensitive
  - True for most enzymes, temperature sensitivity of proteins in water and also in organic solvents.
  - Some enzymes can be very stable and tolerate temperatures above 100 deg.C. Also in org. solvents

- Enzymes are expensive
  - True for some, but if they are produced for technical applications they are available at reasonable prices.
  - Enzymes can be reused when immobilised.
  - Immobilisation is a very good means of stabilisation inn org. Solvents.
  - Most chemical reactions can be done with crude enzyme preparations

Biocatalysis

- Enzymes are only active on their native substrates:
  - Is true for a few enzymes
  - Is definitely not true for the majority of enzymes
  - Biocatalysis started by the exploitation of the general dogma of biochemistry
  - Enzymes are natures catalysts developed during evolution for the regulation of metabolic pathways.
  - Problem was that man made organic compounds could not be substrates
  - Once this fact was proven wrong, enzymes are no longer regarded to work only on natures substrrates, but also on unnatural substrates and their substrate definition has been widened
  - General trend: the more complex te enzymes mechanism the narrower the limit for the acceptability of foreign substrates.
  - There are a lot of enzymes, where the natural substrate is unknown

Biocatalysis

- Enzymes work only in their natural environment

  Generally true for enzymes that they display their highest catalytic power in water
  This is something of a nightmare for an organic chemist, if ti si the solvent of choice.
  Biocatalysts can function in organic solvents
  The key rules have been established only a decade ago
  Even the lowered activity of an enzyme in organic solvents can account for the efficiency increase of the reactions.

Biocatalysis

Advantages and disadvantages of Biocatalysts

Advantages of Biocatalysts

- Enzymes are very efficient catalysts
  - Enzyme mediated procecsses are accelerated by a facor 10^8-10^{10}, compared to non enzyme mediated processes
  - Enzymes are more effective catalysts by a few orders of magnitude
- Enzymes are environmentally acceptable
  - Enzymes are environmentally friendly, because they are fully biodegradable, heavy metals are not environmentally friendly
- Enzymes act under mild conditions
  - Enzymes are active in a pH range between 5-8, and 20-40 deg C
  - This minimizes problems with side reactions, such as
  - Decomposition
  - Isomerisation
  - Racemisation
  - Rearrangement
  - These are often problematic in classical synthesis

Biocatalysis

- Enzymes are commpatible with each other
  - Enzymes generally work under simmilar or same conditions, several biocatalytic reactions caan be carried out in a reaction cascade in one flask.
  - Sequential reeactions can be carried out using multienzyme systems
  - This simplifies reaction processes, and unstable intermediates do not necessarily be isolated.
  - Unfavorable equilibria can be shifted towards the products.

- Enzymes are not bound to their natural role
  - Enzymes do accept a large variety of natural and unnatural substrates,
  - They do not need to work in water
  - Replacing the aqueous medium by organic solvents can be advantageous

- Enzymes can catalyze a broad spectrum of reactions
  - All catlysts only accelerate a reaction but they have no impact on the position of the thermodynamic equilibrium
  - Some reactrions can be run in both directions

Biocatalysis

- Hydrolysis-synthesis of
- Esters
- Amides
- Lactones
- Lactams
- Ethers
- Acid anhydrides
- Epoxides
- Nitriles

- Redox reactions
- Addition-elimination
- Halogenation-dehalogenation .....

Biocatalysis

- Enzymes display three major types of selectivities:

- Chemoselectivity
  - Enzymes are designed to work only on one substrate, therefore laborious purification due to side reactions is not necessary
- Regioselectivity and Diastereoselectivity
  - Due to their complex 3D structure enzymes may distinguish between functional groups which are chemically situated in different regions f the same substrate molecules.

- Enantioselectivity
  - Due to the L-aminoacids in all proteins, they are chiral catalysts.
  - Any kind of chirality in the substrate molecule is thus recognised.
  - Due to this one achieves kinetic resolution in the reaction.
  - These features are explored for selective and asymmetric reactions.

Biocatalysis

Advantages and disadvantages of Biocatalysts

Disadvantages of Biocatalysts

- Enzymes are provided by nature in only one enantiomeric form

  No way of inverting the amino acids in a protein.
  Sometimes an enzyme can be found with exactly the opposite stereochemical selectivity
- Enzymes require narrow operation parameters

  - Enzymes are operating within their activity limits and these can not exceeded easily (t, pH, salt concenration)
- Enzymes display heir highest catalytic activity in water

  - Due to its physical properties water is the worst solvent, organic compounds ae not very soluble in water
  - Shift to organic solvents from water costs one order of magnitude in enzyme activity

Biocatalysis

- Enzymes are bound to their natural cofactors
  - Cofactors are not very stable compounds,
  - Are excessively expensive when used stoichiometrically
  - Can not be substituted by cheaper non natural molecules
  - Recycling of cofactors is problematic

- Enzymes are prone to inhibition phenomena
  - Enzymes show often substrate or product inhibition
  - Substrate inhibition can be circumvented easily by aadding substrate dropwise and keeping the substrate concentration low throughout the reaction
  - Product is not so easily removed during the reaction

- Enzymes may cause allergies
  - When handled with care, they are not problematic. Removal of enzymes fromm the product is not problematic and can be achieved easily

Biocatalysis

Two main strategies in applying enzymes in biotransformations

Isolated enzymes Versus Whole Cell Systems

The physical state of biocatalysts wich are used for biotransformations can be very diverse.

Isolated, crude purified, pure enzymes or whole microorganisms
Either in free or immobilised form depends on many factors:

i)The type of reaction
ii)Whether there are cofactors to be recycled
iii)The scale in which the biotransformation has to be performed.

Biocatalysis

Table 1.1. Pros and cons of using isolated enzymes and whole cell systems

Biocatalyst	Form	Pros	Cons
isolated enzymes	any	simple apparatus, simple workup, better productivity due to higher concentration tolerance	cofactor recycling necessary
	dissolved in water	high enzyme activities	side reactions possible, lipophilic substrates insoluble, workup requires extraction
	suspended in organic solvents	easy to perform, easy workup, lipophilic substrates soluble, enzyme recovery easy	reduced activities
	immobilized	enzyme recovery easy	loss of activity during immobilization

Biocatalysis

whole cells	any	no cofactor recycling necessary	expensive equipment, tedious workup due to large volumes, low productivity due to lower concentration tolerance, low tolerance of organic solvents, side reactions likely due to uncontrolled metabolism
	growing culture	higher activities	large biomass, more by-products, process control difficult
	resting cells	workup easier, fewer byproducts	lower activities
	immobilized cells	cell re-use possible	lower activities

Biocatalysis

Fermantation together with biochemical and genetic engineering has led to a huge number of specialty chemicals based on cheap substrate molecules. They constitute de novo synthesis in a biological sense.

Microbially mediated biotransformations

often start from complex organic molecules

Uses only a single or few biochemical synthetic steps by using the microbes enzymatic potential to convert a non natural substrate into the desired product.

This process is called enzymation

Biocatalysis

Table 1.2. Characteristics of 'enzymation' and 'fermentation'

	Enzymation	Fermentation
microorganism	resting cells	growing cells
reaction type	short, catalytic	long, life process
number of reaction steps	few	many
number of enzymes active	few	many
starting material	substrate	C + N source
product	natural or nonnatural	only natural
concentration tolerance	high	low
product isolation	easy	tedious
by-products	few	many

Biocatalysis

Proteins are delicate and soft 3D structures.
Proteins are instrinsically unstable in solution and can be deactivated by denaturation

Types of reactions that lead to enzyme deactivation:

Rearrangement of peptide chains:

Unfolding typically starts at around 40-50 degrees.
Process is typically reversible and thus reelatively harmless

Hydrolysis of peptide bonds in the backbone:

in particular adjacent to asparagine, occurs at higher temperatures.
Functional groups can be hydrolysed (ASN or GLN residues)
→ ASP, GLU residues
These reactions are associated with irreversible rearrangements.

Biocatalysis

Thiol groups:

S-S group interchange will modify the folding pattern and thus lead to an inactive form of the protein.

Elimination and oxidation reactions:

often involving cysteines cause teh final destruction of the protein

Thermostable enzymes often differ only vey slightly form their mesophilic counterparts. They possess less ASN, GLN residues and more salt bridges and disulfide bonds.

AS a general rule increased thermostability leads to higher tolerance for organic solvents

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Mechanistic aspects:

Lock and key mechanism
Induced fit mechanism

Desolvation and solvation substitution theory

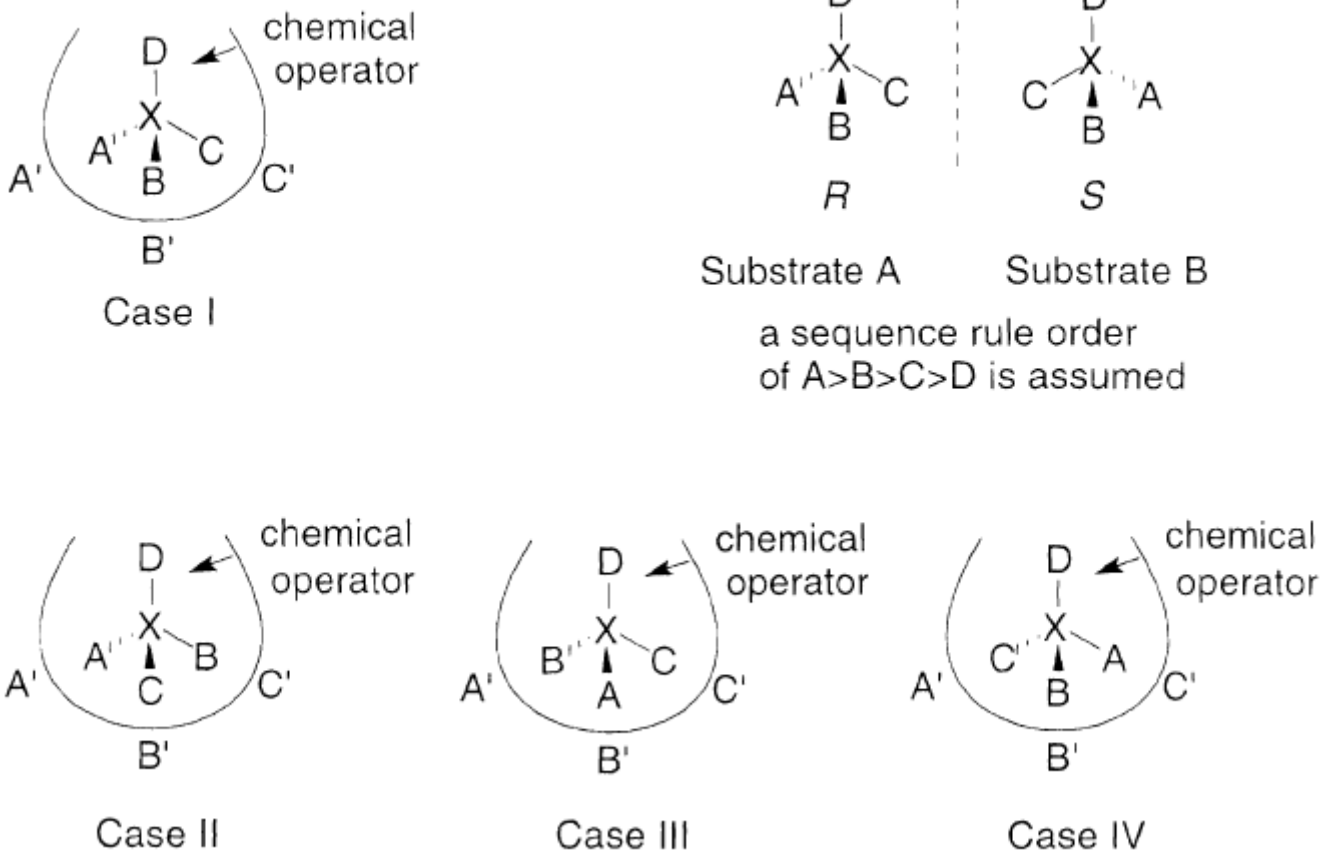
This theory takes into account that water is replaced by the substrate molecule, thus no solvent is present during the reaction and the partners are reacting in a quasi gas phase with each other.
If the reation is promoted by a desolvation or solvation substitution mechanism it has to involve a tight binding of the substrate to the active site, leading to a maximum entropy change.

Three-Point attachment rule

Chirality is a quality of space, meaning that a substrate moelcule to be reecognised needs to be attached firmly at 3 points.

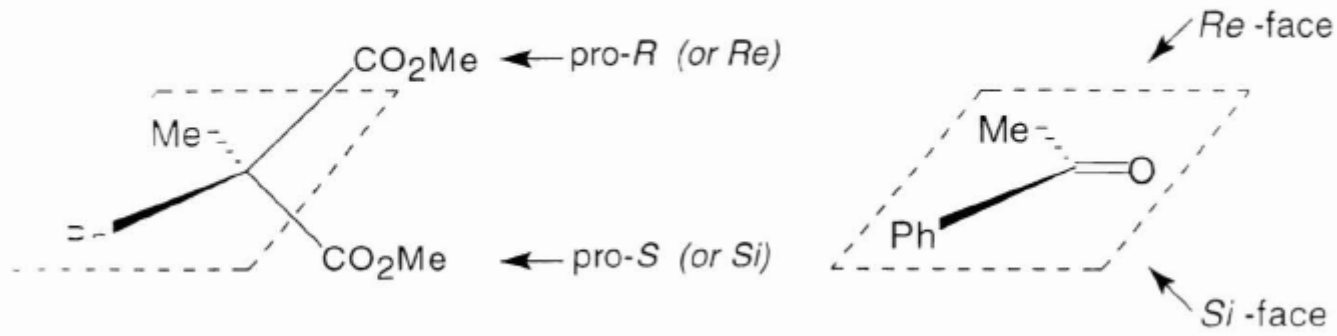
Biocatalysis

Figure 1.3. Schematic representation of enzymatic enantiomer discrimination



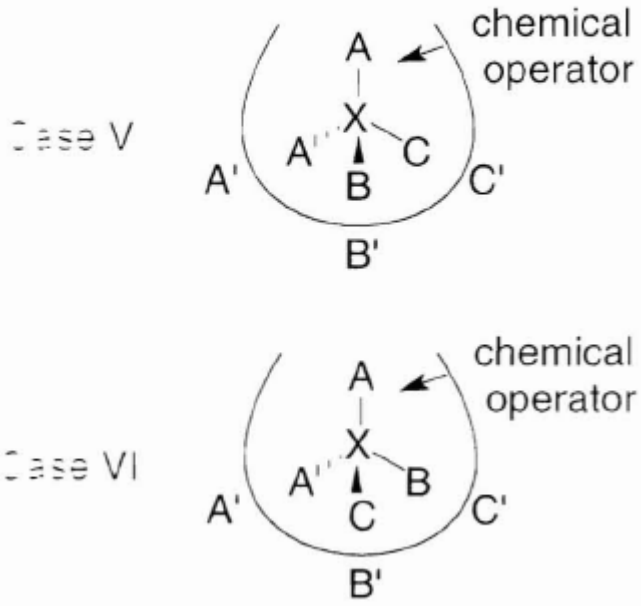
Biocatalysis

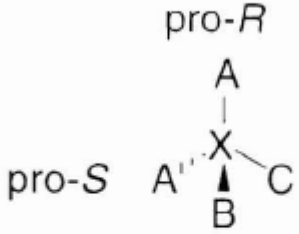
Scheme 1.3. Enantiotopos and -face nomenclature



Biocatalysis

Figure **1.4.** Schematic representation of enzymatic enantiotopos discrimination



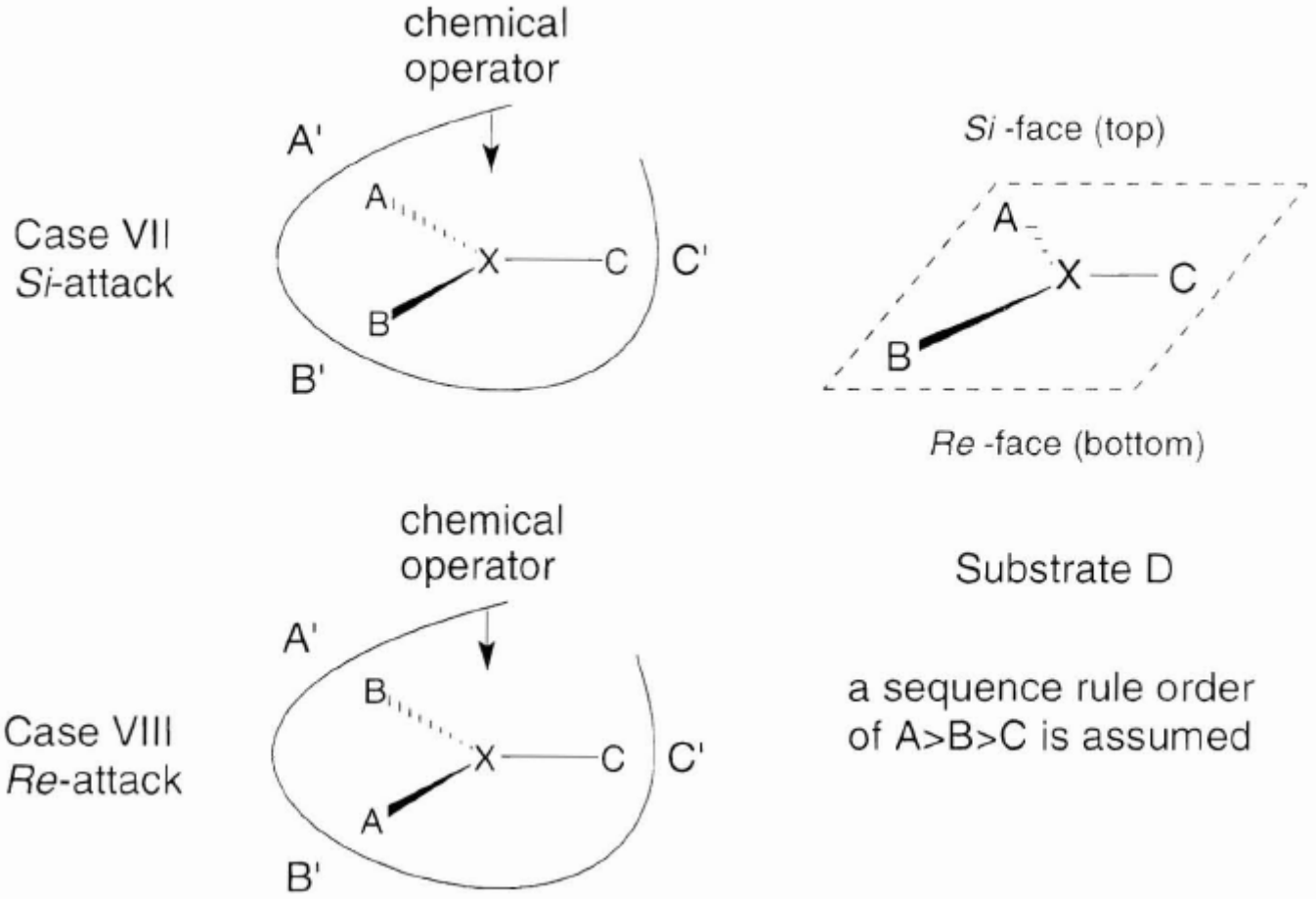


Substrate C

a sequence rule order of A>B>C is assumed

Biocatalysis

Figure 1.5. Schematic representation of enzymatic enantioface discrimination



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Kinetic reasons for selectivity

Enzymes are the catalysts in the reactions.
Their action is best described by the stabilisation of the transition state of the reaction.

An assumption is that the catalyst is binding more strongly to the transition state than to the ground state of the substrate, by a factor approximately equal to the rate acceleration.

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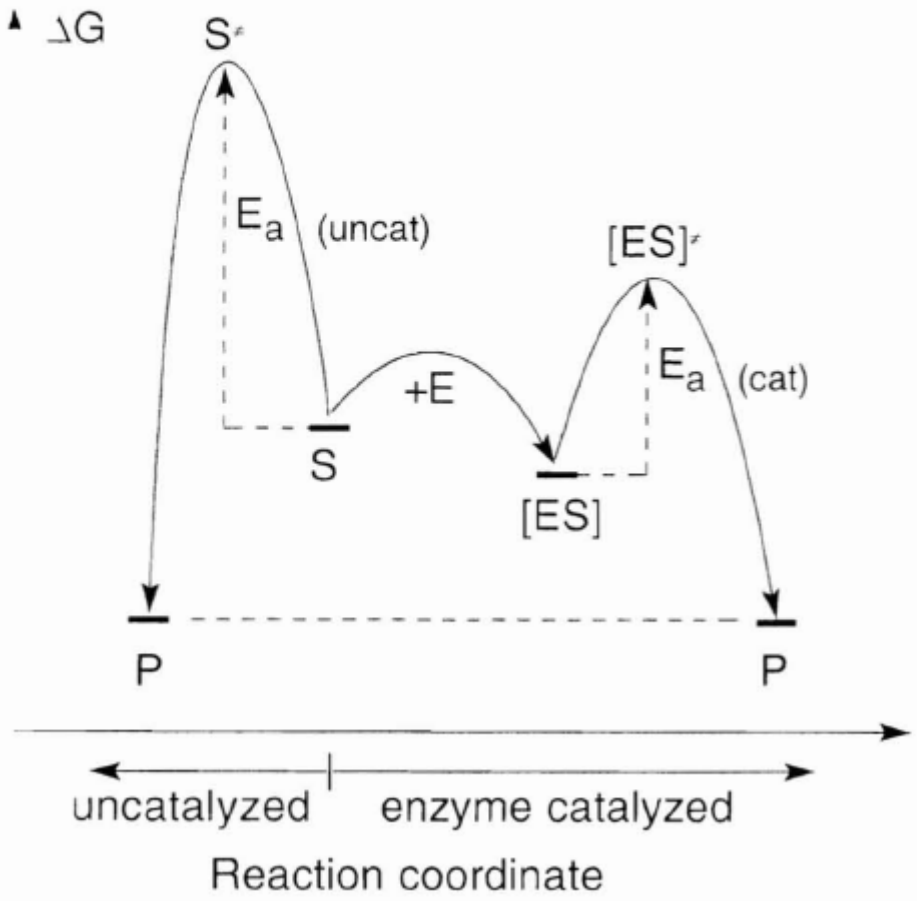
Figure 1.6. Energy diagram of catalyzed versus uncatalyzed reaction

$$E + S \underset{}{\overset{K}{\rightleftharpoons}} [ES] \xrightarrow{k_{cat}} E + P$$

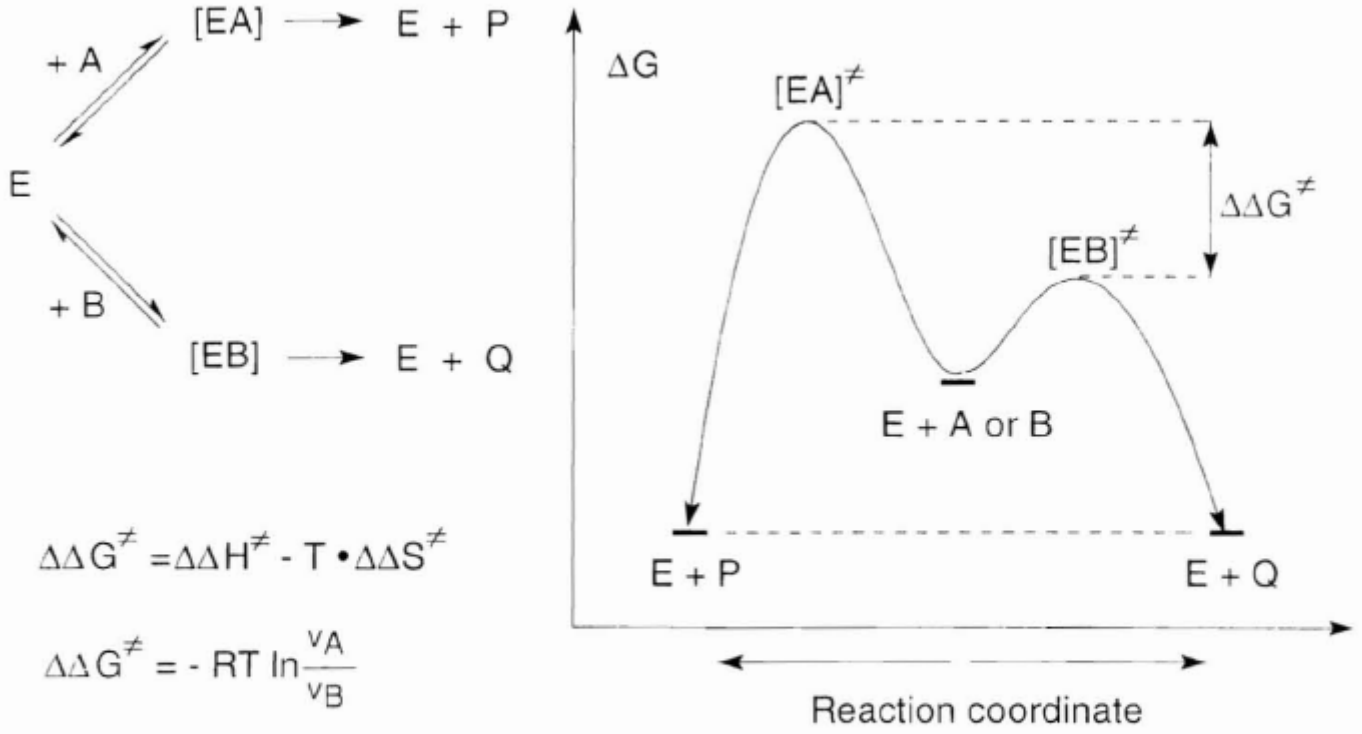


$$K = \frac{[ES]}{[E] \bullet [S]} = \frac{1}{K_M}$$

$$v \sim k_{cat} \bullet [ES] \sim \frac{k_{cat}}{K_M}$$

Biocatalysis

Figure 1.7. Energy diagram for an enzyme-catalyzed enantioselective reaction



$$\Delta\Delta G^{\neq} = \Delta\Delta H^{\neq} - T \bullet \Delta\Delta S^{\neq}$$

$$\Delta\Delta G^{\neq} = - RT \ln \frac{v_A}{v_B}$$

E = enzyme; A and B = enantiomeric substrates, P and Q = enantiomeric products; [EA] and [EB] = diastereomeric enzyme-substrate complexes; ≠ denotes a transition state; $\Delta\Delta G$, $\Delta\Delta H$ and $\Delta\Delta S$ = free energy, enthalpy and entropy difference, resp.; R = gas constant, T = temperature, v_A and v_B = reaction velocities of A and B, resp.

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Cofactors:

A remarkable proportion of synthetically useful enzyme catalysed reactions require cofactors.
They provide either:
Chemical reagents

redox-equivalents
hydrogen, oxygen, electrons, carbon units

Chemical energy:

energy rich functional groups, such as acid anhydrides.

In general enzymes are bound to their natural cofactors, which can not be replaced by synthetic cheaper substitutes.

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Table 1.5. Common coenzymes required for biotransformations

Coenzyme	Reaction type	Recycling[a]
NAD^+/NADH	removal or addition of	(+) [++]
$NADP^+$/NADPH	hydrogen	(+) [+]
ATP[b]	phosphorylation	(+) [+]
SAM	C_1-alkylation	(+) [±]
Acetyl-CoA	C_2-alkylation	(+) [±]
Flavins[c]	oxygenation	(-)
Pyridoxal-phosphate	transamination	(-)
Biotin	carboxylation	(-)
Metal-porphyrin complexes[c]	peroxidation, oxygenation	(-)

[a] Recycling of a cofactor is necessary (+) or not required (-), the feasibility of which is indicated in square brackets ranging from 'feasible' [++] to 'complicated' [±].

[b] For other triphosphates, such as GTP, CTP and UTP, the situation is similar.

[c] Many flavin- and metal-porphyrin-dependent mono- or dioxygenases require additional NAD(P)H as an indirect reducing agent.

Biocatalysis

Enzyme sources:

Most enzymes used in organic chemsitry are crude preparatoins and relatively inexpensive.
Main part is stabilisers, other proteins, inactive proteins, salts, carbohydrates from the fermentations.
Crude preparations are often more stabel than pure enzymes.

Main source for enzymes:
Detergent industry:
Proteases, lipases
Food industry:
Proteases, lipases, Glycosidases (baking industry)
Slaughter waste, mammalian organsMicroorganisms
Plants, (fruits and vegetables), sensitive
Pure enzymes:
Generally very expensive, sold by the unit. Crude preparations sold by the kg. Enzyme purification from crude enzymes is feasible.

Biocatalysis

Questions:

1)What are the common arguments againnst the use of enzymes?
2)Pro and cons in the use of enzymes?
3)Isolated vs. Whole cells?
4)What are the common types of reactions leading to enzyme deactivation?
5)What are the common models to explain the mechanism of cartalysis?
6)What is the three point rule?
7)What is the kinetic reason for selectivity?