Enzymes in organic solvents

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Enzymes in organic solvents

- Enzymes work very well in water because
- A) They were evolved in water.
- B) The major constituent of most organisms are water.
- C) Water is essential for maintaining their structure in solution.
- However, many reactions that are difficult to occur in water can be performed in nonaqueous systems.

Early approaches

- Water- organic solvent (such as acetone or ethanol) mixture: Predominantly water. So many enzymes can work in this environment.
- Biphasic mixture: Use of water phase for enzymes and organic phase for substrates. Subtrate diffuses from organic phase to water, gets converted to product and goes back to organic phase. Reducing the size of aqueous droplets allows easy mass transfer. Reverse micelles, micro emulsions are all variation of this approach.
- Nearly Nonaqueous phase: They contain very few percentage of water. Ultimate step is to use enzymes in organic solvents.

Enzymes are active in nonaquesous solutions

- In all previous cases, enzymes are predominantly located at the aqueous phase. So they are not in totally different situation from aqueous environment.
- However, enzymes can also work predominantly in nearly anhydrous environment (membrane enzymes for example).

Enzymes can act in organic solvents that contain less than 0.01% water (typically anhydrous).

- Both chymotrypsin and subtilisin work in organic solvents although they are proteolytic enzymes needing water as a co-substrate.
- So if they can work in anhydrous solvents other enzymes can also work.

Water - How much does an enzyme really need?

- Water is essential for maintaining the correct structure of the enzymes. Therefore, it is natural to expect that removal of water will result in denaturation of enzyme activity.
- However, the question is how much water a protein really needs?
- Proteins need only a few monolayers of water. Beyond that, they do not have any serious contact with the water molecules.
- So if we provide the first one or two monolayers of water necessary fo rthe protein, then it should exhibit biological activity in anhydrous solvents.

Conditions for nonaqueous enzyme catalysis -1

- Nature of solvent is critical - Hydrophobic solvents are the best.
- Hydrophilic solvents remove the essential water from the protein and hence reduce the enzyme activity.

Chymotrypsin catalysis

In anhydrous solvent such as octane, chymotrypsin works well. Water on the enzyme is also quite high in this hydrophobic solvent. Surprisingly in hydrophilic solvents, enzyme activity is lowered due to stripping of essential water molecules from the enzyme.

Solvent	k_{cat}/K_m (M^{-1} min^{-1})	% water in enzyme
Octane	63	2.5
Toluene	4.4	2.3
Tetrahydrofuran	0.27	1.6
Acetone	0.022	1.2
Pyridine	<0.004	1.0

Addition of water where stripping took place, restores the enzyme activity dramatically.
By adding water to the enzyme in the following case, one could regain as much as 1000 fold increase in activity.

Chymotrypsin in:	K_{cat}/K_m (M^{-1} min^{-1})	% water in enzyme
Octane	63	2.5
Acetone (without added water)	0.022	1.2
Acetone [with added water (1.5%)]	22	2.4

How much water needed?

- Calculations show that about 50 molecules of water per molecule of chymotrypsin is needed.
- This is lot less than what is needed for a monolayer (500 water moelcules)
- Apparently you need only a few clusters of water molecule around charged amino acids at the surface of the enzyme.
- The water molecules allow the enzyme confromational flexibility to perform catalysis.

Conditions for nonaqueous enzyme catalysis -2

- Enzymes used in organic solvents should be either precipitated (or lyophilized) from aqueous solutions with appropriate pH in which it shows optimal activity. So that the enzyme can have pH memory.
- At pH optimum, the group at and around active site are appropriately ionized to perform the catalysis. By precipitating or lyophilizing the enzyme under this conditions, we allow the enzyme to remember this structure when placed in organic solvent.

Conditions for nonaqueous enzyme catalysis -3

- In organic solvents, enzyme is suspended rather than dispersed. So for it to catalyze the reaction, you need to bring the substrate closer to it.
- Vigorous stirring or sonication is needed to make sure that the enzyme and substrates are brought together in organic solvents.

General characteristics of enzymes in organic solvents

- Follow typical Michaelis Menten kinetics indicating ES complex formation.
- Rate enhancements are typically in the range of 10^{10} to 10^{11}
- Enzymes previously inactivated by active site titrants did not show activity.
- The rate of active site titration in aqueous and organic solvent was comparable.
- Thus enzyme seems to use the same active site mechanism in organic solvents also.

Increase in thermal stability

- Heat induced unfolding of proteins requires confromational mobility. To inactivate a protein you also need free water. Since both these processes are restricted in organic solvents, they should increase the thermal stability of enzymes.

- Accordingly, chymotrypsin is more stable in octane than in water.

Chymotrypsin in	Temperature ˚C	Half life
water	60	Few minutes
Octane	100	Few hours

Storage

- In addition to thermal stability, enzymes in organic solvents also show remarkable capacity for storage.
- Chymotrypsin retains full activity even after 6 months in octane at 20 ˚C , where as its half life is only a few days in water.

Liagnd induced enzyme memory

- Lyophilization of subtilisin from aqueous solutions containing competitive inhibitor followed by their removal results in a rate enhancement of 100 fold in organic solvent.

Alteration in substrate specificity

- Substrate specificity is dictated by the free energy of binding. Since, the free energy of binding is altered in organic solvents dramatically substrate specificity is also altered.
- Chymotrypsin substrate specificity in water Phe >> Ser. In organic solvent: transesterification of Ser is three times faster than Phe.
- Naphthalene is 18 time more potent competitive inhibitor of chymotrypsin than 1-naphthoic acid in water. In octane, 1-naphthoic acid is 370 times more effective than naphthalene.
- Histidine ester hydroysis is 0.5% of that of Phenylalanine in water. In Octane, His is 20 fold more active than Phe.

Alteration of stereospecificity

- Stereoselectivity of L- isomers over D -isomer for hydrolysis of N-acetyl alanyl chloroethyl ester for elastase, chymotrypsin, subtilisin, trypsin and α-lytic protease is of the order of 10^3 to 10^4 in water. In nonaqueous solvents it does not exceed even 10. (more hydrophobic the solvent is less stereospecific the reaction is)

Hallmarks of enzymes in organic solvents

- Lipases - catalyze esterification, transesterification, aminolysis, thiotransesterification and oximolysis in organic solvents while in water only perform hydrolysis.
- Chymotrypsin catalyzes the synthesis of peptides in organic solvents. In water it catalyzes only the hydrolysis.
- Tyrosinase catalyzed conversion of catechols to quinones results in eventual polymerization in water. In chloroform, one can recover the quinone nearly quantitatively at the end.

References

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