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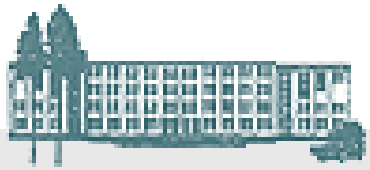

INFLUENCE OF THE PHYSICO-CHEMICAL CONDITIONS ON THE SUBSTRATE SPECIFICITY OF THE ENZYMES

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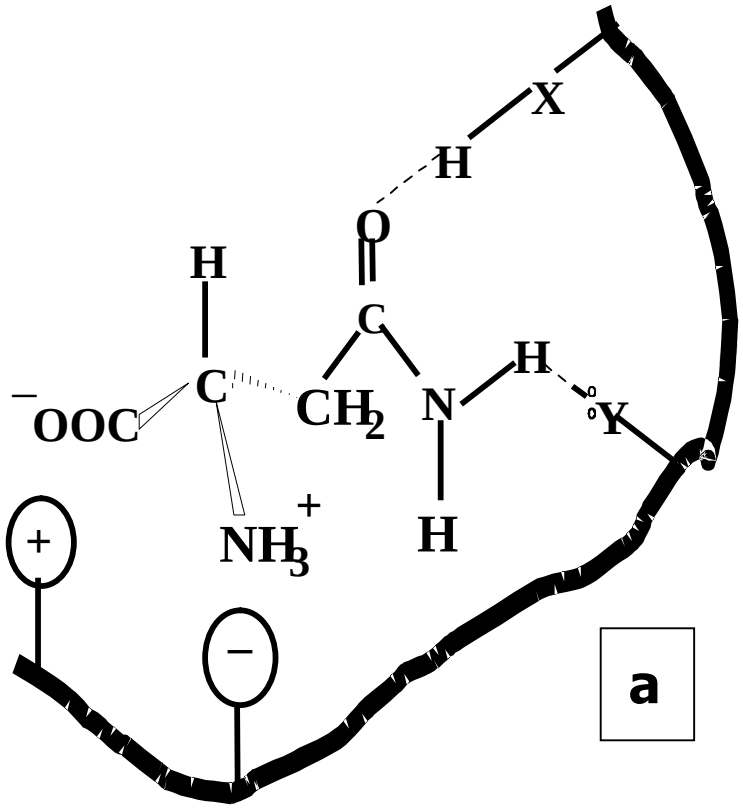
GENERAL

- One of the main features of enzymes as catalysts is their extremely high specificity to the substrates
- This is very important in the synthesis of asymmetric products in fine chemicals and pharmaceuticals production

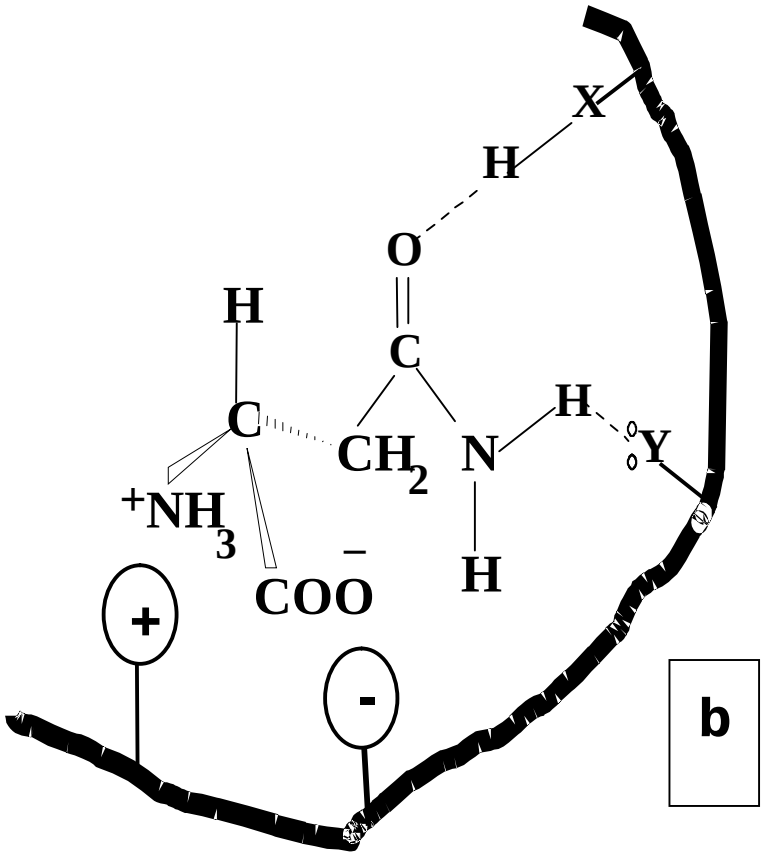
SOME MODEL



The asymmetry of active center of the enzyme catalyzing the L-asparagine hydrolysis

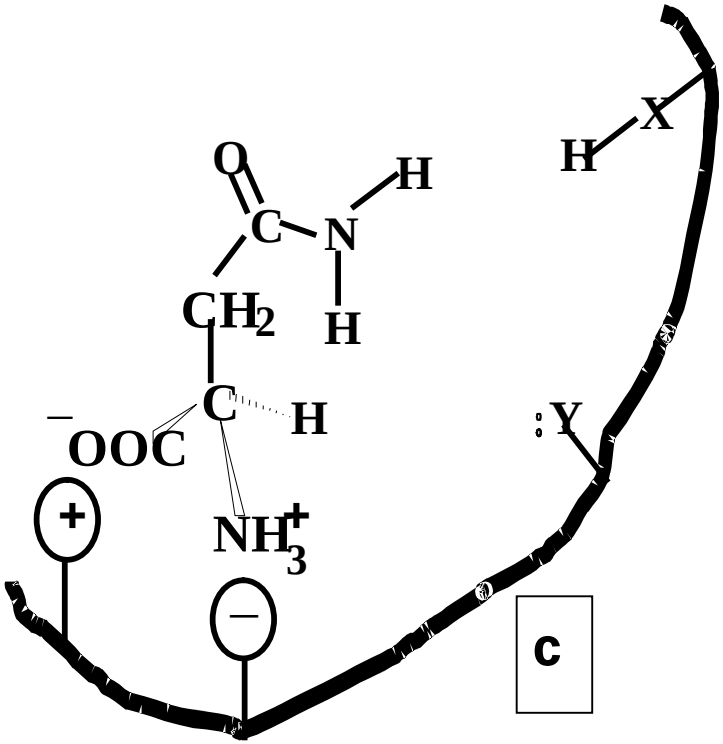
a. Binding of the substrate In accordance with the active center geometry

SOME MODEL



b. Binding of the D-isomer doesn't correspond to the active center configuration which confers the electrostatic repulsion between the carboxyl and α-amino groups of the substrate and side chains of the amino acid residues on the enzyme The stable enzyme-substrate complex doesn't form

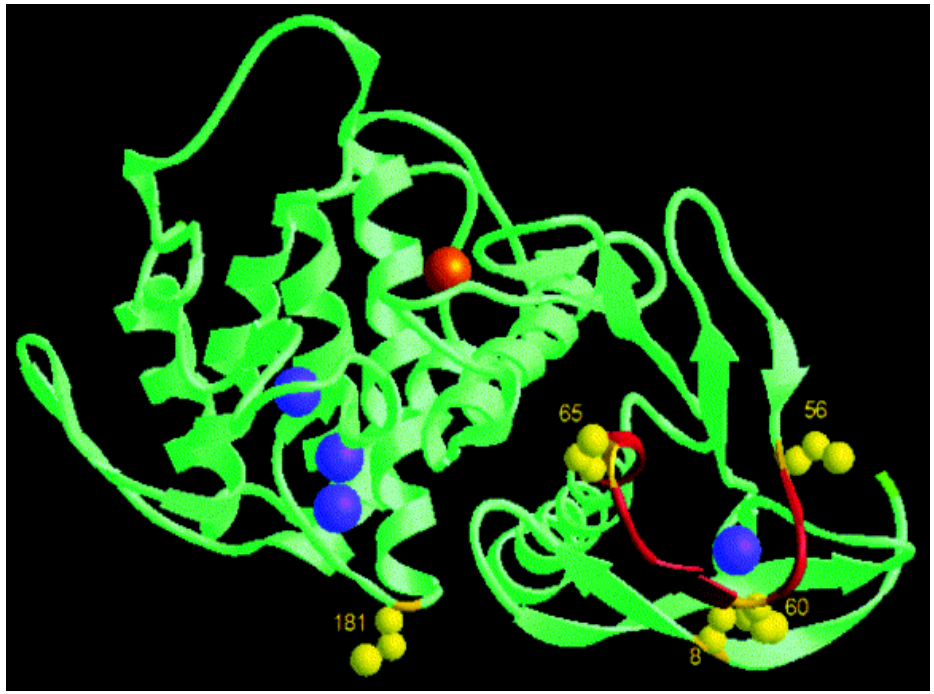
SOME MODEL



If the D-asparagine binds to the enzyme so that its charged groups locate properly, the amide group to be hydrolyzed becomes inaccessible to catalytic residues of the enzyme and reaction doesn't occur

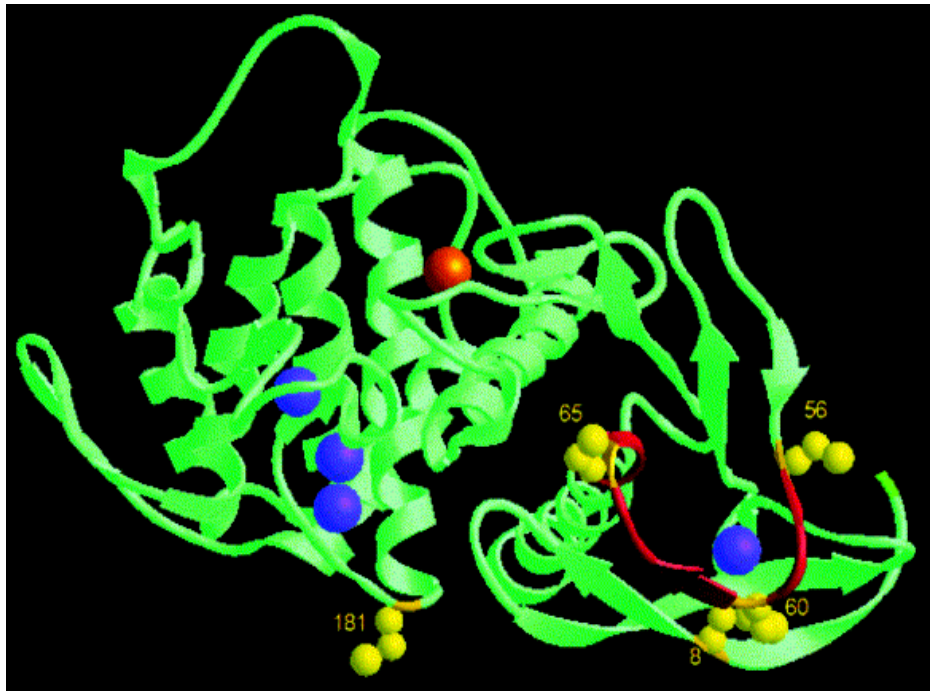
EXAMPLE OF 3D STRUCTURE OF ENZYME

- **The model of tertiary structure of thermolysin molecule (*Bacillus stearothermophilus* protease). The segments of various types of secondary structure could be seen (helices, β-sheets, loops). The colored balls – different metal ions.**
- **This molecule could change its 3D structure depending on physico-chemical conditions**



EXAMPLE OF 3D STRUCTURE OF ENZYME

- **Because of those changes the structure of substrate binding center could also change, so one may expect that the substrate specificity could be altered depending on physico-chemical conditions.**

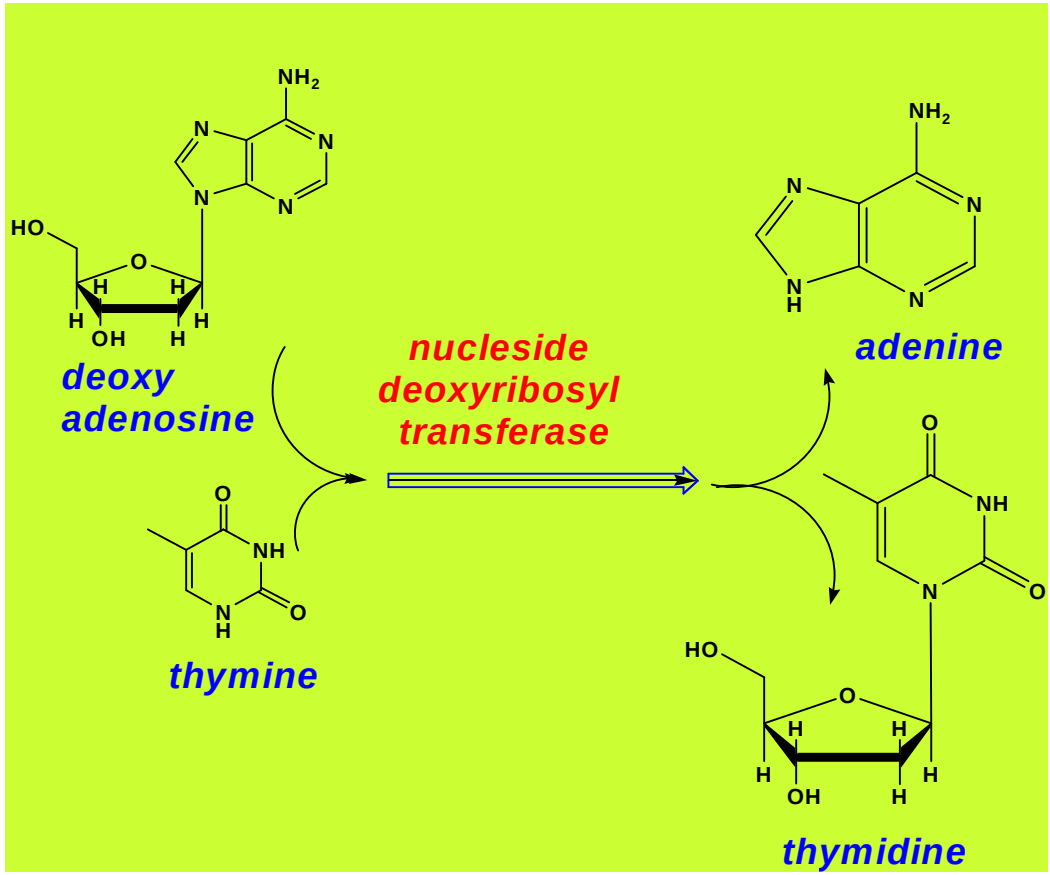


EFFECT OF TEMPERATURE

- Utagawa and coworkers investigated the possibility of enzymatic synthesis of unnatural nucleosides as potential inhibitors of polymerase reactions in pathogenic microorganisms. They synthesized chemically arabino uracyl and showed such an inhibitory effect. But the chemical synthesis of nucleosides is rather difficult task and the products are very expensive. They tried to synthesize arabino nucleosides enzymatically.

EFFECT OF TEMPERATURE

There is well known reaction of transfer deoxyribose moiety from one nucleic base to another one. If we take deoxyribo adenosine and thymine and the enzyme preparation the reaction could occur with formation of deoxyribo thymidine and free base adenine
Utagawa and coworkers tried to learn if the similar reaction could occur with arabino nucleosides will be taken



EFFECT OF TEMPERATURE

GENERA	Number of strains examined	Number of strains forming Ara-A at elevated temperature
Escherichia	33	31
Enterobacter	13	10
Proteus	11	10
Kluyvera	5	5
Salmonella	3	3
Citrobacter	2	2
Ervinia	7	6
Flavobacterium	31	4

ENZYME SPECIFICITY MANAGEMENT

- Molecular imprinting – adaptation of the enzyme to particular substrate.
- The enzyme capable of catalyzing the reactions with different substrates is bound to the complex with that substrate, which should be converted. This complex is fixed in some manner and then is dissociated. In this way the desired substrate is “imprinted” into the enzyme molecule which is tuned to the desired substrate.

ENZYME SPECIFICITY MANAGEMENT

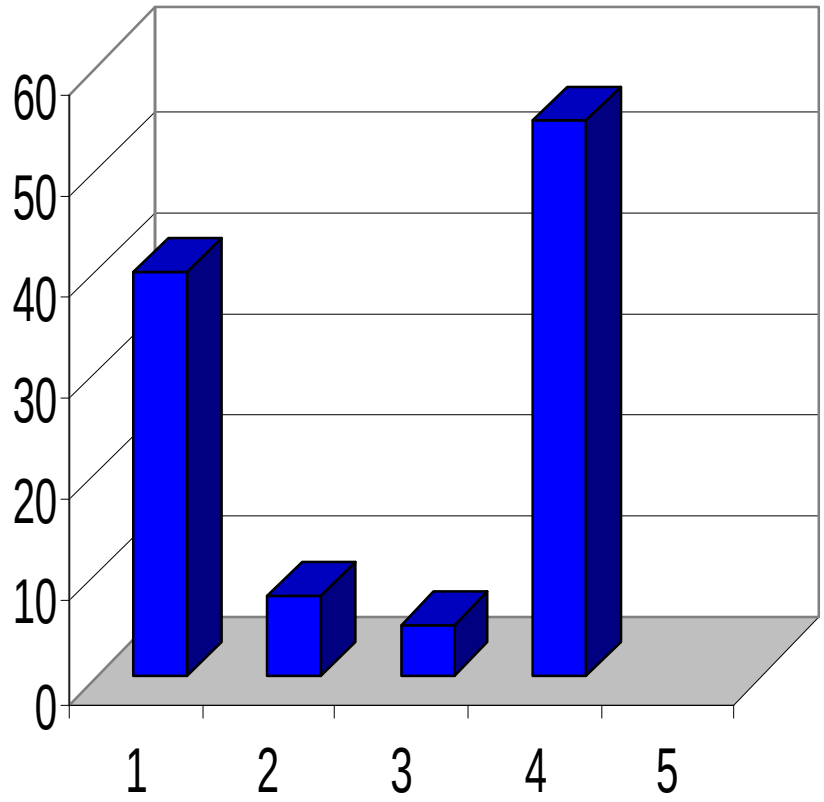
- The example:
- The reaction was the transesterification of sucrose or thymidine with vinyl butyrate in the presence of subtilysine Carlsberg.
- The solutions containing the enzyme and one or another substrate have been frozen and then freeze dried. The conformational flexibility of the enzyme in frozen state is extremely low, so the protein maintains the conformation formed during the formation of enzyme – substrate complex.
- When the reactions were performed the ratios of the conversion strongly depended upon the substrate imprinted.

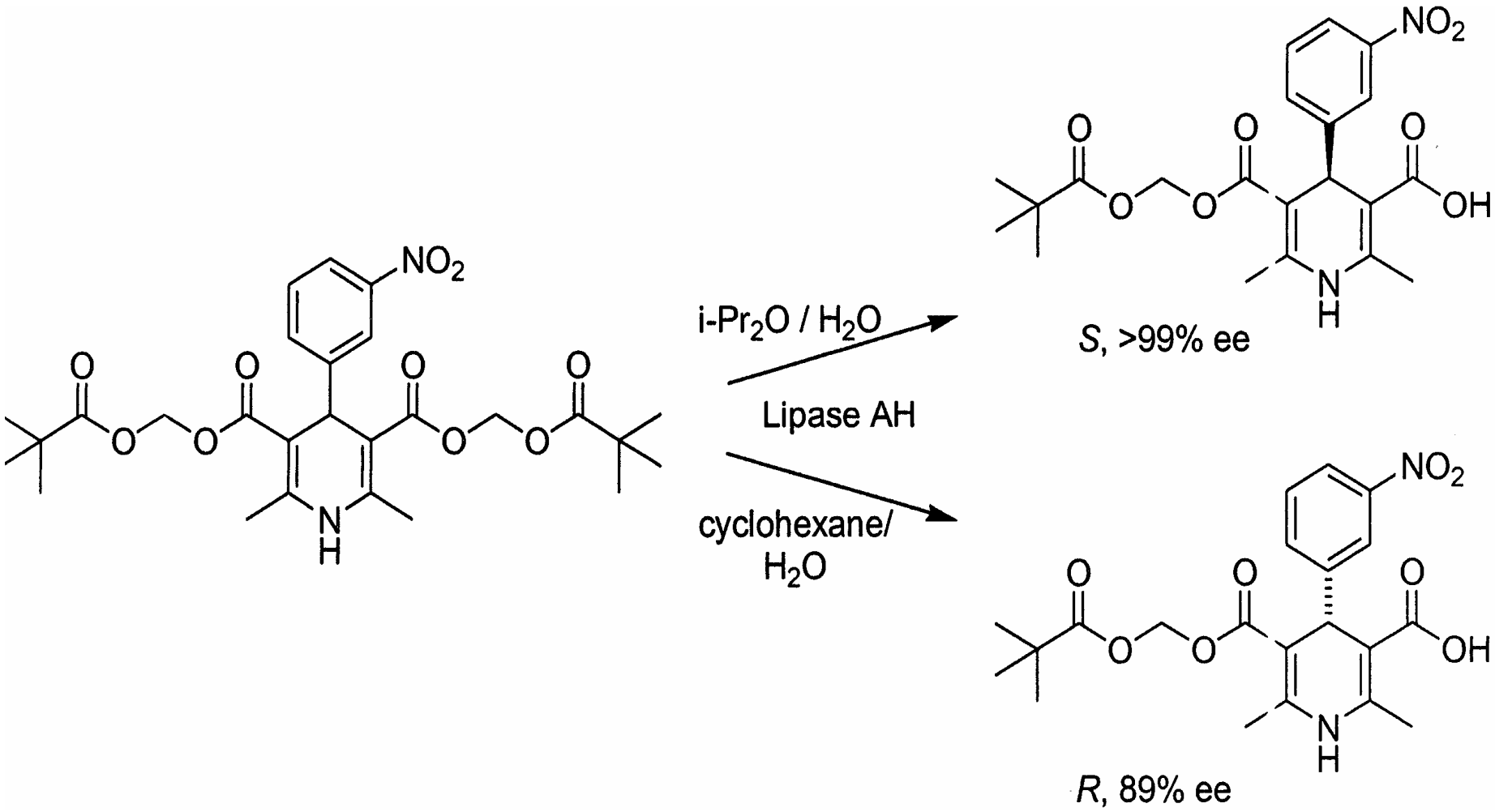
ENZYME SPECIFICITY MANAGEMENT

1,2 – acylation of sucrose in pyridine;

3,4 – acylation of thymidine in tetrahydrofurane.

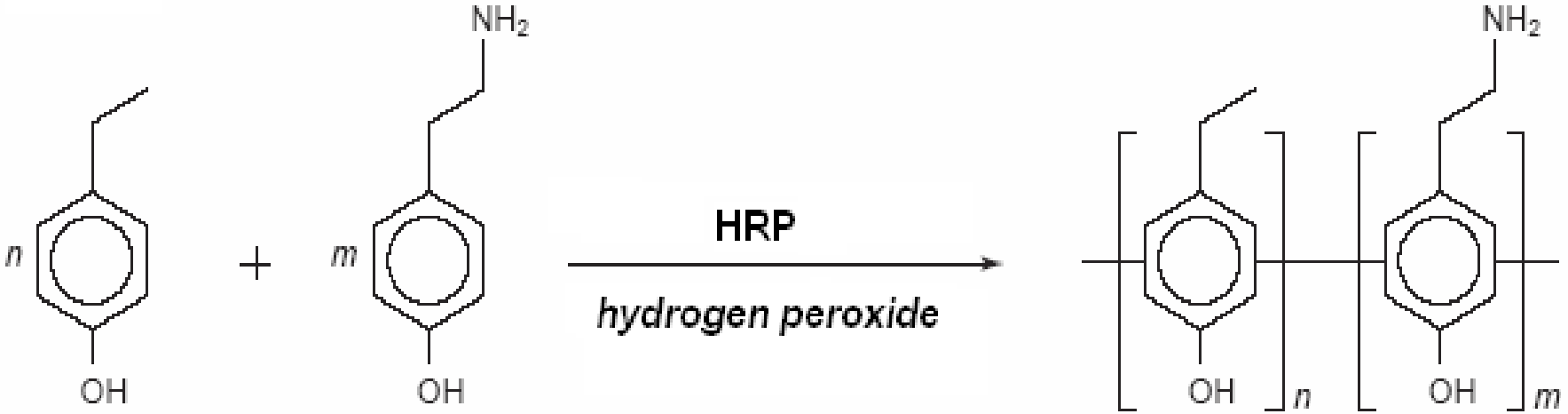
Imprinting: 1, 3 – with sucrose, 2, 4 – with thymidine. On the ordinate axis – the enhancement of reaction rate with imprinted enzyme compared to unimprinted





The influence of the solvent nature on the stereospecificity of hydrolysis by ***Pseudomonas sp.*** lipase as example of the approach to substrate specificity management

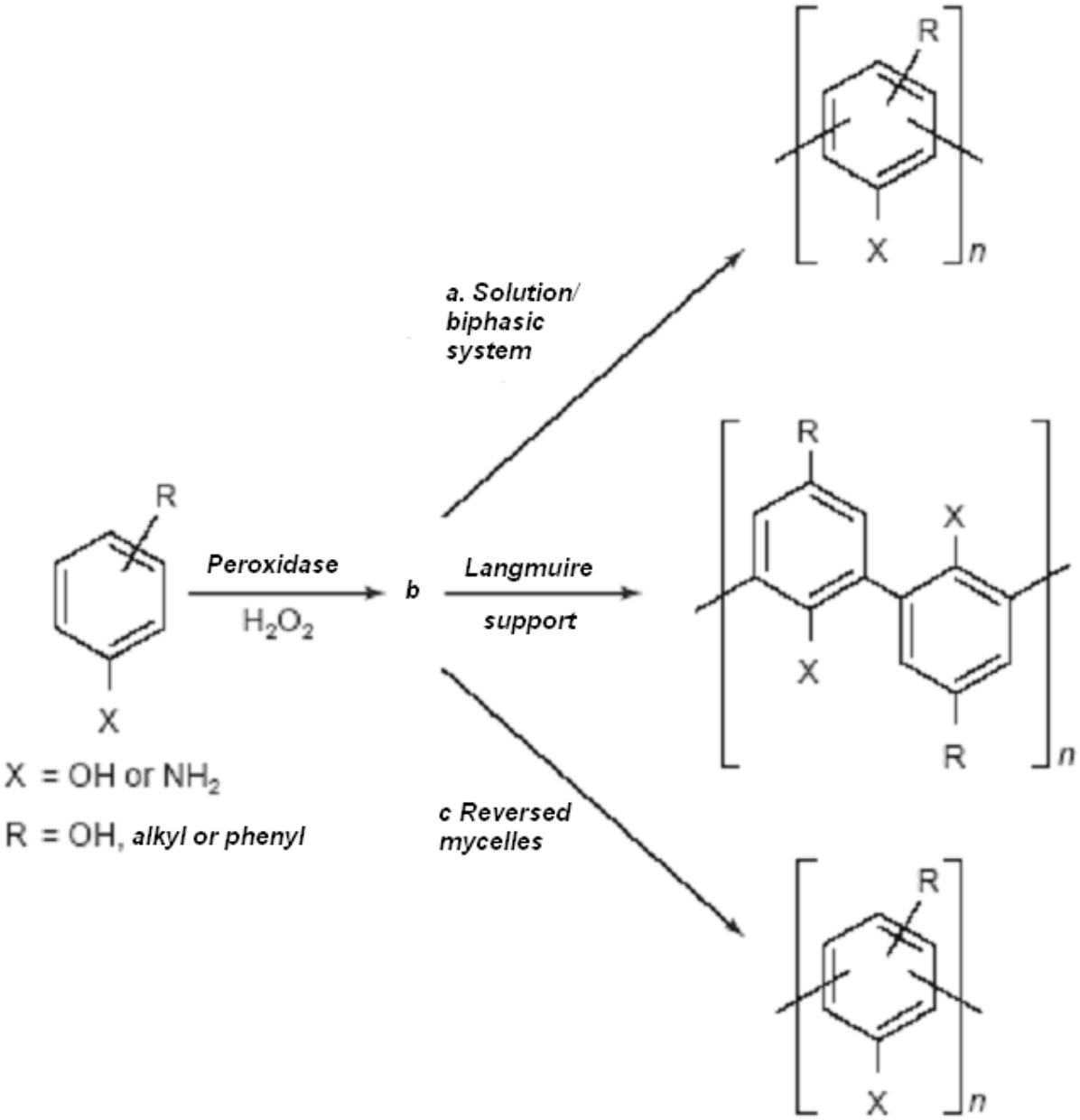
POLYMER SYNTHESIS



- Horse reddish peroxidase catalyses the formation of copolymer of ***p***-ethyl phenole and tyramine – oxidative copolymerization

Depending upon the reaction conditions the products of different structure are formed

When the reaction is performed in monomolecular layer (on some support) the product is stereoregular polymer



Conclusion

It is possible to present even more examples of marked influence of reaction conditions on the product structure

Only in some cases one can predict what will happen and manage the results

In most cases we only observe the results and can explain them

The reason is still limited knowledge about the structure of enzymes and fine details of their interaction with substrates and other ligands

But the accumulation of such information and its analysis using the bioinformatics could improve our understanding how these rather complicated systems work

Thank you for attention