

Biocatalysis

Code: 100997
ECTS Credits: 6

| Degree | Type | Year | Semester |
|----------------------|------|------|----------|
| 2500502 Microbiology | OT | 4 | 0 |

Contact

Name: Josep Antoni Biosca Vaqué
Email: Josep.Biosca@uab.cat

Use of languages

Principal working language: catalan (cat)
Some groups entirely in English: No
Some groups entirely in Catalan: Yes
Some groups entirely in Spanish: No

Teachers

Xavier Parés Casasampera

Prerequisites

There are no official prerequisites. In any case, some of the contents of the 1st year course, Biochemistry, are necessary to follow the subject correctly.

Objectives and Contextualisation

The subject Biocatalysis focuses on the study of enzymes, their properties and applications. The knowledge of enzymes is key in the context of Biochemistry, Molecular Biology and related sciences, given their role as catalysts of biological reactions and their applications in biotechnological processes. The subject analyzes enzymes from different perspectives: their activity, kinetics, mechanisms and applications. The general objective of the subject is to provide the foundations for the analysis, characterization and use of enzymes from the point of view of research and from their biotechnological and biomedical applications.

Specific objectives of the subject:

Knowledge of the general characteristics, classification and testing methods of enzymatic activity.
Analysis of enzyme kinetics and determination and meaning of kinetic parameters.
Knowledge of enzyme inhibition and its applications, especially in the field of drugs.
Analysis of the active center and knowledge of the methods of characterization.
Analysis of enzymatic and regulatory mechanisms.
Biomedical and biotechnological applications of enzymes.

Content

Lesson 1

Concept of biocatalysis. Historical perspective. Factors to consider in a biocatalytic process: source of the biocatalyst and optimization of the process. Cellular and enzymatic systems: properties.

Lesson 2

General properties of the enzymes: Concept and biological significance, chemistry and practice. Definitions Enzyme-substrate complex. Decrease of activation energy. Transition state. Regulation. Enzymatic cofactors. Classification of enzymes.

Lesson3

The obtention and characterization of enzymes. Sources of collection. Techniques for the extraction of enzymes. Methods of determining the enzymatic activity. Initial rate concept, determination, representation. Units of enzymatic activity. Effect of enzyme concentration.

Lesson 4

Enzyme kinetics. Reactions with a substrate. Effect of substrate concentration: Michaelis-Menten equation. Pre-steady and steady states: concepts. Hypothesis of steady state: treatment of Briggs-Haldane. Pre-steady state. Methods of study. "Bursts" and "lags".

Lesson 5

Determination of K_M and V_{max} . Methods of Lineweaver-Burk and Eadie-Hofstee. Other methods to determine the kinetic parameters. Meaning of the kinetic parameters k_{cat} and K_M . Concept of k_{cat} / K_M : catalytic efficiency and enzymatic specificity. Michaelis-Menten's equation for reversible reactions: Haldane's relationship.

Lesson 6

Inhibition of enzyme catalysis: types of inhibitors. Reversible inhibitors: competitive and non-competitive inhibition; Acompetitive and mixed inhibition. General model of inhibition. Graphic analysis of the different types of inhibition. Determination of constants of inhibition. Concept of IC_{50} and its relation to constants of inhibition. Substrate excess inhibition. Discrimination between competitive substrates. Pseudoirreversible and irreversible inhibitors. Use of inhibitors as drugs. Affinity markers. Suicide substrates as irreversible inhibitors.

Lesson 7

Reactions with more than one substrate: Cleland's notation. Double displacement mechanism (ping-pong); Ordered sequential mechanism; Statistical sequential mechanism. Mathematical treatment and graphic analysis. Methods for determining the type of mechanism. Isotopic exchange and isotopic effect concepts and applications.

Lesson 8

Action of the temperature on the enzyme kinetics. Arrhenius representation. Enzymes of extremophile organisms. Effects of pH on the enzyme kinetics. Ionization of essential residues. Influence of pH on the kinetic parameters. Evaluation of ionization constants. Identification of the ionizable groups involved in the processes of binding and catalysis. Effects of microenvironment on the pK . Examples

Lesson 9

Binding of ligands to proteins. Concept and types of cooperativity. Analysis of the cooperativity. Binding of oxygen to hemoglobin. Models of cooperativity. Model of Monod, Wyman and Changeux. Explanation of the homotropic cooperative effects by the MWC model. Allosteric enzymes. Systems K and systems V. Model of Koshland, Nemethy and Filmer. Determination of the cooperativity model that follows an enzyme. Example of enzyme with allosteric regulation: aspartate carbamoyltransferase.

Lesson 10

Enzymatic specificity. The active site, specificity and tridimensional structure. Definition of active site. Characteristics of the active site. Theories on enzyme-substrate binding. The Fisher model (lock-and-key). The Koshland model (induced fit). The hexokinase as induced fit example. Hypothesis of binding to three sites. Hypothesis of strain effect. Stabilization of the transition estate. Evidence supporting the theory of the transition state. Catalytic antibodies. Applications of catalytic antibodies

Lesson 11

The active site. Identification of the binding and catalytic sites. Labeling with a part of the substrate. Use of artificial substrates. Chemical modification with irreversible specific inhibitors. Affinity labeling. Suicide inhibitors, examples of pharmacologic interest. Site directed mutagenesis. The serine-proteases: subtilisin.

Comparison of mutagenesis and chemical labeling. Investigation of the tridimensional structure of proteins: X-rays, NMR, molecular modeling. Evolutionary invariability of amino acid residues. The alcohol dehydrogenase.

Lesson 12

Mechanisms of catalysis. Introduction to the mechanisms of the enzyme action. Acid-base catalysis. Covalent catalysis. Mechanism of subtilisin. Metal ion catalysis. Mechanisms of alcohol dehydrogenase la carbonic anhydrase. Effect of the environment: electrostatic catalysis. Lysozyme. Superoxide dismutase. Effects of proximity and orientation. Energetics of the enzyme catalysis. Optimal values of the K_m . Triosephosphate isomerase as an energetically efficient enzyme.

Lesson 13

Enzymatic cofactors and ribozymes. Structure and mechanism of cofactors. Catalytic activity of RNA: ribozymes. Types of ribozymes. The ribosome is a ribozyme. Biologic significance of ribozymes. Applications of ribozymes.

Lesson 14

Regulation of the enzymatic activity. Modification of the enzyme concentration. Regulation of the synthesis and degradation of enzymes. Mechanisms of degradation. Variation of the enzymatic activity as a function of the concentration of substrate, product and cofactors. Activation by precursor and feedback inhibition. Functional significance of cooperativity and allosterism. Control linked to energy. Hormonal control. Isoenzymes. Multienzymatic clusters. Multienzymatic complexes. Systems bound to membranes. Multifunctional enzymes. Polymerization-depolymerization. Binding to other proteins. Irreversible covalent modification. Reversible covalent modification. Systems of enzymatic cascade. Regulation of photosynthesis. Regulation of the metabolic pathways.

Lesson 15

Enzymes in clinical biochemistry and biotechnology. Enzymes as therapeutic agents. Enzymes as indicators of pathologies. Plasmatic enzymes. Origin of the plasmatic enzymes. Factors affecting the plasmatic enzyme levels. Examples of enzymes with diagnostic interest. Aminotransferases. Creatine kinase. Lactate dehydrogenase. Indicators of myocardium infarction. Enzymes as reagents in clinical biochemistry. Enzymes and metabolism inborn errors. Examples. Enzymes in the industry. Large scale production of enzymes. Applications: drugs, alimentary industry, detergents, textile industry. Immobilized enzymes. Enzymes as biosensors

Lesson 16

Methods to improve biocatalysis. Design and synthesis of new catalysts. Directed evolution. Generation of mutants. Selection and screening of the desired enzymatic activity. Redesigning of enzymes to modify their thermostability and enantioselectivity.

Problems

The problems that are proposed refer to some aspects of the syllabus, emphasizing the determination of kinetic parameters in different situations: presence of inhibitors, bisubstrate reactions, non-homogeneous preparations, etc. The statements of the problems will be delivered through the Virtual Campus in advance to the problem sessions in which they will be solved.

Practical sessions.

In the practical sessions different methodologies will be applied aimed at the characterization of a biocatalyst overexpressed in yeast (*Saccharomyces cerevisiae*). The stereo-specificity of the reaction will be determined and different computer programs will be used to determine its kinetic parameters and to study its tridimensional structure.