Universitat Autònoma de Barcelona

2017/2018

Biocatalysis

Code: 100867
ECTS Credits: 6

Degree	Type	Year	Semester
2500252 Biochemistry	OB	2	2

Contact

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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

Marc Torrent Burgas

Prerequisites

There are no official prerequisites. In any case, some of the contents of the 1st and 2nd year subjects (first semester) are necessary to be able to follow the subject correctly. In particular, those of the following subjects: Thermodynamics and Chemical Kinetics, Organic Chemistry of Biochemical Processes, Biochemistry I, Biochemistry II, Chemistry and Protein Engineering, Basic and Advanced Instrumental Techniques.
For some activities, a basic level of reading comprehension of English is required.

Objectives and Contextualisation

The subject Biocatatalization focuses on the study of enzymes. The knowledge of enzymes is key in the Biochemistry and related sciences given their role as catalysts of biological reactions and applications in biotechnological processes. The subject analyzes enzymes from different perspectives: 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 the biotechnological and biomedical applications.
Specific objectives of the subject:
Knowledge of the general characteristics, classification and testing methods of enzymatic activity.
Analysis of enzymatic kinetics and determination and meaning of kinetic parameters.
Knowledge of enzymatic 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. **Introduction to enzymes.**

Historical perspective. General properties of the enzymes. Enzyme activity. Efficiency. Specificity. Enzyme-substrate complex. Transition state. Decrease of activation energy. Regulation. Enzymatic cofactors. Other biocatalysts.

Lesson 2. **Classification and nomenclature of enzymes.**

International Commission of Enzymes. Nomenclature and classification of the six main classes of enzymes. Other characteristics to identify an enzyme. Databases with information on enzymes.

Lesson 3. **Methods for determining enzymatic activity and purification of enzymes.**

Initial rate: concept, determination and representation. Units of enzymatic activity. Effect of enzyme concentration. Methods of determining the enzymatic activity. Enzyme purification procedures.

Lesson 4. **Analysis of enzyme kinetics (1).**

Reactions with a substrate. Effect of substrate concentration: Michaelis-Menten equation. Pre-steady state and steady state: concepts. Hypothesis of steady state: treatment of Briggs-Haldane. Enzyme reactions with more than one intermediate complex.

Lesson 5. **Analysis of enzyme kinetics (2).**

Determination of kinetic parameters (K_M, V_{max} and k_{cat}). Methods of 1) Lineweaver-Burk, 2) Eadie-Hofstee, 3) Hanes-Wolff, 4) Eisenthal-Cornish-Bowden and 5) Integrated equation of Michaelis-Menten. 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, uncompetitive and mixed (including non-competitive) inhibition. General model. Graphic analysis of the different types of inhibition. Determination of constants of inhibition. Concept of IC_{50} and its relation to constants of inhibition. Tight-binding or pseudo-reversible inhibitors. Irreversible inhibitors. Use of enzyme inhibitors as drugs.

Lesson 7. **Analysis of enzyme kinetics in reactions with more than one substrate.**

Overall description of reactions with more than one substrate: Cleland notation. Mechanisms of bisubstrate: ordered sequential mechanism, statistical sequential mechanism and double displacement (ping-pong) mechanism. Mathematical treatment and graphic analysis. Methods for determining the type of mechanism. Isotopic exchange and isotopic effect.

Lesson 8. **Kinetics of transient states.**

Characteristics of the analysis methods. Mixing methods: continuous flow, stopped-flow (quenched-flow). Relaxation methods: temperature jump (T-jump), pressure jump (P-jump). Analysis of the "Burst" of a reaction: determination of the enzyme concentration.

Lesson 9. **Effect of pH and temperature on enzymatic reactions.**

Effect of pH on the activity of enzymes. Kinetic treatment in the steady state. Effect of pH on the enzyme kinetics. Ionization of specific groups Evaluation of the pK of groups involved in the processes of binding and catalysis. Effect of temperature. Enzymes of extremophile organisms.

Lesson 10. **Cooperativity and allosteric enzymes.**

Kinetic analysis of cooperativity. Equations of Hill and Adair. Model of Monod, Wyman and Changeux. Model of Koshland, Némethy and Filmer. Activity and regulation of the aspartate transcarbamoylase.

Lesson 11. **Enzyme specificity.**

The active site: definition, characteristics, specificity and 3D structure. 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 state. Catalytic antibodies

Lesson 12. **Study of the active site.**

Identification of the binding and catalytic sites. Chemical modification with specific irreversible inhibitors. Labelling with a part of the substrate. Use of artificial substrates. Affinity labelling. 3D structure of proteins: X-rays, NMR. Site-directed mutagenesis. Comparison of mutagenesis and chemical labelling. Substrate Assisted Catalysis (SAC).

Lesson 13. **Mechanisms of catalysis.**

Introduction to the mechanisms of the enzyme action. Energetics of the enzyme catalysis. Proximity and orientation effects. Acid-base catalysis. Covalent catalysis. Metal ion catalysis. Effect of the environment: electrostatic catalysis. Substrate channelling. Multifunctional enzymes. Enzymes with additional non-enzymatic functions "moonlighting enzymes".

Lesson 14. **Cofactors and ribozymes.**

Structure and mechanism of cofactors: examples. Ribozymes. Catalytic activity of the RNA. Types of ribozymes. Biological significance of ribozymes.

Lesson 15. **High fidelity enzymes.**

Specificity of restriction endonucleases. Editing mechanisms of aminoacyl-tRNA synthetases. Recognition of chiral centers.

Lesson 16. **Regulation of enzymatic activity.**

Review of the different strategies for regulation of enzyme 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. Control linked to energy. Hormonal control. Isoenzymes Polymerization-depolymerization. Binding to other proteins. Irreversible covalent modification. Reversible covalent modification.

Lesson 17. **Biomedical and biotechnological applications of enzymes.**

Enzymes in the industry. The biocatalysis cycle. Applications of enzymes: drugs and diagnostics, detergents, textile industry and food industry.

Problems.

The problems that are proposed refer to the analysis of enzyme activity and determination and interpretation of kinetic parameters. The statements of the problems will be delivered through the Virtual Campus.