

Chem*4570 Applied Biochemistry Lecture 33

Review

Final exam will cover the whole semester but will emphasize the second half (70-75%)

Applications of Enzymes

Cross linking

glutaraldehyde as crosslinking agent - how it reacts

Lifetime versus activity - reduced mobility of polypeptide prevents unfolding

- some residual flexibility may be necessary for catalytic function

Stability and reusability - lifetime extended by orders of magnitude

Thermal tolerance

- other effects that improve behaviour at high temperature
- enhanced reaction rate at high temperature

Tolerance to harsh clean up

- solvent, acid and base resistance (some limits to base resistance)
- avoiding biological contamination in prolonged usage

Immobilization

Change in mode of application - flow through versus batch, benefits

Immobilized cells - what about all the other enzymes in the cell

Not always applicable e.g. insoluble substrates - processing starch or cellulose, proteins

Oxidases and oxygenases

FAD dependent oxidases

analytical applications - variety of clinically or industrially relevant enzymes

Suitability for automated detection in analysis: O₂ electrode,

- substrate as analyte vs. enzyme as analyte
- enzyme electrode
- flow microcalorimetry
- oxidases have good thermal yield

Monooxygenases

Cytochrome P450 types versus non-heme iron types

- functions of monooxygenases
- reaction at inert C, suitability for drug processing
- Biotransformation versus purified enzyme

Steroid drugs

- biological action
- drug characteristics

Antibodies and their application

Structure of immunoglobulin	<ul style="list-style-type: none">- Domain organization- CDRs- Effector functions- Classes
Immunogens and antigens	<ul style="list-style-type: none">- Molecular characteristics
Immunogen preparation	<ul style="list-style-type: none">- Macromolecular and repetitive- persistence in host
Epitopes	<ul style="list-style-type: none">- sequence, conformational, limitations
Hapten-Carrier techniques	<ul style="list-style-type: none">- making a small molecule macromolecular, repetitive- affinity purification
synthetic immunogens	<ul style="list-style-type: none">- predicting antigenic epitopes from the sequence- Hoppe-Woods method
Gene organization of immunoglobulins	<ul style="list-style-type: none">- Heavy chain and light chain genes
Recombination as a method for generating diversity	<ul style="list-style-type: none">- recombination signal sequences
The immune response	<ul style="list-style-type: none">- Lymphocyte maturation- clonal selection- class switching- affinity maturation- primary and secondary responses- affinity and avidity
Hybridoma methods	<ul style="list-style-type: none">- myelomas- somatic cell hybridization- basis of HAT selection- dilution cloning
Immunoassays	<ul style="list-style-type: none">- Elisa etc