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NOTES

Immobilisation by covalent bonding :

Enzyme immobilization by covalent binding is one of the most widely used methods, in which stable complexes between functional groups on enzyme molecules and a support matrix are formed through covalent bondings.

The functional group present on enzyme, through which a covalent bond with support could be established, should be non-essential for enzymatic activity which usually involves binding via the side chains of lysine (ϵ-amino group), cysteine (thiol group) and aspartic and glutamic acids (carboxylic group).

The binding procedure of enzyme to the solid support generally goes through two stages:

(1) activation of the surface using linker molecules such as glutaraldehyde or carbodiimide and

(2) enzyme covalent coupling to the activated support.

Linker molecules are multifunctional reagents (glutaraldehyde or carbodiimide) While the first group matches the immobilization surface and forms a so-called Self-Assembled Monolayer (SAM), the second group bound to preactivated support then forms a covalent bond with the enzyme.

Different linkers are used for different surfaces (inorganic material, natural or synthetic polymer, membranes) and immobilization protocols (directly onto the transducer surface or onto a thin membrane fixed onto the transducer).act as the bridge between surface and enzyme via covalent bonding. Covalent immobilization provides strong bindings between enzymes and support matrix and therefore little leakage of enzyme from the support may occur. In addition, high uniformity of the SAM layer and good control of the immobilized enzyme amount are the other advantages.

In covalent attachment, there is a high risk of enzyme denaturization when most enzymes must go through chemical modifications to possess functional group. The immobilization procedure largely increases enzyme stability but decreases enzyme activity in affinity reaction and is poorly reproducible .

In comparison to adsorption, covalent bonding requires longer incubation time, since the formation of the SAM and the subsequent linkage of the enzymes to it take several hours. The process is also more complex and care has to be taken to ensure chemical purity so that the SAM is obtained in high homogeneity. The process is also more complex and care has to be taken to ensure chemical purity so that the SAM is obtained in high homogeneity. The most used procedures to covalently immobilize enzyme on functionalized surface (through the activations of carboxylic group and amino group) are briefly described,

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2.1. Activation of carboxylic groups:

A carbodiimide is a functional group (formula RN=C=NR) which allows the binding between the carboxyl groups (-COOH) of a support and the amino function (-NH2) of an enzyme. In order to improve immobilization efficiency, N-hydroxysuccinimide (NHS) could be associated to carbodiimide prior to enzyme covalent coupling step.

2.2. Activation of amino groups : The binding between an amine functionalized support and carboxyl functionalized enzyme could also be done with carbodiimides. Alternatively, glutaraldehyde could be used as the activating agent for enzyme immobilization.

Firstly, Schiff-base reaction occurs between amine functionalized support and an aldehyde group of glutaraldehyde and then, the second aldehyde group of glutaraldehyde covalently bind to an amine functionalized enzyme.

2.3. Chemisorption:

The principle of this immobilization method based on a strong affinity and semi-covalent bond between thiol group (-SH) and gold substrates (Au). Thus, thiol-containing enzymes, such as oxidoreductases and isomerases which contain double-catalytic site cysteine residues, could be immobilized on gold surface via the thiol groups of their amino acid residues.

The various methods used for entrapment include:

1. Electrochemical polymerization:

Electrochemical polymerization (or electropolymerization) is a simple approach in which an appropriate potential or current is applied into a solution containing both enzyme and monomer molecules.

The oxidization or reduction reactions of monomers occurred in the solution at electrode surface could then generate reactive radical species which couple together and finally form an adherent polymer at the electrode surface. Enzyme molecules that are present in the solution close by the electrode surface are trapped inside the growing polymer network as polymerization process propagates

2. Photopolymerization:

In photopolymerization process-based enzyme immobilization the use of liquid, photopolymers (radiation curable resins) and enzyme solution are required.

The photopolymerization reactions are chain-growth polymerizations which are initiated when the photopolymers exposed to light in the ultraviolet or visible region of the electromagnetic spectrum. Upon light exposure, these photopolymers undergo chemical reactions for cross-linking of molecules resulting in the hardening of the material.

3. Sol-gel Process:

The sol-gel process is based on the ability to form metaloxide, silica, and organosiloxane matrices of defined porosity by the reaction of organic precursors at room temperature . There are two generic methods of the sol-gel technique depending on the types of starting materials (precursors) used:

colloidal method, and

polymeric (or alkoxide) route.

In enzyme immobilization the latter method is commonly employed.