Immobilized enzymes

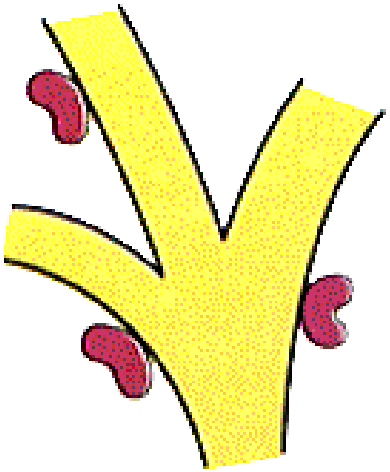
An **immobilized enzyme** is an enzyme which is attached to an inert, insoluble material. This can provide increased resistance to changes in conditions such as pH or temperature. It also allow enzymes to be held in place through out the reaction, following which they are easily separated from the products and may be used again.

There are a number of advantages to attaching enzymes to a solid support and a few of the major reasons are listed below:

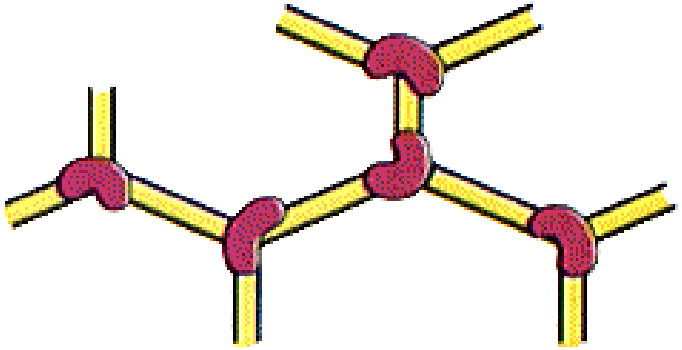
- Multiple or repetitive use of a single batch of enzymes
- The ability to stop the reaction rapidly by removing the enzyme from the reaction solution (or vice versa)
- Enzymes are usually stabilized by bounding
- Product is not contaminated with the enzyme (especially useful in the food and pharmaceutical industries)
- Analytical purposes: Long 1/2-life, predictable decay rates, elimination of reagent preparation, etc.

Methods used for the immobilization of enzymes fall into four main categories:

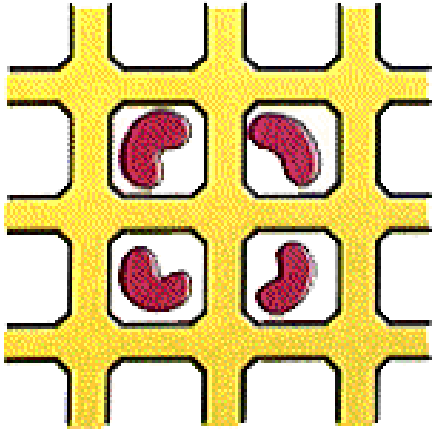
- Physical adsorption onto an inert carrier,
- Inclusion in the lattices of a polymerized gel (microencapsulation),
- Cross-linking of the protein with a bifunctional reagent
- Covalent binding to a reactive insoluble support.



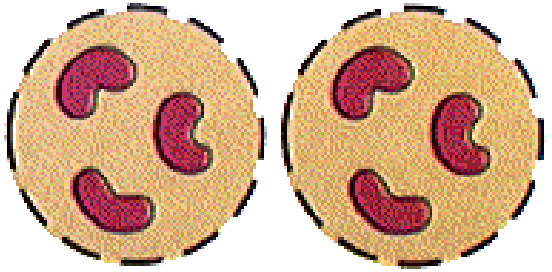
Carrier-bound enzyme



Cross-linked enzyme



Enzyme inclusion



Microcapsule

Adsorption

Physical adsorption of an enzyme onto a solid is probably the simplest way of preparing immobilized enzymes. The method relies on non-specific physical interaction between the enzyme protein and the surface of the matrix, brought about by mixing a concentrated solution of enzyme with the solid.

Advantages

A major advantage of adsorption as a general method of insolubilizing enzymes is that usually no reagents and only a minimum of activation steps are required. As a result, adsorption is cheap, easily carried out, and tends to be less disruptive to the enzymic protein than chemical means of attachment, the binding being mainly by hydrogen bonds, multiple salt linkages, and Van der Waal's forces.

Disadvantages

Because of the weak bonds involved, desorption of the protein resulting from changes in temperature, pH, ionic strength or even the mere presence of substrate, is often observed. Another disadvantage is non-specific further adsorption of other proteins or other substances as the immobilized enzyme is used. This may alter the properties of the immobilized enzyme or, if the substance adsorbed is a substrate for the enzyme, the rate will probably decrease depending on the surface mobility of enzyme and substrate.

Substrates for adsorption

1. Charcoal (catalse)
2. Aluminium hydroxide
3. Titanium dioxide
4. Silica
5. Calcium Phospphate (α-amylase)
6. Cellul,ose (Subtilisin)
7. Cellophane (glutaraldehyde)

Occlusion

Confining enzymes within the lattices of polymerized gels. This allows the free diffusion of low molecular weight substrates and reaction products. The usual method is to polymerize the hydrophilic matrix in an aqueous solution of the enzyme and break up the polymeric mass to the desired particle size.

Advantages

As there is no bond formation between the enzyme and the polymer matrix, occlusion provides a generally applicable method that, in theory, involves no disruption of the protein molecules.

Disadvantages

The free radicals generated on the course of the polymerization may affect the activity of entrapped enzymes. Another disadvantage is that only low molecular weight substrates can diffuse rapidly to the enzyme, thus making the method unsuitable for enzymes that act on macromolecular substrates, such as ribonuclease, trypsin, dextranase.

The broad distribution in pore size of synthetic gels of the polyacrylamide type inevitably results in leakage of the entrapped enzyme, even after prolonged washing. This may be overcome by cross-linking the entrapped protein with glutaraldehyde. Alternatively, ultrafiltration membranes of well-defined pore size may be used to occlude the enzyme.

Cross-Linking

Immobilization of enzymes has been achieved by intermolecular cross-linking of the protein, either to other protein molecules or to functional groups on an insoluble support matrix. Cross-linking an enzyme to itself is both expensive and insufficient, as some of the protein material will inevitably be acting mainly as a support, resulting in relatively low enzymatic activity. Generally, cross-linking is best used in conjunction with one of the other methods. Preventing leakage from polyacrylamide gels has already been mentioned, but it is used much more widely as a means of stablilizing adsorbed enzymes.

Since the enzyme is covalently linked to the support matrix, very little desorption is likely using this method. Marshall (1973), for example, reported that carbamyl phosphokinase cross-linked to alkylamine glass with glutaraldehyde lost only 16% of its activity after continuous use in a column at room temperature for fourteen days.

Covalent Binding

The most intensely studied of the immobilization techniques is the formation of covalent bonds between the enzyme and the support matrix. When trying to select the type of reaction by which a given protein should be insolubilized, the choice is limited by the fact that the binding reaction must be performed under conditions that do not cause loss of enzymatic activity, and the active site of the enzyme must be unaffected by the reagents used.

The functional groups of proteins suitable for covalent binding under mild conditions include

i. The alpha amino groups of the chain and the epsilon amino groups of lysine and arginine,
ii. The alpha carboxyl group of the chain end and the beta and gamma carboxyl groups of aspartic and glutamic acids,
iii. The phenol ring of tyrosine,
iv. The thiol group of cysteine,
v. The hydroxyl groups of serine and threonine,
vi. The imidazile group of histidine, and
vii. The indole group of tryptophan.

The wide variety of binding reactions, and insoluble carriers with functional groups capable of covalent coupling, or being activated to give such groups, makes this a generally applicable method of insolubilization.

The activated polymers used are hydrogels incorporated with diazo, carbodiimide or azide groups.

Advantage

This an advantage that the attachment in not reversed by pH, ionic strength or substrate.

Disadvantage

The active site may be blocked through the chemical reaction rendering the enzyme inactive.

Application of immobilized enzymes:

Antibiotics Production

Immobilized enzymes are used to produce 6-aminopenicillin acid, penicillin, cephalosporin.

Penicillin amidase immobilized by covalently binding with amberlite and crosslinked by glutaraldehyde, or physically adsorbed to bentonite is used for production of 6-APA, penicillin V. also ampicillin and amoxicillin are produced from 6-APA.

Cepholosporins are produced by cephalosporins amidase. Cephalexin and cephalosporin C can also be produced.

- **Steroid Production**

Synthesis of hydrocortisone and prednisolone

3. **Amino Acid Production**

Immobilized Amino acid acylase is used to resolute DL amino acid. the production of L-aspartic acid, L-tryptophan and L- alanine, L-DOPA (β-tyrosinase)

4. **Acids production**

Acetic acid, Citric acid, L-Malic acid, 2-ketogluconic acid and 12-ketochenodeoxycholic acid.

5.Other organic Compounds

Coenzyme A, FAD, pyridoxal 5 phosphate, Vitamin B_{12}, Proinsulin, prostaglandin, interleukin-2., anthraquinones.