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Mini Review

Alkaline Phosphatase: Different Method for Immobilization

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Enzyme immobilization is an important Technique to improve enzyme properties and stability. Immobilized protein increases reuse of enzyme in numerous cycles, thermal stability and periode time of activity of enzyme. Untile now, several research have focused on alkaline phosphatase immobilization. Alkaline phosphatase (*ALp*) is a hydrolase enzyme which removes phosphate groups from many kinds of molecules and degrades inorganic pyro phosphate (*pp_i*) to inorganic phosphate. These reactions are valuable in research and so is main concern in enzyme engineering. Accordingly, this mini review concentrate exclusively on different method of alkaline phosphatase immobilization.

Key words: Alkaline phosphatase, Immobilization, Matrix, Enzyme activity

The enzyme stability is an important subject with many applications in varied fields. Enzyme engineering via immobilization techniques such as adsorption, multipoint and multi subunit covalent binding and entrapment are suitable approches to improve enzyme properties including stability, specificity, activity, inhibition by reaction products (Betancor et al., 2006) (Fig 1). The immobilized enzyme shows operational activity such as rapid termination of reaction, control of product formation and easy isolation from the reaction mixture. Usually the immobilized enzymes have much better functional properties than the free ones in the harsh condition Also, the immobilized enzyme is able to remain active for a long time and be reused in industrial reactors for several times (Cesar et al., 2007; Fernandes et al., 2003). There are different organic and inorganic supports such as polymers and nanoparticles which can be chosen as suitable supports for enzyme immobilization to ensure the highest retention of enzyme activity and its stability (Fernandes et al., 2004; Gerard et al., 2002; Gomez et al., 2006).

Alkaline phosphatase (*Aps; EC* 3.1.3.1) are homodimeric enzymes that are widely distributed in nature, and are found in many organisms from procaryotes to eukaryotes (Coleman, 1983). Alkaline phosphatases are metalloenzymes with three metal ions in each catalytic site, two *Zn* and one *Mg*, which catalyzes the cleavage of orthophosphate from orthophosphoric monoesters under

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alkaline conditions. And also trans-phosphorylation reaction is catalyzed by *Aps* in the presence of large concentrations of phosphate acceptors (Millan, 2005) (Fig2). This reaction is ueful in research and therefore alkaline phosphatase could be employed commonly in several ways. It is labeling enzyme which is used in electrochemical enzyme immunoassays and immunosensors (Montornes, 2006). In addition, it is a beneficial tool in molecular biology laboratories for keeping DNA molecules; another important application of alkaline phosphatase is as a marker of pasteurization in cowmilk. Therefore, alkaline phosphatase immobilization is a main issue in Enzyme engineering and this mini review will focous on the most important immobilization techniques.

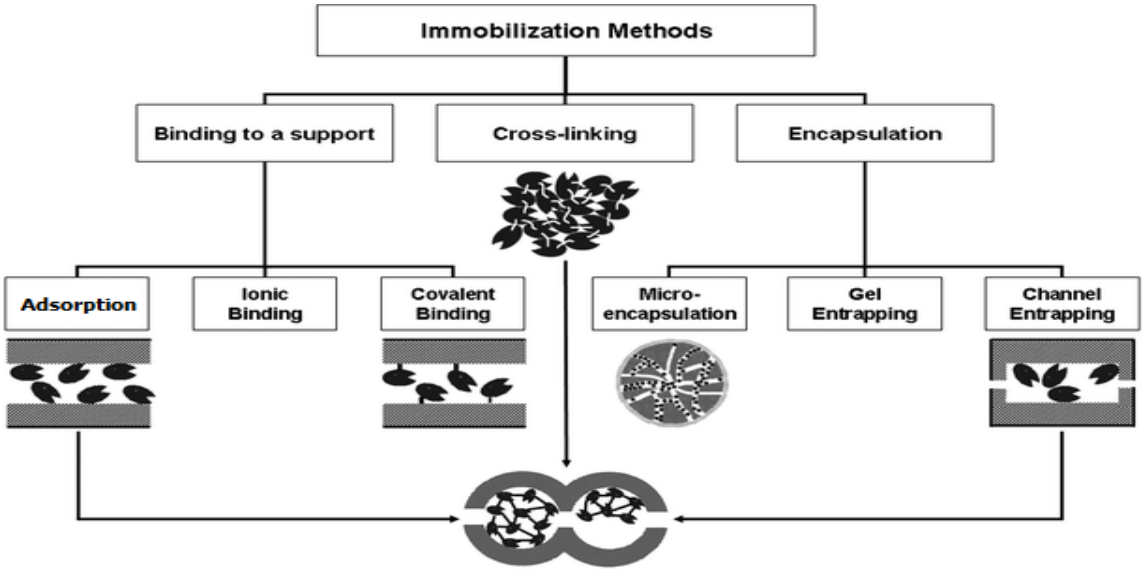

Fig 1. Overview on different Kind of immobilization method (Hartmann et al., 2010)

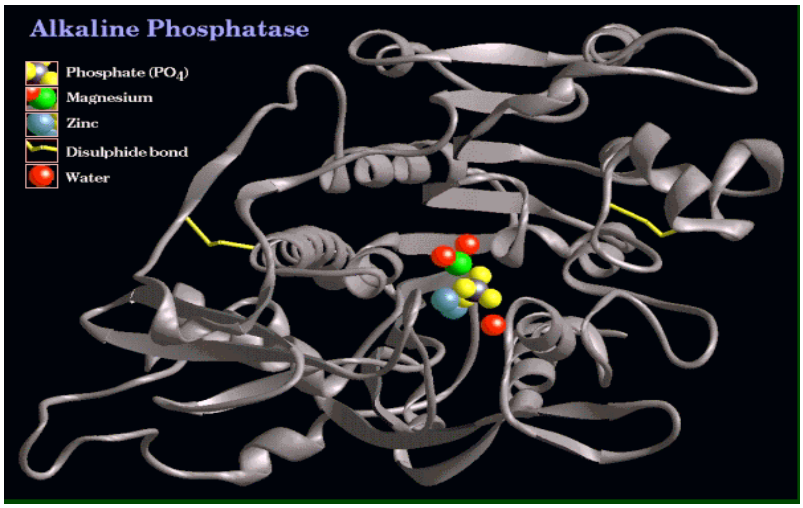

Fig 2. Structure of alkaline phosphatase (biochem.szote.u-szeged.hu)

Enzyme Immobilization

There are different kinds of matrix including beads, fibers, films, and membranes and also varied alternative coupling reaction which have been used in alkaline phosphatse immobilization. The most common of coupling reactions are adsorption, covalent bonding, and cross-linking that we aim to describe in this review.

1. Cross-linking Method

Cross-linking or co-polymerization is covalent bonding between enzyme and various matrixes via a polyfunctional reagent. Weetall in 1969 repotred that 0.74 mg of *ALPase* per gram of glass could be immobilized by adding the enzyme to glass beads chemically activated with diazo groups (Weetall, 1969).

Although alkaline phosphatase was immobilized on aminated glass surface using cross-linking method without chemical reagent. This process is based on intraction between ammonium and carboxylate ions to easily form an amide linkage under vacue. This research demonstrated that vacue immobilization process was more effective than traditional chemical method especially for small amounts of protein. Its result investigated that about 60% of the *ALPase* activity was removed after the first trial but there was no further loss of activity in later trials (Taylor et al., 2005).

Sharmin and coworkers in 2007 provided new reagent for enzyme immobilization. They employed Glycidoxy-propyle trimethoxysilane and so amide bonds were formed between carboxyls groups on the enzyme and amino group on the glass surface in vacuum condition with the loss of hydrochloric acid. In this method immobilized and free enzyme have a same ativity in different pH and about 16% of activity was eliminated after the first trial which is less than other method(Sharmin et al., 2007) .

This enzyme was immobilized on microscope glass slides which were coated with unstructured or mesoporous silica films. The protein was attached to the surface by trialkoxysilanes with different functional groups like amino, epoxy, and urea functions. Especially, combination of a mesoporous with amino group resulted in the highest activity of bound *ALP* (Ehlert et al., 2010). Alkaline phosphatase was also immobilized on fibrin scaffolds using carbodiimide hydrochloride (*EDC*) and N-hydroxysuccinimide (*NHS*) (Osathanon et al., 2009).

2. Covalent Method

This method is based on forming covalent bond between the chemical groups of enzyme and chemical groups on surface of matix. Alkaline phosphatase was covalently immobilized on low-density polyethylene (*LDPE*) films which were cografted with vinylene carbonate (*VCA*) and N-vinyl-N-methyl acetamide (*VTMA*). This matrix was used for enzyme immobilized in both direct fixation and inserting spacer. Water –soluble alkyldiamines such as diaminoethylene, diaminobutane, diethylenetriamine, and diaminohexane were utilized as agent for crosslinking method (Chen et al., 1993).

Poly venylene carbonate (*PVCA*) was also cross-linked with jeffamines to produced microspheric hydrogel beads with a high water content and a high intensive of reactive cyclic carbonate (*CCA*) groups for the immobilized enzyme. Immobilization of *ALP* on hydrogel beads showed a higher thermal stability and residual activity after repeated use in comparison with native enzyme (Chen et al., 1993).

Alkaline phosphatase was also immobilized on polystyrene surface using the enzymatic reaction of microbial trans-glutaminase (*MTG*) by Kamiya. MTG catalyzed ε-(γ-glutamyl) lysine bond formation between the recombinant

Escherichia coli alkaline phosphatase and β-casein or *BSA* which were physically coated on polystyrene. Their result reported that this method could be sutable for devising of functional solid biomaterial such as protein microarrays and immobilized enzyme (Kamiya, 2007).

Moryiama in 2011 used *MTG* for immobilization of bacterial alkaline phosphatase (*BAP*) on magnetic particles (*MPs*). *MTG* is recognized a *Gln* residue on peptide tage and the surface of the *MPs* is changed with diethyleneglycol bis (3-aminopropyl) ethr (*DGBE*). This modification suppressed physical adsorption of *CQ6-BAPs* by the change with hydrophilic molecules such as *DGBE* and *EA* on the surface of *MPs*. Their result illustrated that the immobilized *BAP* preserved more than 90% of the initial activity after 10 rounds of assaying the activity of the tethered *CQ6-BAP* (Moriyama et al., 2011).

3. Adsorption Method

An enzyme may be immobilized to either external or internal surface of a matrix. Low energy bonds such as ionic interaction, hydrogen bond, vander Waals forces are involved in adsorption method. In general Michaelis behaviour of the enzyme conserves during the adsorption process but kinetic constants may be changed (V_{max} is normally decreased and Km rose) (Boyd, 1990).

Alkalne phosphatase was adsorbed on Na-sepiolite, which is an insoluble carrier, similar to the clay-enzyme complexes. There was not appreciably change in its kinetic and operative characteristics but, immobilized enzyme had lower stability against storage at 30 ^{0}C, thermal denaturation and proteolysis in compered with free enzyme (Carrasco et al., 1995).

The enzyme was adsorbed on top of or be embedded in layer-by-layer(*LBL*) film made of poly-l- glutamic(*PGA*) acid and poly-l-lysine(*PLL*) with an extension to the functionalization of Affi-gel heparin beads. As a result of this research, no further desorption is observed over storage time larger than 3 months when *ALP* is immobilized on the top of the *LBL* architecture. Moreover adsorbed or embedded *ALP* molecules remain partly active for such long storage periods (Derbal et al., 2003).

Sedaghat et al used modified and unmodified Na-sepiolite as matrix for *ALP* immobilization. Sepiolite discloses difference attractive properties such as high specific surface area, high porosity, and surface activity. Hence, it can be a sutable matrix for organic and inorganic materials immobilization. Sepiolite was covered with bilayer surfactant (*SBS*). *SBS* is an effective sorbent for *ALP* enzyme because it cases to forming electrostatic attraction between cationic head groups of the surfactant molecules and the anionic groups of the enzyme. Their results illustrated that the stability of *ALP* increased whilst the activity reduced after immobilization on matrix (Sedaghat et al., 2009). The enzyme was also immobilized on Na-bentonite and modified bentonite. Modification of bentonite was related to adsorption of surfactant monolayer and bilayers. They reported that, the activity of immobilized enzyme after 20-25 days was lower than that of the free enzyme and it reached maximum activity but it remained even after 70 days. However, immobilized phosphatase remaied undenatured during storage at 30 ^{0}C (Ghiaci et al., 2009)

Conclusion and Further Remarks:

A variety of matrixes and methods were used for alkaline phosphatase immobilization. They mostly increased the storage stability and reusability of ALP. The research in the field of enzyme immobilization is ongoing. What usually is sought is that a newly-found matrix functions better in alkaline phosphatase immobilization than ever before ones. A substantial part of this review is committed to various matrixes applied to

alkaline phosphatase Immobilization that will direct researchers to use new matrices with greatest effect on immobilization.

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