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Immobilization of α-amylase onto a calix[4]arene derivative: Evaluation of its enzymatic activity

Irshad Ali Veesar [a], Imam Bakhsh Solangi [b,*], Shahabuddin Memon [a]

[a] National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan
[b] Dr. M.A. Kazi Institute of Chemistry, University of Sindh, Jamshoro 76080, Pakistan

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ABSTRACT

In order to enhance the cost-effectiveness practicability of enzymes in many industries such as pharmaceutical, food, medical and some other technological processes, there is great need to immobilize them onto a solid supports. In this study, a new and efficient immobilization of α-amylase from *Saccharomyces cerevisiae* has been developed by using the surface functionalization of calix[4]arene as support. A glutaraldehyde-containing amino group functionalized calix[4]arene was used to immobilize α-amylase covalently. In this procedure, imide bonds are formed between amino groups on the protein and aldehyde groups on the calix[4]arene surface. The surface modified support was characterized using Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM). The effect of various preparation conditions on the immobilized α-amylase process such as immobilization time, enzyme concentration, temperature and pH were investigated. The influence of pH and temperature on the activity of free and immobilized α-amylase was also studied using starch as substrate. The optimum reaction temperature and pH value for the enzymatic conversion catalyzed by the immobilized α-amylase were 25 °C and 7, respectively. Compared to the free enzyme, the immobilized α-amylase retained 85% of its original activity and exhibited significant thermal stability than the free one and excellent durability.

1. Introduction

During last couple of decades, concentrated investigation in enzyme technology has attended numerous advancements, which assist their numerous useful applications. Catalysts such as enzymes are highly competent and particular under prominent conditions; because of that catalytic methods have found large number of applications in industries. General prospects from enzymes that are employed in industries are operational period enhancement, effective utilization of the reactants and maximization of catalytic velocity [1–4]. The amylases are biocatalysts that are part of an enzyme family most commonly utilized in food industry and fermentation; particularly for the hydrolysis of starch and other related biological compounds like dextrin as well as gradually smaller polymers made up of glucose units. Amylases generally exist in plants, animals and microorganisms. The soluble enzymes are frequently immobilized onto a solid supports in order to enhance their cost-effective practicability in many industries such as pharmaceutical, food, medical and some other technological and industrial processes [5–9]. For the industry, it has become a matter of high interest to immobilize enzymes to solid and insoluble supports [10]. The immobilized enzymes have some common operational benefits such as chance of continuous as well as batch operational modes, rapid termination of reactions, reusability, product formation in controlled manner and separation of the formed product is quite easy [11–13].

In the literature, diverse modes of stabilization of enzymes on different kinds of carriers are present. Immobilization can be a chemical or physical method. In chemical immobilization covalent or ionic bonds are formed between the enzyme and the support. In physical methods, adsorption or encapsulation of the enzyme occurs in or on a solid support substance [14–18]. These supporting materials can be a gel matrix, a fabric or ordinary solid in particle, membrane and/or in microsphere form. While an enzyme is immobilized to the support, the functional moieties situated at the active site of the enzyme are responsible for the catalytic reaction [19]. The advantages associated with enzyme immobilization through covalent binding are;

* Corresponding author. Tel.: +92 22 9213429 30; fax: +92 22 9213431.
E-mail address: imamsolangi@yahoo.com (I.B. Solangi).

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(1) The enzymes do not pour out or separate because of rigid binding from the carrier during utilization.
(2) The attached enzymes can simply get in touch with the reaction media as it is restricted on the surface of carrier.
(3) Elevation in heat stability is often practiced due to strong covalent bonding of the enzyme with support material [20].

It is multitalented to apply the glutaraldehyde technique in various fashions. Conversely, in provisions of stabilization, the primary amino groups offer excellent results in numerous studies by treatment with glutaraldehyde most commonly used as linker through which proteins are bonded with support [21–23]. On the enzyme surface, amino groups are plentiful and reactive and they form Schiff bases with support and the aldehyde groups. The formation of covalent bonds between enzyme and support depend on the degree of activation and concentration of both aldehyde and amino groups [24].

The nature of supports and immobilization strategy greatly affects some of the properties like kinetics and stability of immobilized enzymes. At present a huge number of organic and inorganic substances such as macromolecules and synthetic polymers are being used as supports [25–27].

Calix[*n*]arenes are molecules of synthetic organic compounds formed through a phenol formaldehyde condensation reaction. They have attained a significant attention as valuable building blocks for the synthesis of hosts for many species such as cations, anions and neutral molecules. The growing curiosity in calix[4]arene is encouraged due to straightforward preparative level calix[4]arene synthesis as well as number of ways through which these are selectively functionalized at either the lower rim having phenolic groups or the upper rim containing aromatic nuclei [28]. To the best of our knowledge, there are a few studies on the use of calix[4]arene 1,3-distal glutaraldehyde derivative as a cross linker reagent for alpha amylase immobilization [29–31]. The chief uniqueness of the material support, *i.e.* calix[4]arene derivative is its easy preparation, non-toxic nature, thermal stability and reusability, which can effortlessly be achieved from economical raw material throughout a known organic synthetic tactic as mentioned in Scheme 1.

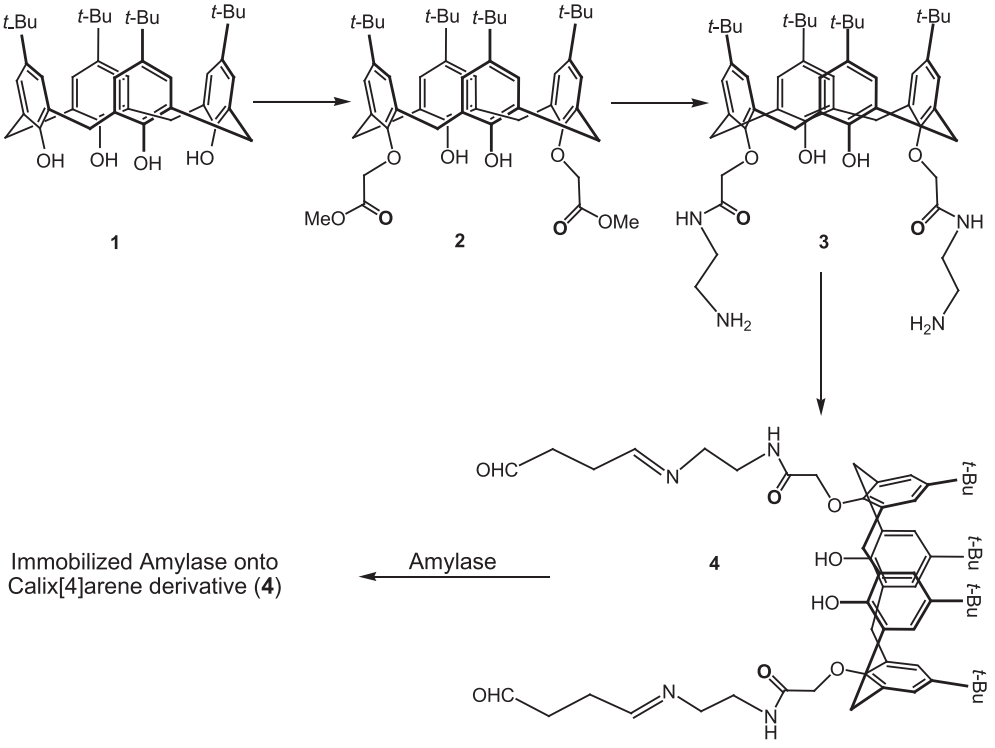
2. Materials and methods

2.1. Materials and instrumentation

A commercial enzyme α-amylase (1,4-alpha-D-Glycan-glycan hydrolase, E.C 3.2.1.1) used for the immobilization, Bovine Serum Albumin (BSA), Triton-X100 reagent, starch and maltose were procured from Sigma Chemical Co. Other analytical grade reagents and chemicals were purchased from Merck, Aldrich or Fluka, and used without any additional purification. All other solvents, which were of commercial grade were subjected to distillation and kept in safe environment. The double distilled water was used to prepare aqueous solutions. For recording UV–vis. Spectra, a Perkin Elmer Lambda 35 model UV–vis. spectrophotometer was used having standard 1.00 cm path length quartz cells. The pH meter (781-pH/Ion meter, Metrohm) was used for pH measurements accompanied with internal reference electrode and glass electrode. Merck prepared plates (silica gel 60 F254 on aluminum) were used for performing analytical TLC. A Centrifuge of WIROWKA Laboratoryjna type WE-1, nr-6933 (speed range 0–6000 rpm with a timer 0–60 min, 220/50 Hz, Mechanika Phecyzyjna, Poland) was used for centrifugation. Thermo Nicolet Avatar 320 FT-IR spectrometer equipped with deuterated triglycine sulfate (DTGS) detector having KBr optics was used to record FT-IR spectra of the compounds.

2.2. Synthesis

The *p-tert*-butylcalix[4]arene (**1**) was synthesized as expressed in previously published procedures [32,33] followed by the preparation of 5,11,17,23-tert-butyl-25,27-ethoxycarbonyl-ethox



Scheme 1. Synthetic pathway for the preparation of **4** and immobilization of α-amylase.