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Maltase entrapment approach as an efficient alternative to increase the stability and recycling efficiency of free enzyme within agarose matrix

Muhammad Asif Nawaz[a,b,*], Asad Karim[b], Zainab Bibi[b], Haneef Ur Rehman[c], Afsheen Aman[b], Dilshad Hussain[d], Midrar Ullah[a], Shah Ali Ul Qader[b]

[a] Department of Biotechnology, Shaheed Benazir Bhutto University, Sheringal, Dir (Upper), KPK, Pakistan
[b] The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi 75270, Pakistan
[c] Department of Chemistry, University of Turbat, Kech, Balochistan, Pakistan
[d] Division of Analytical Chemistry, Institute of Chemical Science, Bahauddin Zakariya University, Multan, Pakistan

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ABSTRACT

Maltase catalyses the hydrolysis of maltose and is widely used in the synthesis of various food and pharmaceutical products. In the current study, matrix entrapment technique was applied to immobilize maltase within agarose beads. Maximum immobilization yield (76.11%) was achieved at 3% agarose concentration and 5.0 mm beads size was found to be optimum combination for maximum catalytic activity of entrapped maltase. It was noticed that entrapment increased the optimum reaction temperature and pH of maltase from 45°C to 60°C and 6.5 to 7.0, respectively with reference to its free enzyme whereas no effect was observed on reaction time. Entrapped maltase showed increase in K_m value from 1.717 to 1.912 mM ml^{-1} and decrease in V_{max} value from 8411.0 to 6214.0 U ml^{-1} min^{-1} as compared to free enzyme. Entrapped maltase displayed broad thermal stability up to 80°C whereas; free enzyme completely lost it activity when temperature reached up to 60°C. Scanning electron microscopy of agarose beads before and after maltase entrapment revealed significant morphological change on the matrix surface. Considering the economic feasibility, the entrapped maltase indicated imperative recycling efficiency up to ten reaction cycles.

1. Introduction

Enzymes have drawn the considerable attention of researchers due to high catalytic efficiency, selectivity and specificity under mild reaction conditions. These properties make them to be used in food, textile, detergents and pharmaceutical industries to catalyse specific biochemical reaction. The use of free enzyme is mostly hampered due to various problems which are faced during downstream processing. Purification of synthesized product and separation of enzyme from reaction mixture increase the total production cost of the process. The denaturation of enzyme under extreme industrial conditions is another factor which limits the utility of free enzyme [1]. Up-to-date, biotechnologists have applied various techniques such as protein engineering, chemical modification, additives treatment and immobilization to improve the catalytic efficiency and stability of enzymes from their native form to industrial bioreactor [2–4]. Among them, immobilization is an encouraging way that not only retains the stability of enzymes against different extreme conditions but also ensures the continuous reusability of expensive enzymes for industrial bioprocesses [5–8].

Immobilization can be characterized into three types such as physical adsorption, covalent binding and entrapment. Physical adsorption and covalent attachment both have disadvantages because they have possibility to alter the substrate binding sites of enzyme and also enforce diffusion restriction on the enzyme which eventually decreases the catalytic efficiency of enzyme. Entrapment is one of the widely investigated immobilization methods where enzymes are enclosed or confined within polymer matrix without altering their native structure and used to develop bioreactors for commercial applications [9–11]. This method is based on the simple and single step procedure which induces no conformation alteration on the active site of enzyme and makes the process easy for the diffusion of substrate and product [12,13]. A variety of matrices such as alginate, agar-agar, carrageenan, agarose, polyacrylamide, pectin, chitosan and gelatin have been evaluated as a polymer matrix sup-

* Corresponding author at: Department of Biotechnology, Shaheed Benazir Bhutto University, Sheringal, Dir (Upper), KPK, Pakistan Tel.: +923449236028.
E-mail address: asif_biotech33@yahoo.com, asif_biotech33@sbbu.edu.pk (M.A. Nawaz).

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port for the immobilization of enzymes. Among them, agarose is believed to be more biocompatible and inert support. It is a linear polymer derived from various marine algae and chemically composed of D-galactose and 3,6-anhydro-L-galactopyranose which are linked through α-(1→3) and β-(1→4) glycosidic bonds [14]. It has porous structure which creates an efficient microenvironment for enzyme-substrate reaction and to release end product into the reaction mixture [15].

Maltase [EC 3.2.1.20] is one of the most economically valuable enzymes that catalyses the hydrolysis reaction of maltose by cleaving α-1,4 glucosidic linkages found between glucose moieties and yields α-D-glucose as an end product. This hydrolase plays a pivotal role in biological systems and also has numerous applications in food processing, brewing, distilling and pharmaceutical industries [16–18]. Maltase is also employed during the process of starch hydrolysis and releases glucose that is used for the preparation of glucose syrups [19]. This product is further utilized as a major ingredient in the process of soft drinks production and in the bakery products synthesis.

The current study is an attempt made to consider the significance of maltase in various industrial processes. Maltase from *Bacillus licheniformis* KIBGE-IB4 was immobilized within porous agarose matrix following entrapment method. Different concentrations of agarose were investigated to attain the maximum immobilization yield of maltase. Immobilized enzyme was characterized on the basis of its catalytic activity and compared to its free counterpart to evaluate the effect of polymer on enzyme catalytic efficiency. Thermal deactivation and reusability of immobilized maltase were also examined to analyse its potential for commercialization applications.

2. Materials and methods

2.1. Production and partial purification of maltase

Bacillus licheniformis KIBGE-IB4 [GenBank Accession: GU216261] was used in this study for the production of maltase. Bacterium was cultivated into previously optimized growth medium containing (%) wheat starch, 2.5; peptone, 1.0; yeast extract, 0.2; meat extract, 0.4; K_2HPO_4, 0.3; and KH_2PO_4, 0.1 with pH-7.0 and incubated at 37°C for 48 h [20]. Cells were harvested by centrifugation at 40,248 × g for 10.0 min and cell free filtrate was subjected to partial purification using 40% ammonium sulfate saturation. Enzyme precipitates were desalted using dialysis tubing (12,000 kDa cut-off, Servapor®) against potassium phosphate buffer (pH-6.5, 0.1 M) for 18 h at 4°C and stored at same temperature for enzyme immobilization within agarose matrix support.

2.2. Maltase activity assay

The catalytic activity of free and immobilized maltase was determined by GOD-PAP method [21,22] using maltose as a substrate and glucose as a standard. Free enzyme (50.0 μl, 9136.0 U^{-1} mg) was incubated with 1.0 ml maltose (7.2 g L^{-1}dissolved in 0.1 M potassium phosphate buffer, pH-6.5) at 45°C for 5.0 min. Afterwards, the enzyme-substrate reaction was terminated by placing the test tubes in boiling water bath for 5.0 min. The glucose released after the enzymatic degradation of maltose was estimated through glucose oxidase (GOD-PAP) method at 546 nm. Slightly modified procedure was used for the entrapped maltase where 0.5 g beads (9136.0 U^{-1} mg) were incubated with 1.0 ml substrate at 60°C for 5.0 min. Then, the reaction mixture was boiled for 5.0 min after separating the maltase entrapped agarose beads. Afterwards, same protocol was followed as mentioned above for free enzyme. One unit of maltase was defined as "*the amount of enzyme required to release 1.0 μmol of glucose per minute under standard assay conditions*".

2.3. Entrapment of maltase within agarose matrix support

The entrapment of maltase was initiated by mixing equal quantity (1:1) of dialyzed enzyme and 3.0% agarose solution. Agarose solution was prepared in potassium phosphate buffer (pH-6.5, 0.1 M) by vigorous shaking at 100°C and was allowed to cool at room temperature until unless 40–45°C achieved. Afterwards, enzyme was incorporated and mixed thoroughly. This mixture was directly casted into glass plates and was kept at 4°C for one hour to completely solidify the gel matrix. Polymer beads with and without immobilized maltase were prepared by using metallic borer (5.0 mm). Beads were washed with double deionized water and potassium phosphate buffer (pH-6.5) three times prior to perform enzyme assay. All experimental work for enzyme characterization was carried out in batch reactor (under continuous stirring) and in triplicate. The results mentioned are the mean values of three observations.

2.4. Agarose concentration effect on the immobilization yield of maltase

Agarose concentration was varied from 1.0 to 6.0% to achieve the optimal level for maximum percent immobilization yield of maltase. The polymer solution was prepared by heating in water bath at 100°C in the presence of potassium phosphate buffer (pH-6.5) and transparent solution was used to prepare agarose beads without and with immobilized maltase. Percent immobilization yield was defined as the total percent of the ratio of immobilized enzyme activity to the activity of free enzyme. It was calculated using the following formula:

$$\text{Immobilization yield (\%)} = \frac{\text{Catalytic activity of the immobilized enzyme}}{\text{Catalytic activity of the free enzyme}} \times 100$$

2.5. Optimum beads size for the maximum activity of immobilized maltase

The effect of different beads size was evaluated to attain the maximum immobilization yield of entrapped maltase. The beads of different sizes ranging from 3.0 to 10.0 mm were prepared using metallic borer and enzyme assay was performed.

2.6. Optimum reaction temperature for free and immobilized maltase

The effect of temperature was analysed on the catalytic activity of free and entrapped maltase by conducting the enzyme assay at different temperatures ranging from 30°C to 80°C at a constant pH, reaction time and substrate concentration.

2.7. Optimum reaction pH for free and immobilized maltase

The optimum pH of free and entrapped maltase was determined by performing the enzyme assay in different pH buffers ranging from 5.0 to 8.0. Buffers system of citrate (pH-5.0 and 6.0) and potassium phosphate (pH-6.5 and 8.0) with same ionic strength (0.1 M) were investigated in this experiment.

2.8. Optimum reaction time for free and immobilized maltase

The influence of different reaction time was examined in order to attain the optimum catalytic activity of free and entrapped maltase. For this purpose, free and immobilized enzyme was incubated with maltose (substrate) for different time (5.0–60.0 min).