IMMOBILIZATION OF CATALASE BY CROSS-LINKING OF PERMEABILIZED YEAST CELLS IN A PROTEIN SUPPORT

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Abstract

Catalase was induced in yeast cells through aeration. The cells were permeabilised for expression of intracellular catalase activity by treatment with toluene. Permeabilised cells were then immobilized in hen egg white using glutaraldehyde as the cross-linking agent. Immobilized cells could be reused for the removal of $\text{H}_2\text{O}_2$ from milk.

Introduction

Immobilized catalase (EC 1.11.1.6) can find applications in the food industry in the removal of excess $\text{H}_2\text{O}_2$ after cold pasteurization of milk and in combination with a variety of oxidase including glucose oxidase in the production of gluconic acid, removal of oxygen and/or glucose and in the treatment of waste water containing $\text{H}_2\text{O}_2$ [1-4]. All these studies have been carried out by immobilization of cell free enzyme of animal and microbial origin [1,2,4]. Major limitation has been the cost and the inactivation of such systems during continuous use. Unlike cell free enzyme preparation, the enzymes have been often found to be more stable inside the whole cells [5]. Even though a number of aerobically grown microbial cells exhibit high catalase activity, not many reports are available on their use in an immobilized form. The present paper describes a simple method for the preparation of immobilized permeabilized yeast cell based catalase by cross-linking in hen egg white, its characterization and its use for the removal of $\text{H}_2\text{O}_2$ from milk.

Materials and methods

Materials

Leghorn varieties of hen eggs were purchased from local market. The media constituents including yeast extract, bactopeptone and agar were procured from Difco Laboratories. Other chemicals were obtained from standard sources.

Culture Conditions and Induction of
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Saccharomyces cerevisiae (ATCC 3177) was maintained on agar slants. Catalase was induced in yeast by the method described by D’Souza [6]. The cells were grown anaerobically in a medium containing 2% glucose; 1% bactopeptone and 0.3% yeast extract and transferred after 24h to “non-growth” medium (pH 6.8) containing 1% glucose, 1% KH$_2$PO$_4$, 0.01% CaCl$_2$ and 0.01% MgSO$_4$. Catalase was induced in these cells by subjecting them to aeration for 6h at 27°C. The cells were then washed with cold saline and suspended in a 0.05 M phosphate buffer pH 7.0.

Cell permeabilization

Yeast cells (1g) in 5ml 0.05M phosphate buffer (pH7.0) were stirred in cold (0-4°C) for 15 min with 5ml of toluene. This treatment was repeated, after which the cells were washed with phosphate buffer. Permeabilization of yeast cells with toluene increased the catalase activity from 300 to 1880U/g of cells.

Method of enzyme immobilization

The yeast cells were immobilized in hen egg white as follows. The egg white (10ml) obtained from fresh eggs was mixed thoroughly with permeabilized yeast cells (2g) and then treated with glutaraldehyde to a final concentration of 2%. The mixture was stirred well and allowed to stand for 2h at 4°C. The hard gel obtained was shattered by passing through a syringe, and washed with water to remove excess of glutaraldehyde and stored in phosphate buffer at 4°C.

Assay of Catalase Activity

Yeast cells (40mg, entrapped or otherwise) were stirred with 20 ml of 0.08 M H$_2$O$_2$ in 0.05M phosphate buffer pH 7.0, and the rate of disappearance of the substrate was measured according to the method described by Sinha [7]. One unit of enzyme activity is defined as the enzyme responsible to degrade one micromole of substrate( H$_2$O$_2$ ) in one minute.

Results and Discussion

Optimum condition for the immobilization

Gel preparations with optimal retention of enzyme activity and mechanical stability were obtained when cross-linking was carried out for 2 h at 4°C using 2 % glutaraldehyde (Fig 1). Higher temperature (27°C) even though gave mechanically stable gels resulted in considerable inactivation of the enzyme activity.

Properties of immobilized catalase

All the cell preparations (intact and permeabilized) in free and immobilized form exhibited pH optima at 7.0. (Fig.2). Optimal activity was obtained with a buffer concentration of 50 mM. (Fig.3). The thermo stability characteristics of catalase in yeast cells was studied by subjecting the cell suspension to the desired temperature for 10 min followed by rapid cooling in ice. The residual enzyme activity was determined at room temperature. Immobilization was found to
enhance the thermostability (Fig. 4). Thus at 45°C the free cells retained only 35% of the original activity as compared to 75% retention of activity seen with the immobilized cells. Immobilized cell preparation can be stored at 4°C for about 40 days retaining over 90% of the original activity.

Operational stability of the immobilized catalase

Reuse of immobilized permeabilized yeast cell catalase for the removal of H₂O₂ was studied in a batch reactor system. The gel particles containing 7g of permeabilized cells was mixed in 400ml of 0.05mM, phosphate buffer pH 7.0 containing 500ppm of H₂O₂ at room temperature for 1 h under stirring. The enzyme gel particles were recovered by filtration using a sieve, washed with buffer and resuspended in a fresh batch of buffer containing 500ppm of H₂O₂. The process was repeated 10 times. It was observed that the immobilized enzyme was stable for repeated use.

Application of immobilized enzyme

One of the major applications of catalase is in the removal of H₂O₂ from milk after cold pasteurization. Milk (350 ml) was mixed with 500ppm of H₂O₂ and then treated with 7.0g of entrapped permeabilized yeast cells. The time required for complete degradation of H₂O₂ from the milk was found to be 1h. The immobilized enzyme was reused in 12 batches over a period of 5days without loss in efficiency. The decomposition of H₂O₂ in milk due to immobilized catalase in a batch process was measured by first precipitating the milk proteins with equal volume of 6% trichloroacetic acid. Residual H₂O₂ concentration was measured at 415 nm after addition of 2ml TiCl₄ solution (4mg/ml, 5.5N HCl) into 5ml of
sample that had been membrane filtered (0.2µm) after protein precipitation [8].

Conclusions

Permeabilization of yeast cells was found to be essential for maximum expression of intracellular catalase activity. Permeabilization of yeast cells with toluene increased the catalase activity from 300 to 1880U/g of cells. Permeabilized cells in turn can act as an economical source of catalase as compared to commercially available purified enzyme preparations. Further the permeabilized cells can be immobilized in hen egg white by cross-linking with glutaraldehyde for its economical reuse. This technique could find potential in the future for immobilization of microbial catalases. The major advantage as compared to the earlier described technique of entrapment in synthetic polymers like polyacrylamide [6] is the non-toxicity of the hen egg white support thus paving its use in food industry as in the treatment of milk. One of the major problems in the treatment of food material such as milk is the possible microbial contamination of the support. Our earlier studies have shown that hen egg white can serve as a self sterilizing support in view of the large quantities of lysozyme naturally present in the egg white which also gets co-cross-linked (9). Glutaraldehyde used for cross-linking has also been classified under Generally Regarded as Safe (GRAS) category.

References

Dr. S. F. D’Souza is currently the Associate Director-A, Biomedical Group and Head of the Nuclear Agriculture and Biotechnology Division of BARC. His major research interests are in the field of Enzyme and Microbial Biotechnology with special reference to immobilized enzymes and cells for use in bioprocessing, biosensors and bioremediation.

Mr. Bansidhar S. Kubal is currently working in the Nuclear Agriculture and Biotechnology Division. His major research contributions have been in the field of immobilized enzyme technology with special reference to invertase, catalase and amylase. He has to his credit 14 research papers in the national, international journals and symposia.