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Immobilization of Cellulase enzyme from *Aspergillus niger* on amyloid nanofibers and its kinetic comparison with the free enzyme

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Introduction

Cellulase enzyme is an industrial enzyme that is produced by bacteria and fungi and is used in various fields such as food, agriculture, textile, detergents, animal feed, pulp and paper industries, as well as in research development. In addition to maintaining the activity of the enzyme during the reaction, cellulase immobilization enables its repeated use. In this research, amyloid nanofibers obtained from bovine serum albumin were used to immobilize cellulase enzyme. Bovine serum albumin, as one of the cheap and available proteins, can be a good model to investigate the process of aggregation and fibrillation. This protein has a potential and important role in the tissue adaptation of the body, and hence its use from this point of view can introduce us to new nanomaterials in the field of nanotechnology. Although amyloid nano–fibrils are known as a pathogenic agent for living systems, but considering their biological nature and their nano–dimensions, they can be introduced as a new biological nanomaterial.

Material and Methods

Bovine serum albumin protein was dissolved in citrate-phosphate mixed buffer with different pH and containing 0.05 % by weight/volume of sodium azide (to prevent microbial and fungal contamination) and the protein concentration was measured by Bradford quantitative assay. For concord spectroscopy, 100 microliters of accumulated protein sample were mixed with 1900 microliters of concord buffer and placed in laboratory conditions for 10 minutes to stabilize the color. A visible spectrometer was used to scan the wavelength between 400-600 nm. The production of amyloid fibers was optimized by Congored absorbance and ThT fluorescence assay

methods and was confirmed by transmission electron microscopy. Cellulase enzyme was immobilized via glutaraldehyde cross–linking bridges on amyloid nano–biofibrils.

Result and Discussion

To investigate the effect of concentration, temperature and pH, the amount of changes in λ_{max} in the range of 400 to 600 nm was investigated using the Concorde absorbance method, and the amount of amyloid fibers production was tracked with changes in λ_{max} in the solution. Also, ThT fluorescence emission method was used to determine the amount of amyloid fibers. To investigate the process of fibril formation, concentrations of 2, 3, and 4 mg/ml, pHs of 3, 4, and 5, and temperatures of 40, 50, and 60°C were investigated. Based on this, the optimal fibrillation conditions for fibrillation of bovine serum albumin protein were determined at a concentration of 4 mg/ml of protein, pH equal to 3, and temperature of 60ºC. The presence of amyloid fibers was confirmed by transmission electron microscopy. The obtained images clearly show the fibrillar structures of amyloid aggregates. The amount of free cellulase activity was equal to 0.08 and the activity of the immobilized enzyme was equal to 0.05 mol/min. Also, the amount of Km in the free and immobilized enzyme was calculated as 0.27 and 0.102 mol, respectively, and the amount of V_{max} in the free and immobilized enzyme was calculated as 7.525 and 2.11 mol/min, respectively. The activity of the free enzyme was slightly higher than the activity of the immobilized enzyme on the nano–biofibrils. The similar trend seen in the specific activity indicates a slight decrease in the enzyme activity due to immobilization on amyloid fibrils. This reduction in specific activity can be considered as the result of reducing the flexibility of the enzyme during the process of covalent immobilization by glutaraldehyde molecules. In this study, the production process of amyloid filaments was optimized at different temperatures, concentrations and pHs. Protein concentration increases protein aggregation by increasing the chance of intermediates hitting each other. In fact, at high protein concentrations, the population of macromolecules increases and occupies a large volume of the whole, and this increases the probability of their collision. At acidic pH, the net charge of the protein changes. Considering that the pI of BSA that is 4.7, in acidic pH the net charge of this protein becomes positive and repulsive forces arise between different parts of the protein, which leads to the relative unfolding of the tertiary structure of the protein. Today, the understanding that amyloid fibers can be used in various ways in nano–sciences, such as the formation of nanotubes, has increased the importance of the discussion.

Conclusions

In this study, in order to form amyloid aggregates under different conditions, protein concentration, temperature and pH were used to investigate the effect of each one separately. In the investigation of the process of fibril formation, the changes of one of them can be carefully and logically

investigated by simultaneously considering two other factors as constant. The reason for this work is to remove any other change factors from the existing conditions in the system of studying and checking the desired variable, especially. To measure the formation of fibrils, Congo red absorbance and ThT fluorescence methods were used, and for the final confirmation of the prepared fibrils, transmission electron microscope images were also used. The results showed that the maximum production of amyloid fibrils is formed at a concentration of 4 mg/ml of protein, a temperature of 60ºC and a pH of 3. With a new approach, amyloid fibers can be introduced as a new nanomaterial for cellulase enzyme stabilization.

Keywords: Bovine serum albumin, Amyloid, Immobilization, Cellulase, Nano–fibrils.

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Declaration of conflict of interest

The authors declare that they have no conflict of interest.

Statement on ethics

There are no human or animal subjects in this article.