



Future Clinical Application of β -galactosidase Stabilized by Magnesium oxide Nanoparticles

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ABSTRACT

The present study demonstrates the application of freshly prepared neem leaf extract as a reducing agent for synthesizing magnesium oxide nanoparticles (MgO-NPs). *In silico* interaction of *Aspergillus oryzae* β -galactosidase with MgO-NPs was observed by using molecular docking program Dock v.6.5 while the visual analyses and illustration of protein–ligand complex were investigated by utilizing chimera v.1.6.2 and PyMOL v.1.3 softwares. The prepared nanomatrix provided 83% immobilization yield, and broadened the biocatalytic activity of immobilized β -galactosidase at higher pH and temperature ranges. Immobilized β -galactosidase exhibited greater activity even at 5.0% galactose concentration as compared to the soluble enzyme under similar experimental conditions. Hence, the use of green nanotechnology makes the process inexpensive, and therefore, immobilization of these enzymes on such nanoparticles can help to recover the enzyme, which ultimately decreases the cost of process.

Keywords: Green nanotechnology, Enzymes, Neem, Magnesium oxide nanoparticles, In-silico characterization.

INTRODUCTION

Twenty-first century witnessed the remarkable upsurge in nanotechnology based research owing to its broader display of utilization in clinical and biotechnological applications¹. Furthermore, the current engineering techniques favor the precise synthesis of nanoparticles on large scale and at less cost, which makes their utilization even more successful in the field of interest². Several

notable features associated with their synthesis include facile incorporation of greater payload of targeted ligand in the same platform due to the desirable magnetic and optical properties³.

In this regard, magnesium nanoparticles have appealed the scientists to exploit them in various sectors as they possess distinctive electronic, optical, thermal, chemical and mechanical properties. It exhibited excessive potential as an



adsorbent for toxic chemical agents⁴. Moreover, magnesium oxide nanoparticles are magnetic as compared to their bulk counterpart. Magnesium nanoparticles are synthesized by various approaches like sol-gel and coprecipitation method. However, synthesis of magnesium oxide nanoparticles via green nanotechnology (plant extract) serves as cost-effective and eco-friendly approach without the involvement of toxic chemicals⁵.

Green nanotechnology is the term used for developing clean technology to reduce the potential human health and environmental risks that are linked with the synthesis of nanoparticles⁶. It encourages the manufacture of nano-products by using non-toxic raw materials in the form of plant extracts. Hence, this process involves inexpensive procedure, thereby favoring the immobilization of enzymes on such nanoparticles which helps in recovering the enzyme, and leads to the ultimate decreases in the cost of biotechnology process^{7,8}.

Solid matrices are increasing the awareness of the enzymologists to immobilize enzymes as offered repeated use of immobilized biocatalysts and that too in a cheaper way⁹. Moreover, certain required characteristics like increase in pH and thermal stability, reduction in product mediated inhibition and leaching out of enzyme is significantly improved as a result of enzyme immobilization on such supports as compared to the soluble enzymes¹⁰. Additionally, magnetic NPs like magnesium nanoparticles add benefits like high surface area to volume ratio for attaching enzyme, minimizing the fouling, and separation of enzyme by a magnetic field. Reduced mass transfer resistance and leaching are other associated advantages that are related by using such nanomatrices for immobilizing the enzymes¹¹. Considerable research has been done on neem tree for developing cost effective and non-toxic products¹². Hence, in the present study, MgO-NPs were synthesized by using neem leaf extract for immobilizing β -galactosidase.

MATERIALS AND METHODS

Magnesium nitrate was obtained from Sisco Research Laboratories Pvt. Ltd., India. ONPG and enzyme was procured from Sigma Aldrich, USA. Neem (*Azadirachta indica*) leaves were collected from nearby garden. All reagents were used without further purifications.

Green synthesis of magnesium nanoparticles (MgO-NPs) by *Azadirachta indica* leaves

Neem leaves (100 g) were cleansed by distilled water thrice and left for drying at room temperature. The obtained dried leaves were boiled in a beaker at 100°C for 1 hour. The freshly prepared neem leaf extract was used as reducing agent for synthesizing magnesium oxide nanoparticles (MgO-NPs). Briefly, magnesium nitrate (5 g) was added to the prepared neem leaf extract (5 mL) and the resulting solution was continuously stirred at 80°C for 6 hours.

Characterization of MgO-NPs by TEM

The synthesized MgO-NPs were characterized by JEOL JEM-2100F transmission electron microscope. The process involves the drop coating of suitably diluted magnesium nanoparticles solution on carbon-coated copper grids under the accelerated voltage of 15 kV at normal atmospheric conditions.

In silico interaction of *Aspergillus oryzae* β -galactosidase with MgO-NPs

ChEBI (Chemical Entities of Biological Interest (<https://www.ebi.ac.uk/chebi/>)) was used to obtain the 3-D structure of MgO-NPs with ChEBI ID 36973. Dock v.6.5 was used for docking¹³⁻¹⁵.

Immobilization of β -galactosidase on MgO-NPs

MgO-NPs was used as a matrix to adsorb the enzyme (1000 enzyme units) was adsorbed on in the assay buffer under slow stirring at 30°C overnight. The resulting mixture was centrifuged for 20 min at 200 rpm to collect the precipitate. The precipitate was washed three times with the assay buffer and the resulting β -galactosidase immobilized on the activated MgO-NPs was used to carry out the experiments¹⁶.

Measurement of enzyme activity

The reaction mixture containing 100 μ L β -galactosidase (2 U), 200 μ L ONPG and 1.7 mL of assay buffer was analyzed for enzyme activity at 50°C. Na_2CO_3 (2 mL) was used for terminating the reaction post 15 minute. Production of o-nitrophenol was analyzed spectrophotometrically at 405nm and analyzed¹⁷.

pH stability

The buffers of various pH values (4.0 to 8.0) were used to observe the enzyme activity and for determining the optimum pH of soluble and immobilized enzyme. The activity is expressed as the absorbance developed in the solution at the wavelength of 405nm¹⁷.

Temperature stability

The activity of soluble and immobilized form of enzyme was evaluated at temperature ranges between 30 and 70°C¹⁷.

Product inhibition

Soluble and immobilized enzyme (100 μ L) was evaluated for their activity in varying concentration of galactose (1.0%-5.0%, w/v) by incubating in 100 mM assay buffer for 1 hours. The control experiment was run simultaneously in which the enzyme activity was analyzed without adding galactose. The result was compared with the control in order to calculate the activity of enzyme preparations under the effect of galactose^{16,17}.

Statistical analysis

Data was expressed by Sigma Plot-9 software. All the experiments were performed in triplicates and results with average standard deviation <5% were considered as satisfactory.

RESULTS AND DISCUSSION

Importance of green nanotechnology

Synthesis of materials at the nanoscale level is constantly aspired for utilization in biotechnology and biomedical sectors with an aim of improving their chemical and physical properties. Eco-friendly technology which utilizes the application of plants in manufacturing such nanomaterials presents a better way of expanding their biological applications. Hence, efforts were raised to synthesize various nanoparticles via green nanotechnology with distinct morphology and size, and chemical composition, for demonstrating their use in diverse research applications¹⁸⁻²⁰.

Synthesis and characterization of MgO-NPs

The yellowish-brown confirms the synthesis of MgO-NPs. TEM image confirms the shape and size of the prepared MgO-NPs as 33nm (Figure1).

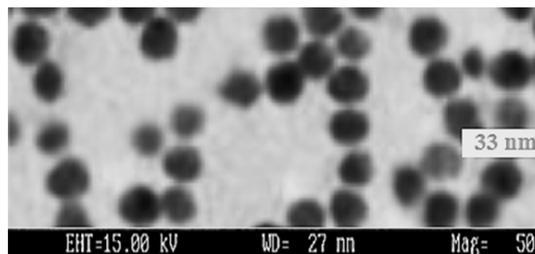


Fig. 1. Transmission electron microscopy of MgO nanoparticles

Adsorption of *Aspergillus oryzae* β -galactosidase on MgO-NPs

MgO-NPs serve as excellent matrices by immobilizing greater percentage of enzyme. The percent of enzyme immobilized on the developed nanoparticles is critical in suggesting their possible applications in biotechnological sectors.

In silico studies

PDB ID with reference no. 4IUG was used for crystal structure of β -galactosidase. Dock v.6.5 software¹³ confirmed the probe and the region around 10 Å of galactose [Fig. 2]. The protein-ligand complex was obtained by Chimera v.1.6.2¹⁴ and PyMOL v.1.3¹⁵ for visual analyses. Ligplot v.1.4.5 program showed the ligand interaction plots of protein-ligand complexes. While observing the binding of MgO-NPs to galactose, four interacting residues Phe-304, Glu-142, Asn-140 and Tyr-260 were found overlapping (Figure 3).

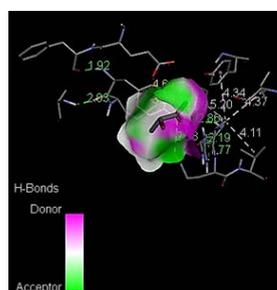


Fig. 2. Display receptor surface–H bonds

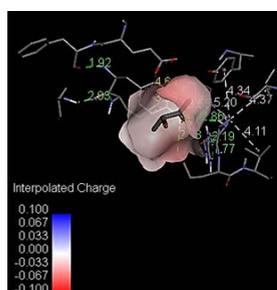


Fig. 3. Display receptor surface–charges

Physical stability studies

Proteins with proper conformations are accountable as biocatalysts i. e. enzymes. Hence forth a minute alteration in tertiary form is an indication of diminished catalytic activity²¹. Fig. 4 showed profile of free and conjugated enzyme reflecting up on the pH. The pH based activity profile of an immobilized enzyme is remarkably increased that of the native enzyme. It may be due to more distortions in the structure resulting due to acidic and basic environment for the free and immobilized enzyme. It resulted in a greater shift in enzyme activity from the free (62%) to immobilized (90%) enzyme at pH 4.0 (Fig. 4). Under varying temperature conditions, free and conjugated enzyme exhibited 70% and 94% of its activity at 60°C, respectively. Enzyme denaturation at higher temperatures is responsible for the significant downshift in enzymatic activity of free β -galactosidase (Figure 5).

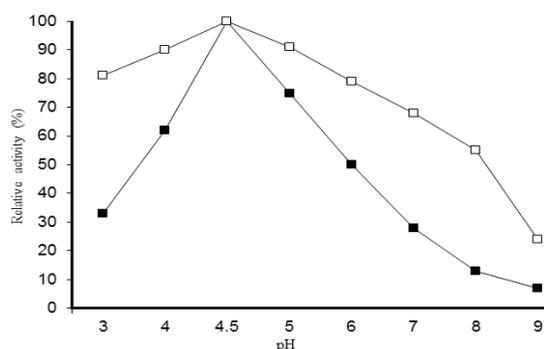


Fig. 4. pH-activity profiles for soluble and MgO-NPs bound β -galactosidase

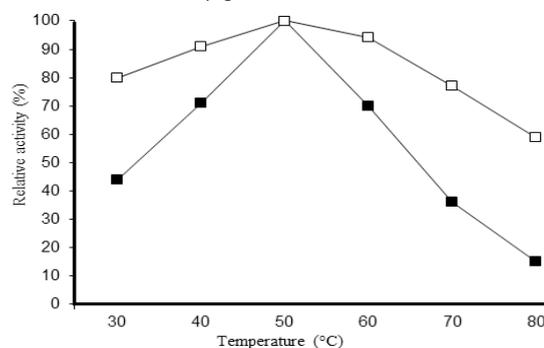


Fig. 5. Temperature-activity profiles for soluble and MgO-NPs bound β -galactosidase

Product inhibition

Activity of β -galactosidase through galactose significantly affects the hydrolysis of lactose, and this challenge can be reflected positively by immobilizing enzyme on MgO-NPs as it counters the activity of β -galactosidase as a protector of the active site access by the galactose²²⁻²⁵. Fig. 6 suggests 60% activity of immobilized enzyme at 5%

galactose even after one hour incubation. On the other hand, 24% activity of free enzyme was evident under identical conditions. Hence, with the above mentioned data it can be predicted that the conjugate enzyme exhibited resistance to the actions of galactose as inhibitor in comparison soluble enzyme.

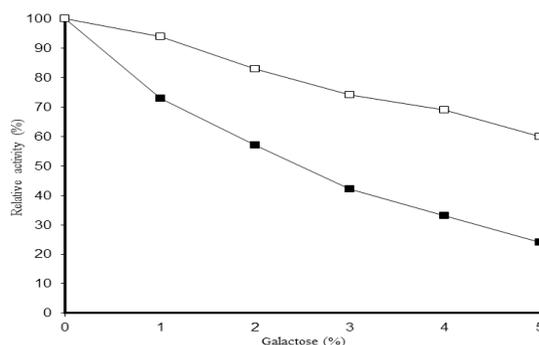


Fig. 6. Effect of galactose on soluble and MgO-NPs bound β -galactosidase

Reusability studies

Enzyme conjugated to MgO-NPs exhibited 91% and 87% of the initial activity, after its 5th and 6th repeated use, respectively (Fig. 7). Henceforth, it is evident that the synthesized MgO-NPs are promising candidates for biotechnological relevance. The biotechnological application of β galactosidase conjugated on various nanomatrices has been excellently reviewed recently²⁶.

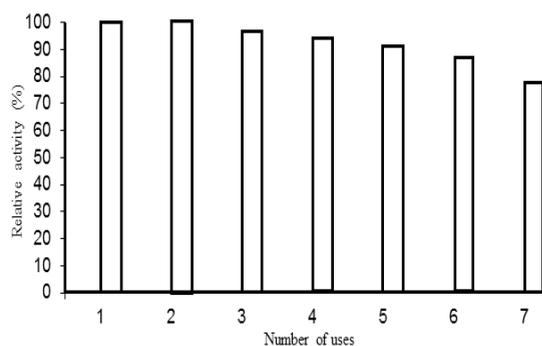


Fig. 7. Reusability of MgO-NPs bound β -galactosidase

CONCLUSION

Herein, neem leaves were used to synthesize magnesium oxide nanoparticles by using green nanotechnology approach without using toxic and expensive chemicals. Enzyme immobilization on magnesium oxide nanoparticles resulted in improving the catalytic performance under various chemical and physical effects. The synthesized nanoparticles were able to adsorb the enzyme in significant amount. It improved the enzyme

reusability for several runs and hence could be exploited in biotechnology industries with improved results and less cost.

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Conflict of interest

The authors declare no conflict of interest.

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