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A Green Approach towards the Synthesis of Enantio Pure Diols Using Horse Radish Peroxidase Enzyme Immobilized on Magnetic Nanoparticles

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ABSTRACT

Enantiopure epoxides and their corresponding chiral vicinal diols serve as valuable intermediates in the synthesis of biologically active pharma and agro-compounds and also value added fine chemicals. Biocatalysts are well known for their selective hydrolysis of racemic epoxides to give optically pure chiral diols. This study highlights an efficient process of synthesis of chiral vicinal diols in good yields and enantioselectiviy using horse radish peroxidase enzyme immobilized on the amine functionalized magnetic nano particles (Fe_3O_4 nanoparticles) as enzyme carriers. It also facilitates separation of MNP-immobilized enzymes by applying external magnetic field. The immobilization of magnetic nano particles was confirmed by transmission electron microscope (TEM) and scanning electron microscope (SEM). The MNP-immobilized peroxidase enzyme improved stability of the enzyme and has shown broader substrate specificity in enantioselective hydrolysis of racemic epoxides, under mild and environmentally friendly conditions. The methodology MNP-immobilized enzyme developed in the synthesis of chiral diols has a potential for use in large-scale applications.

KEYWORDS

Magnetic Nanoparticles; Horseradish Peroxidase; Immobilization; *Vicinal*-Diols

1. Introduction

Chiral compounds play an important role in both chemical and biotech industries. Among the optically active compounds, enantiopure epoxides and their corresponding vicinal diols are important intermediates for pharma, agro and fine chemical industries. Though there are several chemical methodologies for the resolution of epoxides, yet they have their own limitations in terms of efficiency and enantioselectivity. Enzymes/Biocatlysts have been used in the synthesis of chiral alcohols of biological importance. This strategy constitutes an attractive "green chemistry" alternative to the existing chemical methodologies [1-6]. Several epoxide hydrolases (EHs) and Horse radish peroxidase (HRP) have recently been recognized as versatile biocatalysts in synthesis of enantiopure epoxides and their diols. They exhibit high enantioselectivity with broad substrate specificity and these enzymes do not require any cofactors in aqueous/buffer solution [7-9]. However, the use of biocatalysts in the industrial application is limited due to the difficulties associated with the enzyme stability and reuse. Therefore, immobilization of the biocatalysts on certain supports tends to be an efficient solution for the problems associated with the use of free or soluble enzymes [10]. There are many organic and inorganic carriers available to immobilize the biocatalysts. Among them, magnetite (Fe_3O_4) nanoparticles (MNPs) exhibit superparamagnetic proper-

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ties therefore surface modification of such magnetic nanoparticles can make improvements in their surface properties, which may be useful for enhancing the activity and stability of the enzyme [11-14]. The large surface-area-to-volume ratio of magnetic nanoparticles allows serving as an efficient carrier of biocatalyst and also helps in separation of enzymes from the reaction medium using external magnet, thus the magnetic nano particles were employed as supports for the immobilization of the enzymes [15-18].

Therefore, the aim of our present study is to immobilize the enzyme Horseradish peroxidase onto amine functionalized magnetic nanoparticles for the synthesis of chiral vicinal diols from the racemic epoxides. The immobilization of the enzyme on MNPs was characterized by TEM and SEM studies. The enantiomeric excess and yield of the products were confirmed by optical rotation and NMR spectral studies.

2. Results and Discussion

The hydrolysis of the racemic epoxide was carried out with both free and immobilized enzyme and their respective yields are presented in **Table 1**. The structures of the products were confirmed by NMR, LC-MS and IR spectroscopy. The recovered MNPs bound HRP enzyme had shown 5% decrease in enzyme activity. Though the recycled enzyme showed good enantioselectivity, the yields were, gradually decreased to 5% - 10% in each cycle. Mono-substituted racemic aryl epoxides were resolved using MNP-bound peroxidase to obtain the (*R*)-diol, thus the process of enzymatic hydrolysis of the racemic epoxide show (*R*)-selectivity.

Thus the results show that, the peroxidase bound-MNPs can be applied to large-scale reactions with acceptable yields and enantio-purity in ecofriendly environment. The above enzyme-immobilized MNPs methodology may be applicable in the kinetic resolution of a broad range of racemic epoxides in obtaining chiral diols and enantiopure epoxides.

3. Conclusion

Enzymes have long been used in the industry as catalysts in process for production of chiral alcohols of biological interest. MNP-enzyme conjugates (MNP-Es) represent a successful application of immobilized enzyme in the synthesis of value added chiral compounds of biological interest. In this study it is conclude that the enzyme horseradish peroxidase enzyme was immobilized on amine functionalized magnetic nanoparticles in synthesis of enantio pure vicinal diols from corresponding racemic epoxides in environmentally friendly conditions. Thus the MNP-Es methodology developed in synthesis of chiral alcohols has the potential for industrial application.

4. Experimental

4.1. General

All chemicals ($FeSO_4 \cdot 7H_2O$, $Fe_2(SO_4)_3$, ammonium hydroxide, tetraethylorthosilicate, (3-aminopropyl)-triethoxysilane, glutaraldehyde, Horse radish peroxidase; EC 1.11.1.7) were purchased from Sigma Aldrich. The racemic epoxides used in the study were synthesized in our laboratory. Melting points were measured on Mettler-Temp apparatus; uncorrected. IR Spectra were recorded with a Perkin-Elmer-1600 FT-IR spectrometer in KBr; υ in cm^{-1}.^{1}H-NMR spectra were recorded on Gemini-200 spectrometer; in $CDCl_3$. Mass spectra were recorded on ESI-MS: Agilent 6510 Q-TOF LC/MS instrument, ESI-MS: 7070H spectrometer with a direct inlet system.

4.2. Preparation of Fe_3O_4 (Magnetite) Nanoparticles

Magnetic nanoparticles were prepared by co-precipitation method [19-21]. $FeSO_4 \cdot 7H_2O$ (2.78 g) and $Fe_2(SO_4)_3$ (4.0 g) were dissolved in water and ammonium hydroxide (25%) was added slowly to adjust the alkaline pH of the solution. The reaction mixture was then continually stirred for 1 h at 50˚C under nitrogen atmosphere till black magnetite (Fe_3O_4) particles were formed and the mixture was cooled down to room temperature.

4.3. Synthesis of Silica Coated Fe_3O_4 Nanoparticles

To prepare silica coated Fe_3O_4 nanoparticles, tetraethyl orthosilicate (TEOS) was readily added to the above solution and vigorously stirred for 18 h under nitrogen atmosphere. Then the silica coated Fe_3O_4 nanoparticles were separated and washed with water until the solution pH reached to neutral and finally the particles were vacuum dried.

4.4. (3-Aminopropyl) Triethoxysilane Functionalization on γ-Fe_2O_3 @ SiO_2

To prepare (3-aminopropyl)triethoxysilane functionalized γ-Fe_2O_3@SiO_2, 3 g of γ-Fe_2O_3@SiO_2 was dispersed in dry toluene under N_2 atmosphere, to which 2 mL of (3-aminopropyl)triethoxysilane was added and stirred at reflux temperature for 24 h and the material was collected with external magnet, washed with isopropanol and dried under vacuum.

4.5. Immobilization of HRP Enzyme onto Magnetic Nanoparticles and Synthesis of Chiral Diols

For immobilization of the enzyme: MNPs (50 mg) were washed with phosphate buffer of pH 6.0 and 1% (v/v) of glutaraldehyde solution was added under stirring for 1 h.

Table 1. Synthesis of chiral vicinal diols using immobilized enzyme.

Entry	Compound	Conversion %	Yield %		Optical Rotation $[\alpha]_D$
			Free HRP enzyme	Immobilized-HRPenzyme	
2a	(*R*)-1-Phenyl-1,2-ethanediol	42	74	80	−22.5
2b	(*R*)-1-(4-Chlorophenyl)-1,2-ethanediol	34	48	55	−35.6
2c	(*R*)-1-(4-Bromophenyl)-1,2-ethanediol	32	41	46	−37.8
2d	(*R*)-1-(4-Nitrophenyl)-1,2-ethanediol	35	37	42	−21.4

The cross-linker treated MNPs were washed thoroughly with phosphate buffer to remove the un-reacted aldehyde. 4 mL (0.5 mg/mL) of HRP enzyme solution was then added to glutaraldehyde treated MNPs in 6 mL of phosphate buffer and stirred gently for 2 - 3 h to immobilize the enzyme [22-24]. The immobilized enzyme solution was stored in buffer solution at 4˚C until use. The size of nanoparticles and immobilization of the HRP onto magnetic nanoparticles was characterized using TEM and SEM studies (**Figure 1**). The activity of the HRP enzyme before and after immobilization was determined by pyrogallol method [25].

To the enzyme-immobilized onto MNPs taken in buffer, was added 1 mol of racemic epoxide and incubated for optimum conversion period. The product (diol) formation was monitored using TLC at regular intervals of time (**Scheme 1**). Upon completion of the reaction, the enzyme (HRP) bound to the magnetic nanoparticles was separated by using external magnet and thus retained MNP-bound enzyme was re-suspended in the buffer solution containing styrene epoxide for further kinetic resolution. The reaction mixture was separated and extracted with organic solvent and the product was purified using column chromatography.

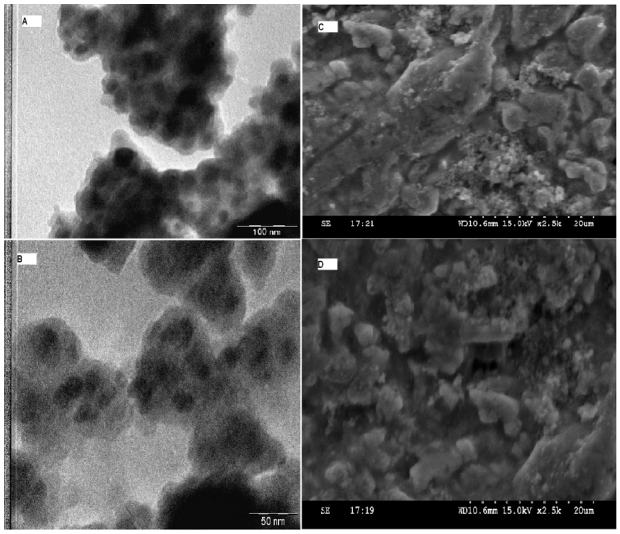
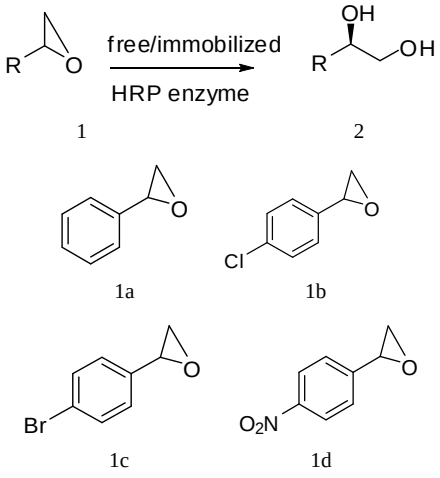


Figure 1. TEM and SEM images of magnetic nanoparticles. TEM (A & B) and SEM (C & D): (A & C) amine functionalized silica coated magnetic nanoparticles; (B & D) HRP enzyme immobilized MNPs.



Scheme 1. Synthesis of vicinal diols.

4.6. (*R*)-1-Phenyl-1,2-Ethanediol (2a)

White solid., m. p. = 58˚C - 60˚C. $[\alpha]_D^{25}$= −22.5 (*c*, 1.0, Ethanol). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.63 (brs, 1H), 3.05 (brs, 1H), 3.55 - 3.70 (m, 2H), 4.73 - 4.77 (dd, 1H, *J* = 3.0, 8.3 Hz), 7.24 - 7.31 (m, 5H. IR (KBr): ν = 3315, 2925, 1600, 1454, 752 cm^{-1}; MS: *m/z* = (M^+, 138).

4.7. (*R*)-1-(4-Chlorophenyl)-1,2-Ethanediol (2b)

White solid, m.p. = 73˚C - 75˚C. $[\alpha]_D^{25}$ = −35.6 (*c*, 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 2.0 (brs, 1H), 2.62 (brs, 1H), 3.65 (dd, 1H, *J* = 8.1, 11.1 H_Z), 3.71 (dd, 1H, *J* = 3.7, 11.1 Hz), 4.77 (dd, 1H, *J* = 3.7, 8.1 Hz) 7.28 - 7.30 (m, 4 H). IR (KBr): ν 3369, 2923, 1595, 1511, 459, 829, 892 cm^{-1}. MS: *m/z* = (M^+, 172).

4.8. (*R*)-1-(4-Bromophenyl)-1,2-Ethanediol (2c)

White solid, m.p = 94˚C - 96˚C. $[\alpha]_D^{25}$ = −37.8 (*c*, 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.88 (brs, 1H), 2.42 (brs, 1H), 3.55 (dd, 1H, *J* = 8.0, 11.0 Hz), 3.70 (dd, 1H, *J* = 3.6, 11.0 Hz), 4.74 (dd, 1H, *J* = 3.6, 8.0 Hz), 7.22 (d, 2H, *J* = 8.8 Hz), 7.45 (d, 2H, *J* = 8.8 Hz). IR (KBr): ν 3368, 2918, 1588, 1487, 894, 830 cm^{-1}. MS: *m/z* = (M^+, 216).

4.9. (*R*)-1-(4-Nitrophenyl)-1,2-Ethanediol (2d)

Light yellow solid. m.p = 70˚C - 72˚C. $[\alpha]_D^{25}$ = −21.4 (*c*

= 1.0, MeOH). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 3.48 - 3.59 (m, 2H, CH_2), 4.33 (m, OH), 4.77 (m, OH), 5.07 - 5.08 (m, 1H, CH), 7.58 (d, 2H, J = 9.0 H_Z), 7.58 (d, 2H, J = 9.0 H_Z), 8.14 (d, 2H, J = 8.2 H_Z). IR (KBr): ν 3304, 2932, 1603, 1514, 854 cm^{-1}. MS: m/z = (M^+, 183).

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