ISSN: 2249 - 7862
Aureole, Vol. VII, December 2015 PP 46-52

CATALYTIC STUDIES OF TSA- IMPRINTED POLYMER CATALYST FOR THE HYDROLYSIS OF AMINO ACID ESTERS

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Abstract

Molecular imprinting technique has come to be regarded as one of the most potentially promising and convenient method to mimic molecular recognition ability of biological molecules such as antibodies, enzymes and receptors [1]. A highly crosslinked enzyme mimic molecular imprinted polymer prepared using transition state analogue (TSA) of phenyl 1-benzyloxycarbonyl amino-4-methoxybenzyl phosphonate as the template and imidazole as the catalytic centre with the use of N-methacryloyl histidine and 4-vinyl pyridine as the functional monomers. The catalytic activities of TSA imprinted polymer and non-imprinted polymer towards the hydrolysis of p-nitrophenyl esters of Z-L-phenylalanine were investigated. Substrate hydrolysis was measured under pseudo first order conditions and compared with those of non-imprinted and uncatalysed reactions. The catalytic activity of the TSA imprinted polymer is found to be substrate selective, solvent, and concentration dependent. The second order rate constant was also evaluated.

Keywords **:** *TSA, Molecular imprinting, Enzyme Catalyst, Kinetic Study, Ester hydrolysis*

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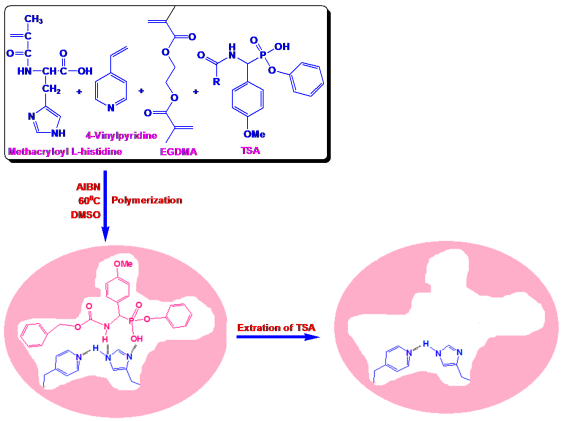
Introduction

Molecular imprinting is a technology providing a new methodology to synthesise materials containing artificial receptors that can be employed in a variety of applications, such as separation, sensors or catalysis[1-3]. Considering the versatility, high level of specificity and recognition that can be achieved, the future of these materials is very promising. Molecular imprinted polymers are synthesized in the presence of specially introduced target template molecules, which are intended for imprinting [4,5]. An important prerequisite for the preparation of these polymers is the formation of a stable pre-polymerization complex between the monomer and template molecules [6]. To obtain this complex, a template and a monomer are mixed in an appropriate (most frequently, aprotic) solvent before polymerization. Because of the formation of a prepolymerization complex, the molecules of a functional monomer are particularly arranged and fixed around the template molecule in the course of the entire process of polymerization, whereas polymerization in the presence of a great amount of a crosslinking agent gives a strongly crosslinked polymer with a rigid structure. After polymerization, the polymer is crushed and sieved to a required particle size, and the template is removed by repeated washing with the use of organic solvents. This imprint is complementary to the target molecule in size, shape, and physicochemical properties and capable of repeated binding and recognizing this molecule among many other molecules. Because of the super crosslinked nature, MIPs are stable to physical and chemical treatment, including heating, organic solvents, acids, and bases. They can be stored in a dry state at room temperature for several years and, if necessary, regenerated and repeatedly used without memory loss of molecular recognition sites.

Enzymes are biological catalysts that exhibit high catalytic activity, high substrate recognition, and stereoselectivity [7]. These properties are the result of a complex three-dimensional arrangement of the functional groups responsible for catalysis. However, the practical application of these biocatalysts faces many difficulties due to their instability against high temperature, organic solvents and drastic pH conditions [8]. As an alternative, the principles of enzyme catalysis have been used to design artificial enzyme analogues. Based on the mechanism of enzymatic catalysis, several requisites must be fulfilled in order to obtain a synthetic material showing enzyme-like behavior [9]. These requisites include the presence of a cavity with the shape of the substrate or the transition state of the reaction, and functional groups that act as binding sites, coenzyme analogues or catalytic groups within the cavity in a defined stereochemistry[10,11]. The technique of molecular imprinting can applied for much more stable polymeric mimic of biological enzymes. To prepare enzyme like polymers by molecular imprinting a stable TSA of a reaction must

be selected as template. The imprint of TSA acts as an active center and shows catalytic The polymerization was carried out in DMSO at 80°C using AIBN as the initiator. The polymer formed was collected by filtration, washed with acetone, soxhlet extracted with chloroform and dried. The non-imprinted polymers were synthesised in the same molar ratios of monomer and EGDMA. The amino capacity of imprinted polymer was estimated by ninhydrin [9]. FTIR cm^{-1}: 3417, 2989, 1728, 1643, 1388, 1257, 1157, 952, 879.



Scheme 1: Preparation of TSA imprinted polymer

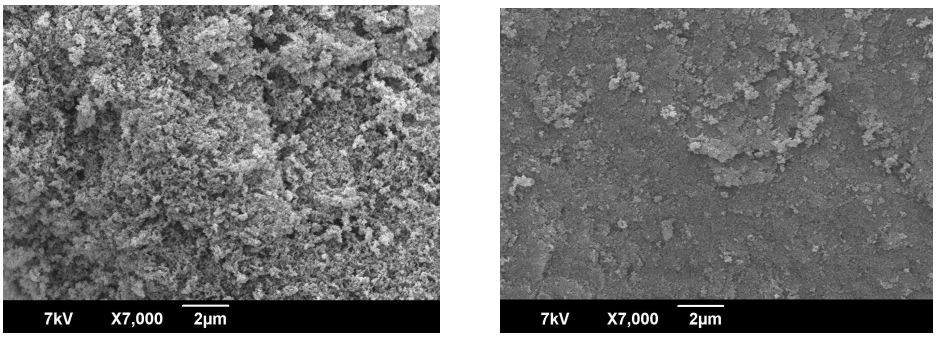
Catalytic Hydrolysis of Esters by TSA Imprinted and Non-imprinted Polymers

Catalytic activities of imprinted and non-imprinted polymers towards the hydrolysis of the p-nitrophenyl esters of Z-L phenylalanine were investigated with ACN-Tris HCl buffer mixture (1:9 by volume) at room temperature and the reaction was followed by monitoring the absorbance of released p-nitrophenolate anion spectrophotometrically at 400nm. A blank reaction without any polymer was also carried out. The conditions of catalytic hydrolysis were optimised.

RESULTS AND DISCUSSION

Preparation of TSA Imprinted Polymer

The TSA imprinted polymer was synthesized using the functional monomer N-methacryloyl histidine and the crosslinking agent EGDMA in the presence of AIBN as the initiator (Scheme1). TSA and functional monomer was dissolved in DMSO and stirred under N_2 atmosphere to form a pre-polymerisation mixture. The polymerisation was carried out at 60°C to produce polymer possessing 90% crosslink density. The solid polymer formed was washed with DMSO, chloroform and acetone gives insoluble macro porous polymer. A non-imprinted polymer was synthesised with functional monomer and crosslinking agent without the TSA. SEM analysis shows the morphological difference in the imprinted and non imprinted polymer specifically.

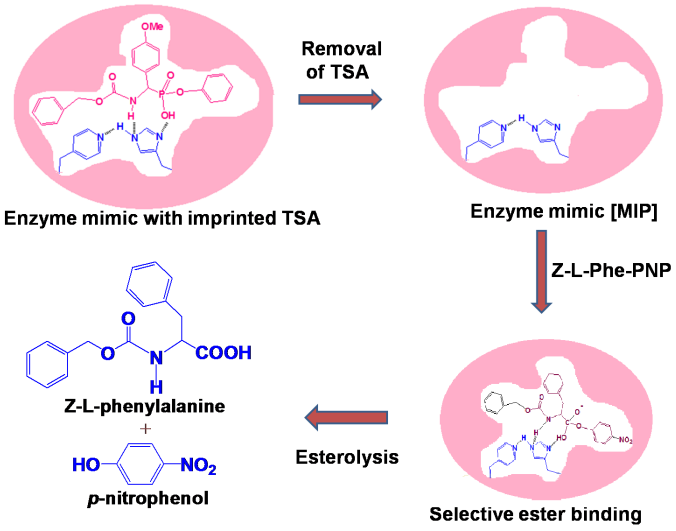


(a) (b)

Figure1: SEM analysis of (a) TSA imprinted polymer (b) Non imprinted polymer

Catalytic Activity of Imprinted and Non-Imprinted Polymers

Hydrolysis of esters catalysed by TSA imprinted, non-imprinted polymers were carried out in 1:9 ACN-Tris HCl buffer at pH 7.25 towards Z-L-phenylalanine p-nitrophenyl ester (Scheme2). Blank reaction without any polymer also investigated. Hydrolysis was followed by measuring the absorbance of p-nitrophenolate ion liberated at 400 nm.



Scheme 2: Catalytic hydrolysis by TSA imprinted polymer

The catalytic activity of molecular imprinted polymer was found to be very much higher compared to that of uncatalysed reaction (Figure 2). The rate of hydrolysis catalysed by TSA imprinted polymer is higher than that of the non-imprinted polymer. During imprinting procedure the phospshonate function provides a site complimentary to transition state and H-bonding between imidazole group and the substrate were responsible for the binding selectivity. In the non-imprinted polymer the imidazole groups are expected to be randomly distributed in the polymer and tetrahedral complimentarity of the TSA is absent. Hence rate of reaction is found to be lower than that of TSA imprinted polymer.

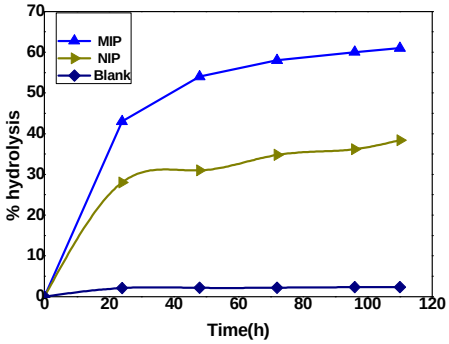


Figure 2: Plot of % hydrolysis of esters vs. Time with TSA imprinted polymer (MIP), non-imprinted polymer (NIP) and uncatalysed reaction.

Evaluation of the Rate of Catalytic Hydrolysis

The rate constant for the hydrolysis of p-nitrophenyl esters of Z-L-phenylalanine were calculated by the equation ln(A -At) = lnA - kt. A = absorbance at infinite time; At = absorbance at time t; k = rate constant. Hydrolysis was found to be psuedo first order (Figure 3). The second order rate constant (k_{cat}^{app}) was evaluated by the equation k_{cat}^{app} = kcat/[Im] where [Im] represents concentration of imidazole in the polymer catalyst.

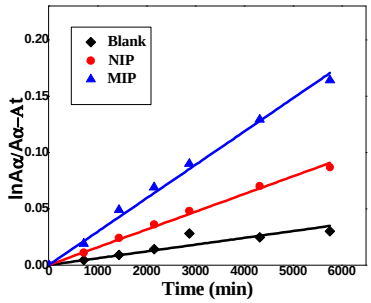


Figure 3: Evaluation of the rate constant for the hydrolysis of esters with TSA imprinted (MIP) and non-imprinted polymer (NIP) and uncatalysed reaction

Effect of Substrate Concentration

In order to investigate the effect of substrate concentration on the catalytic hydrolysis TSA imprinted polymer catalysed hydrolysis was done using different molar concentrations of Z-L-phenylalanine p-nitrophenyl ester. The rate of hydrolysis was found to increase within the lower range of concentration of substrate and as the concentration of substrate is increased very high rate decreases(Figure 4). This is due to the catalytic inhibition of the TSA imprinted polymer. The optimum ratio is fund to be 1:75.

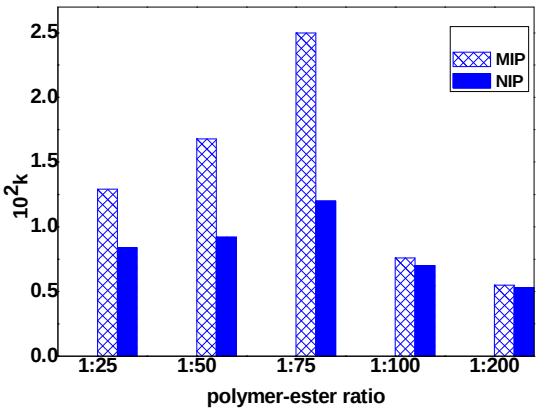


Figure 4: The effect of substrate concentration on the hydrolysis

CONCLUSION

In this work an enzyme mimic molecularly imprinted polymer was synthesised using transition state analogue of phenyl 1-benzyloxy carbonyl amino-4-methoxybenzyl phosphonate, as template. The TSA imprinted polymer exhibits higher catalytic activity towards the Z-L-phenylalanine p-nitrophenyl ester as compared to the non-imprinted polymer. The enzyme mimic molecular imprinted polymer was found to be substrate selective and concentration dependent. The morphological studies using SEM provided important evidences providing the catalytic activity difference between the imprinted and non-imprinted polymers.

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