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# Productive and nonproductive substrate binding in enzyme mimics

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**Abstract**

Hydrolytic activity of molecularly imprinted polymeric mimics of chymotrypsin was evaluated against *N*-acetyl tyrosyl *para* nitrophenyl ester and *N*-benzoyl tyrosyl *para* nitrophenyl ester, respectively. The mimic grafted on hydrophilic support exhibited high $k_{cat}$ and high $K_m$ values for *N*-acetyl tyrosyl *para* nitrophenyl ester. But, for *N*-benzoyl tyrosyl *para* nitrophenyl ester, the mimic exhibited low $k_{cat}$ as well as low $K_m$ values, consistent with the nonproductive binding exhibited by natural chymotrypsin for hydrophobic substrate. The same mimic when grafted on hydrophobic support exhibited trends consistent with Michaelis–Menten kinetics and also higher catalytic activity than that exhibited by the mimic on hydrophilic support. Thus in the case of mimics nonproductive substrate binding could be eliminated by the choice of appropriate support. This helped to enhance the mimic activity towards a specific substrate. This discretion is not available in the case of native enzyme 

*Keywords:* Chymotrypsin mimic; Substrate binding; Hydrophobic support

## 1. Introduction

Significant research efforts have been devoted for over 2 decades to design polymers which can mimic the catalytic activity of hydrolytic enzymes [1]. The choice of molecular imprinting as a methodology to synthesize polymeric mimics is expected to enhance their catalytic activities. We recently exploited this approach to develop a hydrogel exhibiting chymotrypsin like activity [2]. Using this methodology we subsequently reported a polymeric mimic of chymotrypsin which exhibited cooperative effect amongst the functional groups constituting the triad as well as substrate recognition exhibited by chymotrypsin [3]. This mimic was synthesized by grafting Co (II) coordinated assembly of *N*-methacryloyl L-Serine, *N*-methacryloyl L-Aspartic acid, *N*-methacryloyl L-Histidine and a template onto microporous support alongwith crosslinker and then leaching out Co (II) and template. Enhancement in the activity of the mimic through the appropriate choice of functional monomers was also demonstrated [4]. Yet, the activity of the mimic was much lower than that of chymotrypsin.

While the efforts to enhance catalytic activity are being continued, it is worthwhile to explore other features of synthetic mimics that have potential advantages over native enzymes. One such feature is substrate binding to the active site. Proteolytic enzymes like chymotrypsin exhibit more effective binding and higher activity for specific substrates. This has been attributed to the microenvironment of active site binding loci vis-a-vis substrate structure [5,6]. (For more details see next sections.) The microenvironment at the active site of the mimic can be modified to preferentially bind a given substrate by the appropriate choice of the support.

The role of support structure on the activity of polymeric catalysts were reported [7,8]. In this communication we have exploited such effects to enhance the substrate binding and thereby the hydrolytic activity of chymotrypsin mimics. It has been shown that chymotrypsin mimic grafted on hydrophilic support exhibited nonproductive binding and lower activity for hydrophobic substrate, as in the case of native enzyme. However, this nonproductive binding was eliminated by the choice of hydrophobic support for the mimic, which then exhibited higher catalytic activity.

## 2. Experimental section

Functional monomers *N*-methacryloyl L-Serine (MA-Ser), *N*-methacryloyl L-Aspartic acid (MA-Asp) and *N*-methacryloyl L-Histidine (MA-His) were synthesized by reacting methacryloyl chloride with respective amino acids as reported earlier [3]. Substrates containing hydrophilic and hydrophobic *N*-acyl substituent viz. *N*-acetyl tyrosyl *para* nitrophenyl ester (*N*-acetyl-Tyr-PNP) and *N*-benzoyl tyrosyl *para* nitrophenyl ester

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Table 1
1, Data for the feed ratios (columns 1–6) & loadings in mimics (columns 6–9)

| No. | Mimic[a] | Monomers (M) | Template (M) | $CoCl_2 \cdot 6H_2O$ (M) | Support (g) | Functional grs. /50 mg (M) | % grafting | Surface area ($m^2\ g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | P-A | [b] | 0.00125 (*N*-acetyl-Tyr-2AP) | 0.00125 | 0.825 (hydrolyzed Poly (GMA-EGDMA) | $6.04 \times 10^{-7}$ | 1.59 | 11.17 |
| 2 | P-B | [b] | 0.00125 (*N*-nicotinoyl-Tyr(Bzl) | 0.00125 | 0.0825 (-i-) | $1.74 \times 10^{-6}$ | 4.58 | 0.03 |
| 3 | P-C | [b] | 0.00125 (*N*-acetyl-Tyr-2AP) | 0.00125 | 0.825 Poly (PHMA-EGDMA) | $1.55 \times 10^{-7}$ | 0.4 | 0.13 |
| 4 | P-D | [b] | 0.00125 (*N*-nicotinoyl-Tyr(Bzl) | 0.00125 | 0.825 (-i-) | $5.19 \times 10^{-6}$ | 4.39 | 4.75 |

[a] In all the mimics ratio of wt. of support beads to monomers is 1 : 1. Feed of all assemblies contain EGDMA (10% of the wt. of monomers) and AIBN (3% of the wt. of monomers). All mimics are synthesized according to earlier reported procedure [3]. One mole of functional gr. is defined as equimolar composition of all three monomers [3].
[b] 0.00125 M stoichiometric amounts of all functional monomers MA-Ser, MA-Asp and MA-His were used.

(*N*-benzoyl-Tyr-PNP) and their respective templates viz. *N*-acetyl tyrosyl 2 amino pyridinamide (*N*-acetyl-Tyr-2AP) and *N*-nicotinoyl tyrosyl benzyl ester (*N*-nicotinoyl-Tyr (Bzl)) were synthesized by following standard procedures of peptide chemistry as reported elsewhere [9].

### 2.1. Synthesis of support materials

#### 2.1.1. Hydrophilic support: Hydrolyzed poly (glycidyl methacrylate-co-ethylene glycol dimethacrylate) (hydrolyzed poly (GMA-EGDMA))

This was synthesized according to the following modification in the reported procedure [10]. In a three-neck round bottom flask of 500 ml capacity, 180 ml water with 0.6 g poly (vinyl pyrrolidone) (MW 60 000) was placed. This solution was stirred with an overhead constant speed stirrer at 700 rpm. Contents of the flask were heated to 75°C and nitrogen gas was purged inside for half an hour. Then purging was stopped and organic phase containing 4.8 g GMA (60% w/w), 3.2 g EGDMA (40% w/w), 10 ml cyclohexanol and 80 mg azobis isobutyronitrile (AIBN) was added dropwise to aqueous phase at 75°C stirring at 700 rpm. This addition was completed in five minutes. Polymerization was allowed to proceed for 4 h. Contents of the flask were poured in beaker and beads formed allowed to settle. The supernatant solution was decanted off and beads were washed with methanol to remove any unreacted monomers. Beads were then successively washed with methanol, water and dried.

Epoxide ring of GMA was hydrolyzed to vicinal diols according to the following modification in reported procedure [11]. 5 g beads were suspended in 50 ml, 0.1 M $H_2SO_4$ and stirred at 60°C for 10 h to ensure complete hydrolysis of epoxide ring to vicinal diols, as monitored by absence of peak at around 950–990 $cm^{-1}$ in the IR spectrum of beads. After this, beads were filtered off, washed with water and dried. Dry beads were sieved from standard test sieves and only the beads within 45–75 μm range were selected for grafting of mimic.

#### 2.1.2. Hydrophobic support: Poly (Phenyl methacrylate-co-ethylene glycol dimethacrylate) (poly (PHMA-EGDMA))

An organic phase containing 4.8 g phenyl methacrylate (60% w/w), 3.2 g EGDMA (40% w/w), 10 ml cyclohexanol and 100 mg AIBN was added dropwise to the aqueous phase comprising 180 ml water with 0.6 g poly (vinyl pyrrolidone) stirred at 700 rpm at 80°C. Polymerization was carried out for 4 h as described earlier. Worked-up beads were sieved and beads in the range of 75–150 μm were selected for grafting.

In both the supports presence of residual double bonds necessary for grafting the monomers was confirmed by FTIR spectroscopy as reported earlier [3].

Table 2
2, Trends of $k_{cat}$ & $K_m$ for chymotrypsin mimics

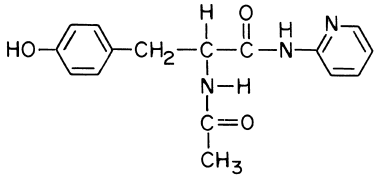
| No. | Catalyst | Support | Substrate | $k_{cat}$, ($s^{-1}$) | $K_m$ (M) | $k_{cat}/K_m$ ($s^{-1}M^{-1}$) |
|---|---|---|---|---|---|---|
| 1[a] | Chymotrypsin | — | *N*-acetyl tyrosyl ethyl ester | 192 | $6.63 \times 10^{-4}$ | $2.8 \times 10^{5}$ |
| 2[a] | Chymotrypsin | — | *N*-benzoyl tyrosyl ethyl ester | 85 | $2.2 \times 10^{-5}$ | $3.8 \times 10^{6}$ |
| 3 | P-A | Hydrolyzed poly (GMA-EGDMA) | *N*-acetyl-Tyr-PNP | 0.45 | $3.33 \times 10^{-4}$ | 1350 |
| 4 | P-B | Hydrolyzed poly (GMA-EGDMA) | *N*-benzoyl-Tyr-PNP | 0.11 | $1.14 \times 10^{-4}$ | 1018 |
| 5 | P-C | Poly (PHMA-EGDMA) | *N*-acetyl-Tyr-PNP | 0.33 | $1.0 \times 10^{-4}$ | 3308 |
| 6 | P-D | Poly (PHMA-EGDMA) | *N*-benzoyl-Tyr-PNP | 0.26 | $2.0 \times 10^{-4}$ | 1338 |

[a] Reported data [2].

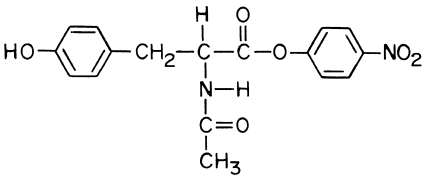
### 2.2. Synthesis of mimics

#### 2.2.1. Mimics on hydrophilic support (P-A and P-B)

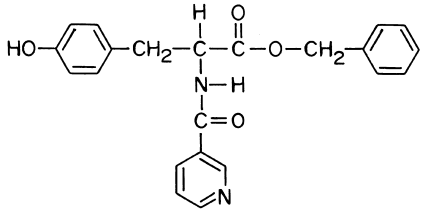
Co (II) coordinated monomers-template assemblies of MA-Ser, MA-Asp and MA-His with *N*-acetyl-Tyr-2AP and *N*-nicotinoyl-Tyr (Bzl) were prepared by dissolving monomers, templates and $CoCl_2 \cdot 6H_2O$ in methanol. Coordination of monomeric ligands with Co (II) was confirmed by ESR spectroscopy as reported earlier [3]. Methanol solutions were stirred under nitrogen for 1 h. Then EGDMA and AIBN were added. Methanol was evaporated in vacuo in presence of hydrolyzed poly (GMA-EGDMA) beads to sorb the assemblies on the support. These materials were then polymerized in an oven at 75°C for 24 h. After this, unreacted monomers, templates and Co (II) were leached out by washing the beads with methanol and HCl. Complete



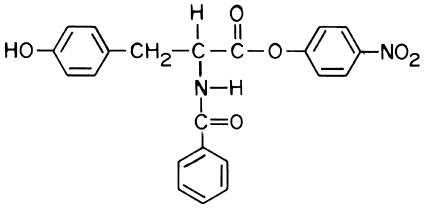
N-acetyl-Tyr-2AP
(Template)



N-acetyl-Tyr-PNP
(Substrate)



N-nicotinoyl-Tyr-OBzl
(Template)



N-benzoyl-Tyr-PNP
(Substrate)

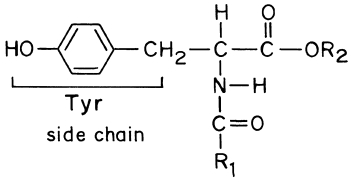



Fig. 1. Structures of substrates and templates.

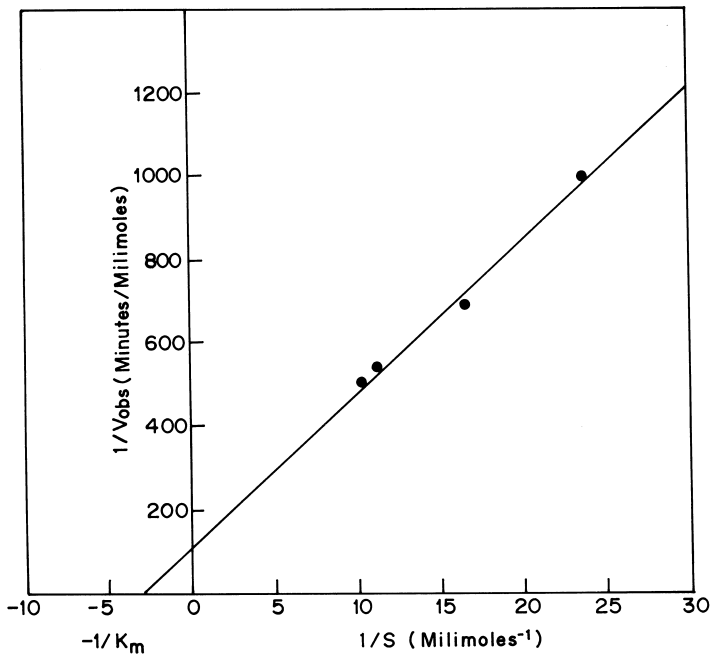



Fig. 2. Lineweaver–Burk plot for hydrolysis of *N*-acetyl – Tyr-PNP catalyzed by P-A (hydrophilic support) at 37°C and pH 7.8. The assay employed 25 mg P-A which contained 0.000302 mmol of functional groups. Substrate concentration was varied between 0.0322 and 0.0959 mmol. $k_{cat} = 0.45\ s^{-1}$, $K_m = 3.33 \times 10^{-4}$ M, $k_{cat}/K_m = 1350\ s^{-1}\ M^{-1}$.

removal of Co (II) and template was confirmed by monitoring the absorbance of elute at 700 nm on UV spectrophotometer. Loading of functional monomers per gram of support beads was determined by hydrolyzing amino acids from methacryl backbone of beads and estimating them quantitatively by Ninhydrin test as reported earlier [3]. Data for the feed ratios of monomers etc. and loadings are listed in Table 1.

### *2.2.2. Mimics on hydrophobic support (P-C and P-D)*

These mimics were synthesized following the procedure

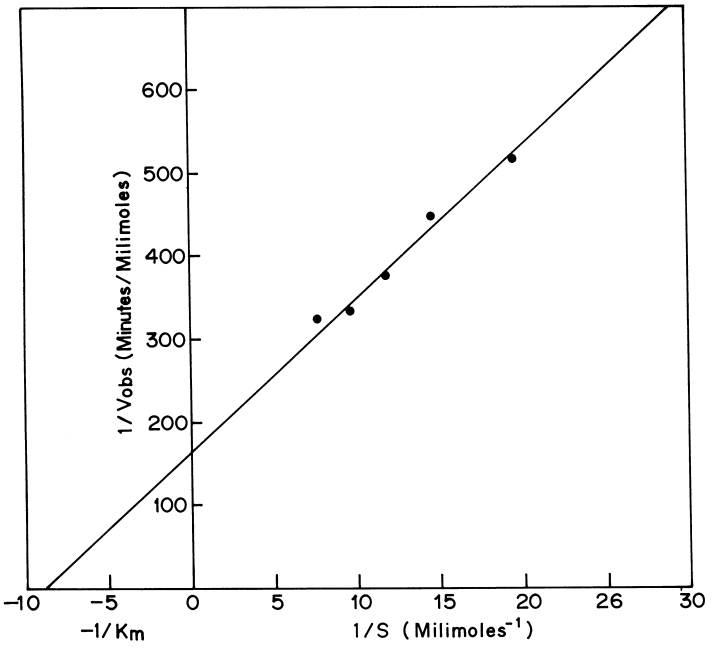
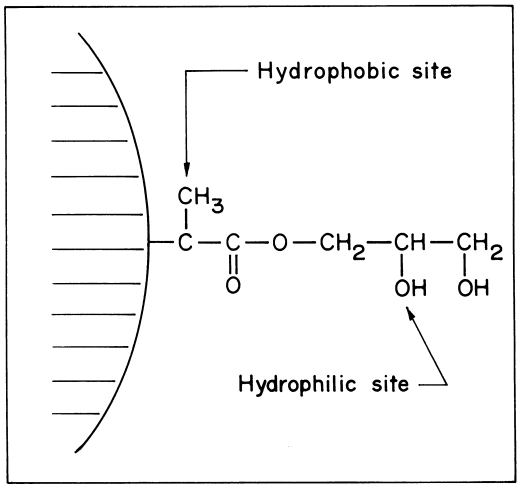
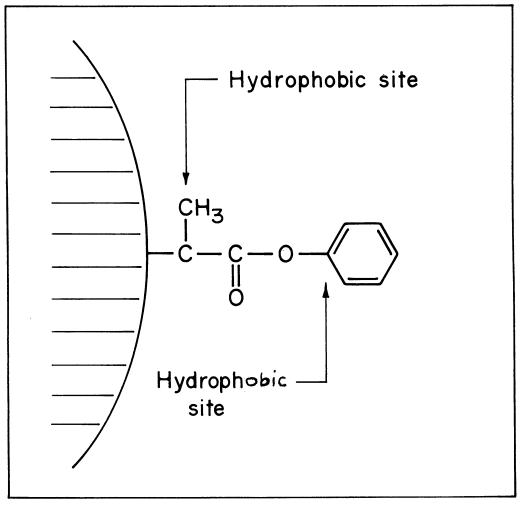



Fig. 3. Lineweaver–Burk plot for hydrolysis of *N*-benzoyl – Tyr-PNP catalyzed by P-B (hydrophilic support) at 37°C and pH 7.8. The assay employed 25 mg P-B which contained 0.00087 mmol of functional groups. Substrate concentration was varied between 0.0522 and 0.1305 mmol. $k_{cat} = 0.11\ s^{-1}$, $K_m = 1.14 \times 10^{-4}$ M, $k_{cat}/K_m = 1018\ s^{-1}\ M^{-1}$.




(a) Binding sites in hydrolyzed poly (GMA-EGDMA)




(b) Binding sites in poly (PHMA-EGDMA)

Fig. 4. Schematic representation of binding sites in hydrophilic and hydrophobic supports.

described earlier using poly (PHMA-EGDMA) support. Relevant data are listed in Table 1.

### 2.3. Evaluation of hydrolytic activity of mimics

In a 25 ml jacketed reactor 50 mg polymeric enzyme mimic was placed. Ten milliliters of 40 : 60 acetonitrile : phosphate buffer (0.05 M, pH 7.8) was added. This suspension was stirred with magnetic needle at 37°C. A predetermined amount of substrate was dissolved in 1 ml acetonitrile and added while stirring. Hydrolysis was followed by monitoring absorbance of released *para* nitrophenol at 400 nm. For generating Michaelis–Menten constants, four to five different substrate concentrations (substrate always excess) were used. Initial observed velocities of reactions were monitored and Lineweaver–Burk plots were obtained.

## 3. Results and discussion

Hydrolytic activity of chymotrypsin depends on whether the substrate binding to the active site is productive or nonproductive. Berezin et al. [5,6] reported the effect of substrate structure on hydrolytic activity of chymotrypsin. Standard substrates, *N*-acyl tyrosine esters, represented by a general structure $R_1$-CO-Tyr-$OR_2$ were used in the study wherein $R_1$ is *N*-acyl substituent, Tyr is phenyl ring of tyrosine and $R_2$ is ester side chain of tyrosine moiety. It was observed that the active site of chymotrypsin comprises three binding loci p1, p2 and p3, complementary to $R_1$, Tyr and $R_2$ groups, respectively. When substrate bindings such as $R_1$ to p1, Tyr to p2 and $R_2$ to p3 take place, it results in ''productive binding'', leading to higher catalytic activity. Hydrophobicity of locus p2 is higher than that of p1. Due to this, with increase in the hydrophobicity of $R_1$ group in substrate, its binding with p2 locus increase. Such $R_1$ to p2 binding results in ''non productive'' binding. This was characterized by low $k_{cat}$ as well as low $K_m$ values in Michaelis–Menten kinetics.

Substrates with hydrophilic and hydrophobic $R_1$ groups used by Berezin et al. [5,6] were *N*-acetyl tyrosyl ethyl ester and *N*-benzoyl tyrosyl ethyl ester, respectively. Chymotrypsin exhibited a high $k_{cat}$ (192 $s^{-1}$) and also a high $K_m$ ($6.63 \times 10^{-4}$ M) value for hydrolysis of *N*-acetyl tyrosyl ethyl ester. But for *N*-benzoyl tyrosyl ethyl ester wherein $R_1$ is hydrophobic, chymotrypsin exhibited nonproductive binding and thus low $k_{cat}$ (85 $s^{-1}$) as well as low $K_m$ ($2.2 \times 10^{-5}$ M) as described earlier. These kinetic data are listed in Table 2.

Thus owing to the fixed hydrophobic levels of binding loci p1, p2 and p3, nonproductive binding for hydrophobic substrates cannot be eliminated in the case of chymotrypsin. In contrast to this, this nonproductive binding in the case of mimic could be eliminated by the choice of appropriate support. In the following sections we discuss these results and show how the activity of the mimic can be enhanced.

### 3.1. Choice of substrates

In the present work we have used *N*-acetyl-Tyr-PNP and *N*-benzoyl-Tyr-PNP as substrates because hydrolytic activity of the mimic is so far limited to activated esters only. Since these substrates could not form coordination complex with Co (II), suitable templates were used for imprinting. Structures of substrates and templates are shown in Fig. 1. The imprinting effect in mimics caused by the templates has been reported earlier [3]. In this work only the effects of support hydrophilicity/hydrophobicity on substrate binding have been studied.

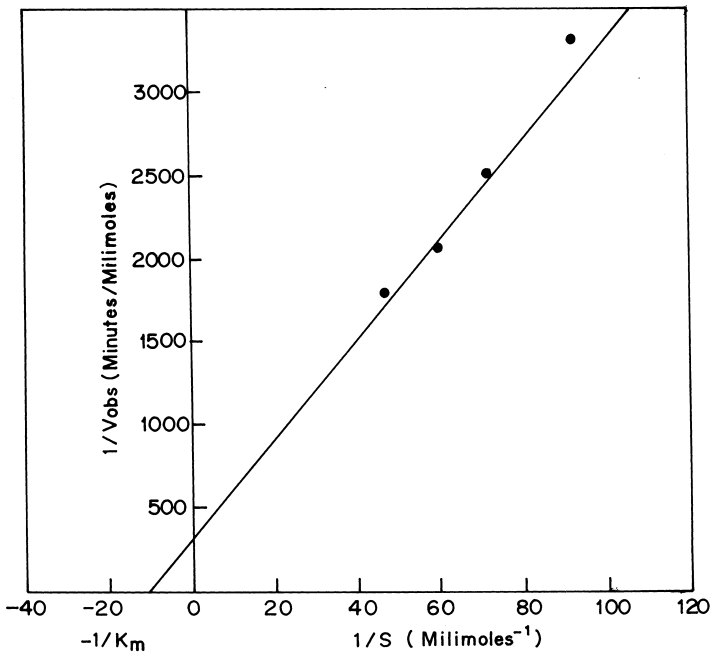



Fig. 5. Lineweaver–Burk plot for hydrolysis of *N*-acetyl – Tyr-PNP catalyzed by P-C (hydrophobic support) at 37°C and pH 7.8. The assay employed 50 mg P-C which contained 0.000155 mmol of functional groups. Substrate concentration was varied between 0.00775 and 0.0217 mmol. $k_{cat} = 0.33\ s^{-1}$, $K_m = 1.0 \times 10^{-4}$ M, $k_{cat}/K_m = 3308\ s^{-1}\ M^{-1}$.

### 3.2. Choice of supports

We chose a hydrophilic and a hydrophobic support for the mimics. The choice of a hydrophilic support was made to simulate overall hydrophilic nature of the enzyme. Hydrophobic support was selected since these are reported to exhibit enhanced hydrophobic interactions with various substrates [7,8,12]. In a prior communication we reported that with increase in the surface area of the support $K_m$ values decrease, in other words, substrate binding increase [4]. Thus in the present case in order to unequivocally establish the effects of hydrophilicity/hydrophobicity on substrate binding, supports with minimal surface area need to be used. Also the supports should contain higher percentage of hydrophilic or hydrophobic comonomer than that of crosslinker in order to achieve desired hydrophilicity or hydrophobicity in the support.

### 3.3. Hydrophilic support (hydrolyzed poly(GMA-EGDMA))

Hydrolysis of epoxide ring of glycidyl methacrylate in GMA-EGDMA support to vicinal diols induces hydrophilicity in the support [11]. Thus we polymerized a composition containing 60% GMA and 40% EGDMA. The polymerization time was limited to 4 h so that complete crosslinking does not take place. This ensured the presence of residual double bonds for grafting functional monomers as well as low surface area of support. Epoxide ring of GMA from the support so synthesized was then hydrolyzed to vicinal diols to induce hydrophilicity. Surface area of this blank support was 28.86 $m^2\ g^{-1}$.

### 3.4. Hydrophobic support [poly (PHMA-EGDMA)]

Incorporation of phenyl methacrylate in EGDMA supports is known to enhance its hydrophobicity [12]. Therefore a composition containing 60% phenyl methacrylate and 40% EGDMA was polymerized as mentioned previously. Surface area of this blank support was 14.64 $m^2\ g^{-1}$.

### 3.5. Mimics on hydrophilic and hydrophobic supports

Mimics P-A and P-B were synthesized by grafting Co (II) coordinated monomers-template assemblies on hydrolyzed poly (GMA-EGDMA) beads. P-A was imprinted for *N*-acetyl-Tyr-PNP and P-B was imprinted for *N*-benzoyl-Tyr-PNP. Similarly, mimics P-C imprinted for *N*-acetyl-Tyr-PNP and P-D imprinted for *N*-benzoyl-Tyr-PNP were prepared on poly (PHMA-EGDMA) beads. Data listed in Table 1 show that although the mimics are synthesized using identical conditions such as monomers to support ratio as well as crosslinker and initiator ratios, the grafting levels differ. For mimics P-B and P-D prepared with hydrophobic templates, percentage grafting was higher than that in the case of P-A and P-C. This indicates that hydrophobic templates increased hydrophobicity of Co (II) complexes which resulted in the efficient grafting. Earlier we reported similar trends for hydrophobic functional monomers [4]. Due to the grafting of monomers on supports, surface area of the resulting mimics decreased significantly. See data listed in Table 1. At these low values of surface area, we

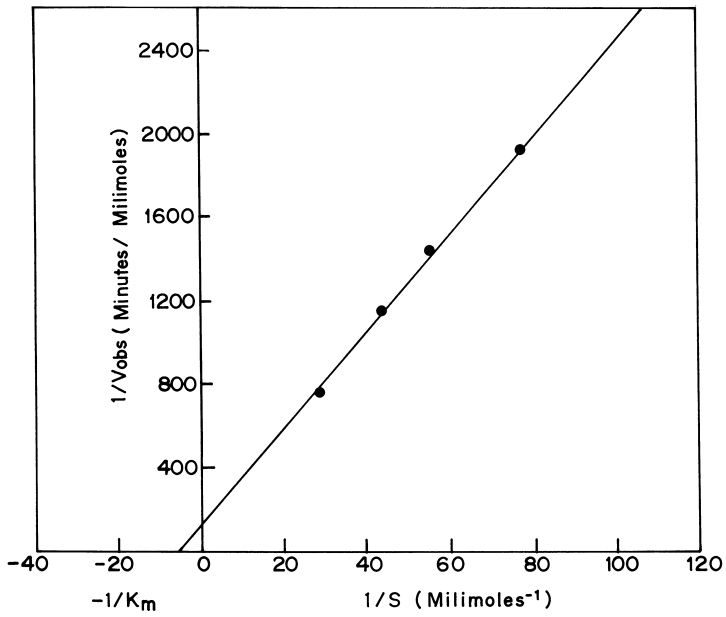



Fig. 6. Lineweaver–Burk plot for hydrolysis of *N*-benzoyl – Tyr-PNP catalyzed by P-D (hydrophobic support) at 37°C and pH 7.8. The assay employed 50 mg P-D which contained 0.00519 mmol of functional groups. Substrate concentration was varied between 0.0129 and 0.03633 mmol. $k_{cat} = 0.26\ s^{-1}$, $K_m = 2.0 \times 10^{-4}$ M, $k_{cat}/K_m = 1338\ s^{-1}\ M^{-1}$.

expect $K_m$ values to reflect only the effects of hydrophilicity or hydrophobicity of supports.

### *3.6. Activity of mimics on hydrolyzed poly (GMA-EGDMA)-hydrophilic support*

Hydrolytic activity of P-A and P-B was evaluated against *N*-acetyl-Tyr-PNP and *N*-benzoyl-Tyr-PNP, respectively. Lineweaver–Burk plots for 1/observed velocity ($1/v_{obs}$) versus. 1/initial substrate concentration (1/*s*) were plotted. The plots are shown in Figs. 2 and 3 and kinetic data are listed in Table 2.

$k_{cat}$ for P-A is 0.45 $s^{-1}$ and $k_{cat}$ for P-B is 0.11 $s^{-1}$. Thus a four-fold decrease in the activity of mimic is observed with increase in the hydrophobicity of $R_1$ group. Hydrophobic $R_1$ is reported to cause steric hindrance for the ester hydrolysis [5,6]. This is consistent with the trend of $k_{cat}$ observed for native chymotrypsin which is shown in Table 2. But, more interestingly, $K_m$ values of mimics P-A and P-B also fall in the trend similar to that of chymotrypsin. $K_m$ value for P-A is $3.33 \times 10^{-4}$ M and $K_m$ value for P-B is $1.14 \times 10^{-4}$ M. In Michaelis–Menten kinetics, a lower value of $K_m$ indicates stronger substrate binding. Thus for substrate with hydrophobic $R_1$ viz. *N*-benzoyl-Tyr-PNP, binding sites on mimic P-B exhibited stronger but nonproductive binding, like native chymotrypsin. In summary, chymotrypsin mimic grafted on hydrophilic support hydrolyzed poly (GMA-EGDMA) exhibited following kinetics. For *N*-acetyl-Tyr-PNP the mimic exhibited high $k_{cat}$ and high $K_m$. For *N*-benzoyl-Tyr-PNP the mimic exhibited low $k_{cat}$ as well as low $K_m$ values. It can be seen from the data listed in Table 2, that kinetic trends of the mimics on hydrolyzed poly (GMA-EGDMA) support are similar to those observed in the case of native chymotrypsin.

The support hydrolyzed poly (GMA-EGDMA) does not contain p1, p2 and p3 loci like chymotrypsin. But it contains hydrophilic binding sites comprising vicinal diols as well as hydrophobic binding sites comprised of methyl groups. Therefore a potential sight for nonproductive binding could be the methyl groups next to vicinal diols. Methyl groups are known to contribute to hydrophobic interactions in monomers like methacrylic acid, GMA etc. A schematic representation of binding sites on hydrolyzed poly (GMA-EGDMA) support is shown in Fig. 4.

### *3.7. Activity of mimics on poly (PHMA-EGDMA)-hydrophobic support*

Hydrolytic activity of P-C and P-D was evaluated against *N*-acetyl-Tyr-PNP and *N*-benzoyl-Tyr-PNP, respectively. Lineweaver–Burk plots are shown in Figs. 5 and 6 and the kinetic data are listed in Table 2.

The $k_{cat}$ for P-C is 0.33 $s^{-1}$ and the $k_{cat}$ for P-D is 0.26 $s^{-1}$. Thus in this case too, the effect of hydrophobic $R_1$ on $k_{cat}$ is similar to that of chymotrypsin as discussed previously. But the trend of $K_m$ values exhibited by P-C and P-D is significantly different than that of native chymotrypsin. The $K_m$ value for P-C is $1.0 \times 10^{-4}$ M, which is lower than $K_m$ value for P-D ($2.0 \times 10^{-4}$ M). Thus the mimics P-C and P-D on poly (PHMA-EGDMA) support exhibited the trend normally observed in the case of Michaelis–Menten kinetics, i.e. ''better the binding, better the reaction''. In other words, chymotrypsin mimic grafted on hydrophobic support poly (PHMA-EGDMA) overcame the nonproductive binding.

This gets further highlighted when $k_{cat}$ values of mimics P-B ($0.11\ s^{-1}$) and P-D ($0.26\ s^{-1}$) for the same substrate i.e. *N*-benzoyl-Tyr-PNP are compared.

This can be attributed to the following. The support poly (PHMA-EGDMA) is comprised of only hydrophobic binding sites viz. methyl groups and phenyl groups. Schematic representation of binding sites is shown in Fig. 4. Thus nonproductive binding of hydrophobic $R_1$ observed in the case of chymotrypsin as well as the mimic on hydrolyzed poly (GMA-EGDMA) support is eliminated when the mimic is grafted on the hydrophobic support viz. Poly (PHMA-EGDMA). The relative hydrophobicities of the loci p1, p2 and p3 are predetermined and cannot be altered.

### *3.8. Enhanced activity caused by hydrophobic support*

It can be seen from the data listed in Table 2 that $k_{cat}/K_m$ values, which are measures of catalysts efficacy, are higher for mimics P-C and P-D ($3308\ s^{-1}\,M^{-1}$, $1338\ s^{-1}\,M^{-1}$, respectively) than those for P-A and P-B ($1350\ s^{-1}\,M^{-1}$, $1018\ s^{-1}\,M^{-1}$, respectively). This increase in the activity of chymotrypsin mimic grafted on hydrophobic support can be attributed to enhanced hydrophobic interactions between substrate and the support. Thus the choice of support provides additional means of enhancing the activity of the mimic towards a given substrate.

## 4. Conclusion

The effect of support composition on substrate binding was studied for molecularly imprinted polymer mimics of chymotrypsin. The mimic grafted on hydrophilic support exhibited low $k_{cat}$ as well as low $K_m$ values and thus nonproductive binding for hydrophobic substrate. This was similar to the native chymotrypsin. But the mimic grafted on hydrophobic support exhibited trends consistent with Michaelis–Menten kinetics i.e. high $k_{cat}$ and low $K_m$ and thus eliminated the nonproductive binding. Also, the activity of mimic on hydrophobic support was higher than that of mimic on hydrophilic support. Thus the activity of the synthetic mimic towards a specific substrate can be enhanced by the choice of appropriate support, a discretion not available in the case of chymotrypsin.

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