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Published in:
Chemical Communications

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
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An enantioselective imprinted receptor for Z-glutamate exhibiting a binding induced color change†

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First published as an Advance Article on the web 9th September 2004

Reagents capable of responding to specific target molecules by a visible color change are highly attractive for fast qualitative analysis. 1,2 This is reflected in the rapidly growing number of chromogenic anion sensors based on ingeniously constructed host molecules with appended chromophores. These typically respond to one or a group of target molecules depending on the host design.

In response to new target molecules, the host needs to be redesigned, often involving elaborate synthesis protocols. Molecular imprinting constitutes a more flexible approach in this context and robust polymeric receptors can now be readily prepared against a large variety of small molecules. 3,5 Although noncovalently imprinted polymers exhibiting a chromogenic or fluorogenic response have been previously reported, 6–9 to our knowledge there continues to date host polymers where the molecular recognising event is associated with a color change visible to the naked eye. This is partly due to the common use of weakly associating monomers, precluding the exact placement of a chromophore in the imprinted binding sites. 10 Stoichiometric imprinting 11 may hold a solution to these problems. In our laboratory we have exploited disubstituted ureas, a functional group which is well established in anion recognition 12 and chromogenic sensing. 12–16 as easily synthetically accessible and potent host motif in monomers for oxyanions. 17

Here we wish to report that 1-(4-styryl)-3-(3-nitrophenyl)urea (I) (Fig. 1) can be used as functional monomer for the imprinting of Z-(o or t)-Glu, a polymeric receptor exhibiting strong enantioselectivity and a change in color intensity upon binding of the guest was obtained.

In order to assess the solution binding ability of the monomer towards carboxylates we first performed a 1H NMR titration in DMF- d6 using benzoxate as its tetrabutylammonium (TBA) salt as guest. Self-association of monomer and/or guest does not occur in these systems and Job plot analysis confirmed the 1 : 1 stoichiometry of monomer-guest interactions as depicted in Fig. 1. Fitting of the raw titration data to a 1 : 1 binding isotherm gave an association constant of 6498 (±170) M−1, in good agreement with previously published results. 11 This implies that carboxylates are quantitatively complexed by this host monomer at typical prepolimerization concentrations and, notably, in a competitive medium. As reported for similar low molecular weight host molecules, 12–13 the titration was accompanied by a bathochromic shift of 15 nm (Fig. 1) leading to a clearly visible increase in the yellow color intensity.

Imprinted (P1) and nonimprinted (P0) polymers were then prepared from I, with ethyleneglycol dimethacrylate (EDMA) as crosslinker, in the presence of 2 equivalents of triethyamine (TEA) in DMF. Elemental analysis performed on the polymers after Soxhlet extraction indicated that the monomer conversion was high, that the monomers had been stoichiometrically incorporated into the polymers and that the template had been successfully removed from the polymer. The molecular recognition properties of the materials were then investigated via chromatography comparing the retention of the template, Z-Glu, with that of more complex biologically active molecules such as methotrexate (MTX), containing the glutamic acid substructure, and structurally related analogues Z-Asp and Z-Glu.

As mechanistically expected, the effect of imprinting depended strongly on the ionization state of the acid groups. Using an aqueous acetonitrile-based mobile phase, all solutes were weakly and similarly retained on both columns in the absence of added base, whereas in the presence of base the glutamate containing solutes were strongly and selectively retained (Table 1).

To gain insight into the binding energy and site density of the polymers we measured the equilibrium binding isotherm on a Z-Glu imprinted polymer in the optimum solvent system described above and in MeCN/TEA (99/1 v/v) where Z-Glu is expected to bind strongly to both the imprinted and nonimprinted polymers (Fig. 2). Z-Glu interacts strongly (Ks > 1000 M−1) with both polymers in the latter system and the difference between the uptake of the solute by them is small. Interestingly, the curve levels off at a value close to the theoretical capacity of P1 based on the amount of template added to the monomer mixture. This shows that the matrix urea groups are functional and fully accessible.

The association to P3 could be selectively suppressed by addition of water, resulting in a large difference in adsorption properties between the materials. Thus, in addition to a preferential

† Electronic supplementary information (ESI) available: experimental procedure for the preparation of the imprinted polymers. Titration data containing 1H NMR C5S curves and Job plot of monomer I. See http://www.rsc.org/suppdata/ccb/b4/0407870e/
Table 1  Chromatographic retention factors (k) and imprinting factors (IF = \( k_{D,\text{imp}}/k_{\text{D,nonimp}} \)) for N-substituted amino acids on P1 and P1

<table>
<thead>
<tr>
<th>Solute</th>
<th>( k_{D,\text{imp}} )</th>
<th>( k_{\text{D,nonimp}} )</th>
<th>IF</th>
<th>( k_{D,\text{imp}} )</th>
<th>( k_{\text{D,nonimp}} )</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-Glu</td>
<td>4.4</td>
<td>2.6</td>
<td>1.7</td>
<td>100</td>
<td>1.4</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Z-Asp</td>
<td>2.8</td>
<td>1.6</td>
<td>1.7</td>
<td>0.6</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Z-Gly</td>
<td>5.2</td>
<td>3.0</td>
<td>1.8</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>MTX</td>
<td>4.0</td>
<td>2.7</td>
<td>1.5</td>
<td>2.5</td>
<td>0.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*The mobile phase was MeCN/H2O: 93/7 (v/v) for P1 (filled symbols) and P1 (open symbols) as solutions in MeCN/H2O/TEA: 92/7/1 (v/v/v) for (−TEA) or MeCN/H2O/TEA: 92/7/1 (v/v) (+TEA).

Notes and references


† The polymers (P1 and P1) were washed as follows: MeOH (× 7); 10% HCl (1 M, aqueous) in MeOH (× 7); 10% HCl (1 M, aqueous) in DMF (× 7); DMF (× 6), THF (× 6), DMF (× 1).
‡ The washed polymers (P1 and P1) were dried in vacuo at room temperature and conditioned in MeCN. Thereafter they were incubated with a solution of Z-L-Glu (10 mM) in MeCN/H2O/TEA: 92/7/1 (v/v/v) for 5 days.

In conclusion, Z-glutamate imprinted polymers made using monomer 1 show strong binding to carboxylates in competitive aqueous-containing environments and the binding can be monitored with the naked eye. Given that a large range of biologically important molecules containing oxygen functionality are compatible with imprinting in such solvent systems as those described above, this type of monomer will significantly expand the scope of imprinted polymer based applications.