ELSEVIER

Biosensors and Bioelectronics 22 (2006) 138–144

BIOELECTRONICS

www.elsevier.com/locate/bios

Concentration dependent atrazine–atrazine complex formation promotes selectivity in atrazine imprinted polymers

Nathalie Lavignac, Keith R. Brain, Christopher J. Allender *

Molecular Recognition Research Unit, Welsh School of Pharmacy, Cardiff University, The Redwood Building, Cathays Park, Cardiff CF10 3XF, UK

Received 16 November 2005; received in revised form 10 February 2006; accepted 22 March 2006
Available online 11 May 2006

Abstract

An atrazine (ATR) molecularly imprinted polymer (MIP) was prepared using a non-covalent strategy. The affinity and selectivity of the polymer was initially evaluated under non-equilibrium conditions and the polymer was shown to possess good template selectivity. The selectivity of the polymer was further investigated under equilibrium conditions and over a range of concentrations using Scatchard plots and Hill plots and by assessing distribution coefficients and normalised selectivity values. It was observed that both selectivity and affinity were dependent on the concentration of the ligand and that unusually selectivity and affinity were better at higher atrazine concentrations. It was concluded that this phenomenon resulted from the formation of atrazine–atrazine complexes during the pre-polymerisation stage and during rebinding and that the polymer demonstrated improved atrazine affinity when the conditions favoured complex formation.

Keywords: Molecularly imprinted polymer; Atrazine; Cooperative interaction; Hill plot

1. Introduction

Molecularly imprinted polymers (MIPs) have routinely been prepared and reported as mimicking the molecular recognition functions of biomolecules such as enzymes, receptors molecules and antibodies (Vlatakis et al., 1993; Allender et al., 1999; Andersson and Nicholls, 2004). The polymeric matrix generated when so called 'functional monomers' (or functional group containing monomers) are copolymerised in the presence of an excess of a divalent 'cross-linking' monomer and a template molecule gives rise to materials that possess specific affinity for the template (Allender et al., 1999). The affinity, specificity and capacity of such MIPs are commonly assessed either chromatographically (non-equilibrium) or in batch binding experiments (equilibrium) and several models have been developed to analyse the arising data (Allender et al., 1999). Although these models can provide useful information on total binding site concentration, binding affinity, affinity distribution and cross-reactivity they do not provide insight into the fundamental process of molecular recognition. Whilst analytical approaches such as NMR, UV and IR spectroscopy have been used to probe the solution phase complex, the structure, orientation and stoichiometry of the imprint site-template complex has proven to be difficult to study. In this study investigations into the binding characteristics of atrazine (ATR) to an atrazine MIP unexpectedly provided insight into the process of template rebinding.

Atrazine is a commonly used broad-spectrum herbicide that has been extensively employed over a period of 30 years. However, recent studies have shown that, due its environmental persistence, it has become a common pollutant of both surface water and groundwater. Gas and liquid chromatography have both been used extensively for the detection and quantification of pesticides at traces levels but these techniques can be time consuming and costly (Pacakova et al., 1996). Immunoassays have therefore become a popular alternative format in environmental analysis but this technique is not without drawbacks. Antibodies can be difficult to generate against non-immunogenic molecule, they can be expensive and a reliance on animals in their production is viewed with some concern. As an alternative, this study uses molecular imprinting in order to develop an alternative analytical tool for use in the analysis of atrazine (Muldoon and Stanker, 1995; Bjarnason et al., 1999; Sergeyeva et al., 1999; Shoji et al., 2003; Matsui et al., 1997; Matsui et al., 2000) and pesticides in general (Surugiu et al., 2000, 2001a,b;

* Corresponding author. Tel.: +44 29 20875824; fax: +44 29 20874149.
E-mail address: allendercj@cf.ac.uk (C.J. Allender).

0956-5663/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.bios.2006.03.017

Haupt, 2001; Pap et al., 2002; Cacho et al., 2004; Zhu et al., 2002; Bastide et al., 2005). These studies have shown that for atrazine-imprinted polymers the recognition process, and hence the selectivity of the polymer, relies on interactions between the polymer and the chlorine atom. In addition, the molecular weight, shape and the basicity and hydrophobicity of cross-reacting molecules strongly influences MIP specificity (Matsui et al., 1997; Siemann et al., 1996; Matsui et al., 1995; Takeuchi et al., 1999). This study uses a number of approaches under both equilibrium and non-equilibrium conditions to investigate MIP performance (Hulme and Birdsall, 1990).

2. Materials and methods

2.1. Chemicals

Atrazine, ametryn (AME), simazine (SIM) and chlorotoluron (CHLO) were obtained from Riedel de Haen (Seelze, Germany). Other herbicides [propazine, prometryn, alachlor, and metribuzine] were obtained as 'gifts' from manufacturers. Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), triazine and 2,2′-azobisisobutyronitrile (AIBN) were from Sigma (Dorset, UK). Chloroform and acetonitrile (HPLC grade) were from Fisher Scientific (Manchester, UK).

2.2. Synthesis of atrazine-imprinted poly(MAA-co-EDMA) and control polymers

The imprinted polymer was prepared by radical polymerisation. The template molecule (ATR, 0.93 mmol; 200 mg), functional monomer (MAA, 5.57 mmol; 0.472 ml), cross-linker (EDMA, 22.3 mmol; 4.2 ml) and initiator (AIBN, 0.35 mmol; 57 mg) were dissolved in chloroform (16.1 ml), in a 20 ml glass vial. Blank non-imprinted polymers (NIP) was prepared using the same protocol, but without atrazine.

The vials were sealed and the solutions were sonicated under vacuum for 5 min at 0 °C and then purged with nitrogen for a further 5 min. Polymerisation was UV initiated ($\lambda = 366$ nm). Polymerisation was carried out at 0 °C for 4 h and then at room temperature for a further 20 h. The resulting polymers were ground with a pestle and mortar. Particles were wet sieved in acetone using a stainless steel sieve ($\emptyset = 45$ μm) and sedimented three times in acetonitrile to remove fines. Template removal was achieved by successive washes in methanol/acetic acid (3 ml × 100 ml; 5%, v/v), methanol (3 ml × 100 ml) and by Soxhlet extraction in methanol for 48 h. The particles were subsequently collected by filtration and dried under vacuum for 48 h.

2.3. Chromatographic evaluation

Polymer particles were pre-swollen in acetonitrile and packed (4000 PSI) into HPLC columns (4.6 mm × 150 mm) using a Jones Chromatography (Hengoed, UK) column packer. Subsequent HPLC evaluation was carried out using a Perkin Elmer (Beaconsfield, UK) ISS-100 system. The columns were equilibrated using 100% acetonitrile as a mobile phase until a steady base line was obtained. For all experiments, the injection volume was 20 μl, the mobile phase was 100% acetonitrile, the flow rate was 1 ml/min and detection was UV (260 nm). The template and a number of potentially cross-reacting molecules where evaluated: atrazine, propazine, simazine, ametryn, prometryn, triazine, alachlor, chlorotoluron and metribuzine.

2.4. Binding assays

Polymer particles (200 mg) were pre-swollen in acetonitrile (20 ml) for 2 h. Five hundred microliters of the suspension (containing 5 mg polymer) was transfered into eppendorf vials, to which was added 500 μl of a pesticide solution of known concentration (3–70 μmol/l). The suspensions were stirred overnight at room temperature then centrifuged to sediment the polymer (5 min/10,000 rpm). Concentrations of the pesticides in the supernatant were determined by HPLC.

2.5. HPLC analysis of supernatant

High-performance liquid chromatography was performed using a Thermo Separation Products system (Hemel Hempstead, UK) [column: ODS 2 column (Spherisorb: 250 mm × 4.6 mm, 5 μm); mobile phase: acetonitrile/water (1:1, v/v); flow rate: 1 ml/min; detection: UV 260 nm].

2.6. Curve fitting

Curve fitting was carried out by non-linear regression using GraphPad Prism 4 (San Diego, CA).

3. Results and discussion

3.1. Non-equilibrium evaluation of atrazine-imprinted poly(MAA-co-EDMA)

HPLC has been commonly employed to investigate and evaluate the molecular recognition process in imprinted polymers prepared by the non-covalent approach. Enantiomers have been used extensively to evaluate the imprinting effect as non-specific binding effects both molecules equally (Sellergren, 2001). For non-chiral molecules, it is customary to compare the retention on the imprinted stationary phase with the retention on a non-imprinted one (Siemann et al., 1996), a difference being the first indicator that a polymer has been successfully imprinted (Sellergren, 2001). Since morphological differences between the imprinted polymer and the non-imprinted polymer can lead to ambiguity a number of related molecules were also evaluated.

Twenty microliters of an atrazine solution (200 μg/ml) was injected onto both imprinted and non-imprinted columns. Retention factors (k') were calculated as $k' = (t_R - t_0)/t_0$ where t_R is the retention time of the analyte and t_0 the retention time of acetone (void marker) (Table 1). For atrazine, retention on the blank column was lower than for the MIP column, which suggested that the polymer had been successfully imprinted. The efficiency of the imprinting process was quantified by determining an imprinting factor (I_f) where $I_f = k'_{MIP}/k'_{NIP}$. In this

Table 1
Retention factors for the control (NIP) and atrazine-imprinted (MIP) polymers

Polymer	Retention time (t_R, min)	Retention factor (k')	Imprinting factor (I_f)
MIP	17.09	6.15	6.68
NIP	3.69	0.93	na

Two micrograms of atrazine was injected on column; mobile phase was acetonitrile; low rate 1 ml/min; detection was UV 260 nm.

equation k'_{MIP} is the retention factor of atrazine on the imprinted column and k'_{NIP} is the retention factor of atrazine on the blank column.

In this study cross-reactant binding profile was used to confirm an atrazine specific imprinted effect. The cross-reactivity of the atrazine-imprinted column for the related compounds was quantified using relative retention factors (K) (Eq. (1)) (Ramström et al., 1998).

$$K = \frac{IF_{atrazine}}{IF_x} \quad (1)$$

where $IF_{atrazine}$ is the imprinting factor for atrazine and IF_x is the imprinting factor of the cross-reacting species.

For the template $K = 1$ and for molecules less-specific for the molecular imprinted polymer compared to the control polymer $K < 1$. The data (Fig. 1) suggests that the cross-reacting molecules can be sub-divided into three classes (high, intermediate and low cross-reactivity) and that this division can be rationalised in terms of the template structural analogy:

- Class 1: High cross-reactivity for propazine and simazine whose structure are extremely close to that of atrazine.
- Class 2: Intermediate cross-reactivity for ametryn and prometryn bearing a thiomethyl group ($-SCH_3$) instead of the chlorine atom.
- Class 3: Low cross-reactivity for triazine, alachlor, chlorotoluron and metribuzine with limited-similarity to atrazine.

Atrazine, simazine and propazine are structurally very similar and it was therefore unsurprising that their retention characteristics on the atrazine-imprinted column were similar. The variation in K observed can be accounted for by small variations in basicity (Dauwe and Sellergren, 1996) and by the additional methyl substitution of propazine.

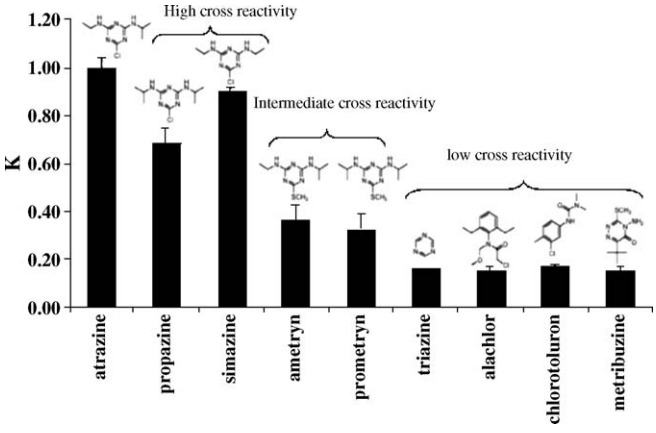

Fig. 1. Relative imprinting factors (K) for a range of atrazine MIP cross-reactants. (Data represents mean ± S.E.M., $n = 3$.)

The relative retention factors for the thiomethyl analogues, ametryn and prometryn, were lower than the values obtained for the chlorine substituted analogues atrazine, propazine and simazine. The chlorine atom appears therefore to be important in providing selectivity. The differences observed in K between class 1 and class 2 compounds would have arisen as a result of changes in the size of the constituent groups, changes in hydrogen bonding or a combination of both. In this case sulphur and chlorine are atoms of similar size, it therefore seems reasonable that the addition of the methyl group may prevent the class 2 compounds from fully docking within the imprinted site. In addition, changing the electronegative atom, from a chlorine atom to a sulphur atom, would also result in a change in hydrogen bonding compared to the class 1 analytes.

The class 3 compounds can be further sub-divided. Triazine is the hetero-aromatic backbone of both the class 1 and the class 2 compounds. It is therefore unsurprising that it is capable of forming the same hetero-aromatic to polymer interactions as these compounds and also that it would be freely able to dock within the atrazine-imprinted site since it possess no additional sterically hindering substitutions. It might therefore have been predicted that triazine would demonstrate a high K value. However, the data showed that despite its apparent ability to fit and establish some reciprocal points of interaction within the atrazine cavity, triazine possessed low affinity for the atrazine molecularly imprinted site. This demonstrates that interactions between the polymer and the 1, 3, 5 side groups of atrazine are key to the recognition process and illustrates the importance of multi-valent binding between MIP and analyte. On the basis of these conclusions it was predictable that the other class 3 compounds would give rise to low K values based on non-reciprocal steric and functional characteristics.

3.2. Equilibrium binding characteristics of atrazine-imprinted poly(MAA-co-EDMA)

Batch binding experiments were carried out in order to assess both qualitatively and quantitatively the equilibrium affinity of the atrazine MIP for the template and for a representative number of cross-reactants and binding isotherms were plotted (Fig. 2).

A common approach for determining affinity constants and binding site concentration is to re-plot the data in the form of a Scatchard plot (Eq. (2)) (a linearised form of the Langmuir equation) where the slope of the graph is equal to $-1/K_D$ and the X axis intercept gives the concentration of binding sites.

$$\frac{B}{F} = \frac{B_{max} - B}{K_D} \quad (2)$$

where B_{max} is the apparent maximum number of binding sites, K_D the equilibrium dissociation constant, B the concentration of analyte bound to the polymer and F the concentration of free analyte.

Importantly this only applies when the binding site population can be described by a single affinity constant and when

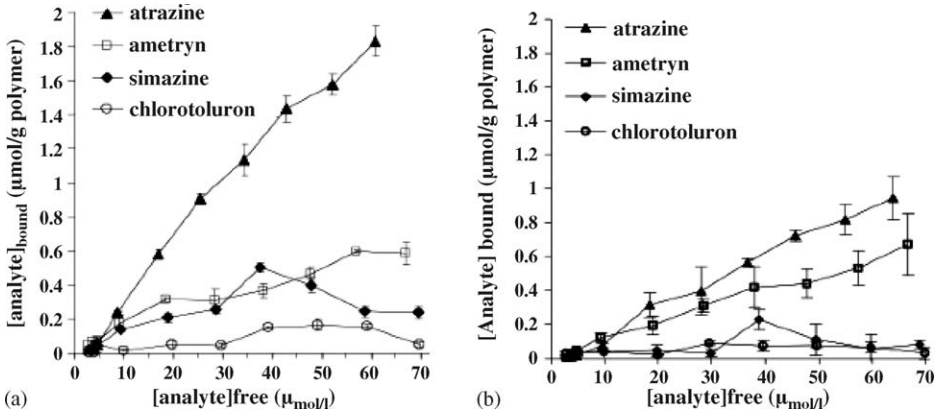

Fig. 2. Equilibrium binding curves for atrazine and a number of potential cross-reactants to the atrazine MIP (panel a) and NIP (panel b). (Data represents mean ± S.E.M., $n = 3$.)

binding sites are independent of each other (Hulme and Birdsall, 1990). However, for binding data arising from MIP-ligand binding studies this is rarely the case and such plots are commonly non-linear concave curves. This phenomena has been attributed to the heterogeneous distribution of binding site affinities (Sellergren, 2001; Ye et al., 1999) and the asymptotes of such curves have, at low and high concentrations, been used to define high and low affinity constants. However, in this study the Scatchard plot for atrazine binding to the atrazine MIP resulted in an unexpected convex plot (Fig. 3).

Although this type of plot has not previously been reported for MIP systems it is commonly observed in biological ligand/receptor systems and can be indicative of binding site cooperativity (Hulme and Birdsall, 1990). An alternative approach used commonly in biological systems for interpreting this type of data is the Hill equation that provides a mechanism to quantify the deviation from linearity (Eq. (3)):

$$\mathrm{Log}\frac{Y}{1-Y} = n_{\mathrm{H}} \times \mathrm{Log}[F] - n_{\mathrm{H}} \times \mathrm{Log}K_{\mathrm{D}} \quad (3)$$

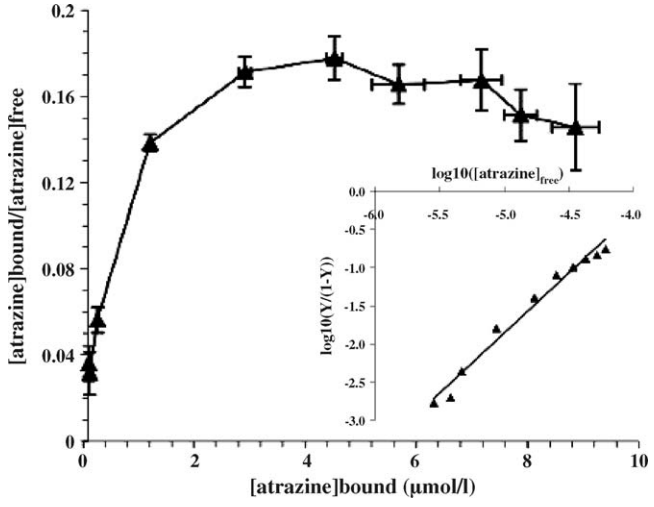

Fig. 3. Scatchard and Hill plots (insert) for atrazine binding to an atrazine MIP. (Data represents mean ± S.E.M., $n = 3$.)

where Y is the binding site occupancy, n_{H} the Hill coefficient and K_{D} is the dissociation constant.

A plot of $\mathrm{Log}(Y/(1-Y))$ versus $\mathrm{Log}[F]$ is called a Hill plot and approximates to a straight line. The slope of the plot gives the Hill coefficient. When $n_{\mathrm{H}} = 1$ this corresponds to a linear Scatchard plot and is indicate of non-cooperative systems, when $n_{\mathrm{H}} > 1$ this indicates positive cooperativity and corresponds to a downward-curved Scatchard plot, and when $n_{\mathrm{H}} < 1$ this indicates negative cooperativity and corresponds with an upward-curved Scatchard plot. Importantly this can also be indicative of heterogeneity in the binding site population.

The Hill equation requires though an estimation of binding site occupation (Y). When the value of $B_{\max}$ is known, it is possible to normalise the binding data [B] by dividing each value by $B_{\max}$ to give an occupancy value (Y) (Eq. (4))

$$Y = \frac{[B]}{B_{\max}} \quad (4)$$

Using the binding to the non-imprinted polymers as an estimation of non-specific, low affinity interactions (Sellergren, 1989; Shea et al., 1993; Haupt, 1999), specific binding was evaluated by subtracting the amount of atrazine bound to the NIP from that bound to the MIP ($\mathrm{MIP}_{\mathrm{specific}} = \mathrm{MIP}_{\mathrm{total}} - \mathrm{NIP}_{\mathrm{total}}$). This specific binding data was then fitted to the Scatchard equation (Eq. (2)) using GraphPad Prism. An apparent K_{D} was determined as 35 µM and $B_{\max}$ as 1.6 µmol/g of polymer. Using Eq. (4), binding site occupation (Y) was determined for a range of concentrations of ligand. Hill coefficient (n_{H}) of 1.62 was determined from the slope of the Hill plot (Fig. 3) (Hulme and Birdsall, 1990).

Conventional interpretation of such data would suggest positive cooperativity, implying that the first molecules of atrazine to bind to the polymer did so with a lower affinity than did subsequent molecules. However, this type of direct interpretation requires that the binding site population can be described by a single affinity constant. However, in a MIP system such as described here, this is not the case. Therefore, the Hill coefficient, rather than a direct indication of cooperativity, is perhaps a useful *indication* of binding site affinity distribution. However, this is not to mean that some form of cooperativity is not involved

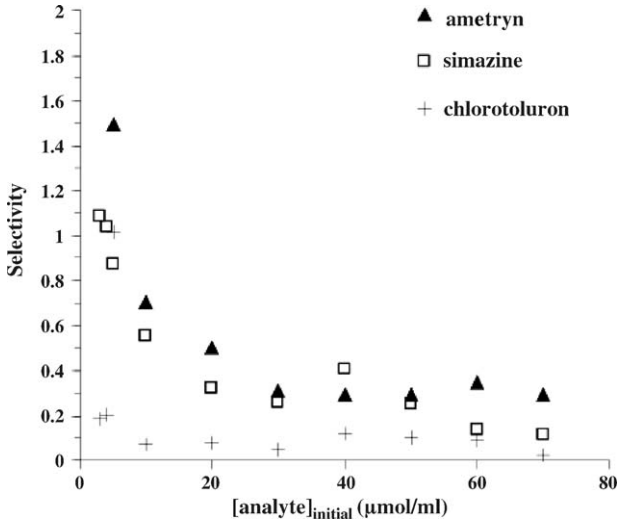

Fig. 4. The selectivity of the atrazine MIP for ametryn, simazine and chlorotoluron.

it is simply not possible to deconvolute the mutual contributions of binding site heterogeneity and positive cooperativity using this approach.

Further indication that both affinity and selectivity of the atrazine MIP changes with the bound analyte concentration was observed when the selectivity of the MIP and atrazine distribution coefficients were considered.

Selectivity over a range of concentrations was studied by comparing the *specific* binding of ametryn, simazine and chlorotoluron against that of atrazine, where specific binding is the difference between the that bound to the MIP ($[\text{analyte}]_{\text{MIP}}$) and to the NIP ($[\text{analyte}]_{\text{NIP}}$). Selectivity was determined using Eq. (5) and is plotted in Fig. 4.

$$\text{Selectivity} = \frac{[\text{analyte}]_{\text{MIP}} - [\text{analyte}]_{\text{NIP}}}{[\text{template}]_{\text{MIP}} - [\text{template}]_{\text{NIP}}} \quad (5)$$

Fig. 4 reveals an interesting and unexpected trend in that relative cross-reactant selectivity *decreases* with concentration. In other words atrazine selectivity appears to increase as concentration increases. This observation opposes the accepted view that template selectivity is highest at low high-affinity binding site occupation, which occurs at low template concentration. This observation suggests that selectivity increases when binding site occupation is high.

This interesting observation is further supported when distribution coefficients (K_d) at high and low concentrations of ligand are considered (Zhu et al., 2002; Jie and Xiwen, 1999) (Eq. (6)) (Table 2):

$$k_d = \frac{C_p}{C_l} \quad (6)$$

where C_p is the pesticide concentration bound to the polymer (μmol/g polymer) and C_l is the concentration of pesticide in solution (μmol/l).

At high concentrations of analyte ([analyte] = 50 μmol/l), the k_d values for atrazine is at least 3 times greater than for ametryn and simazine and 10 times greater than that for chlorotoluron.

Table 2
Binding selectivity of the atrazine-imprinted polymer

Analytes	Distribution coefficient (k_d)	
	Analyte initial concentration: 50 μmol/l	Analyte initial concentration: 5 μmol/l
Atrazine	33.5	11.2
Ametryn	9.8	16.8
Simazine	8.4	9.8
Chlorotoluron	3.4	11.4

Distribution coefficients for atrazine and a number of cross-reactants at determined at high (50 μmol/l) and low (5 μmol/l) concentrations. (Data represents the mean of three experiments, CV < 5%), polymer: 5 mg, solvent acetonitrile, volume: 1 ml.

This correlates with our previous interpretations of both equilibrium and non-equilibrium binding. However, at lower analyte concentrations ([analyte] = 5 μmol/l) the k_d values for atrazine (11.2), ametryn (16.8), simazine (9.8) and chlorotoluron (11.4) are all similar and are much lower than the k_d value for atrazine at 50 μmol/l which was 33.5. Particularly surprising is that the k_d values for ametryn, simazine and chlorotoluron appear to actually increase whilst the value for atrazine significantly falls.

The data clearly suggests that the binding of atrazine to the atrazine MIP does not follow a simple Langmuir binding isotherm and also that the dependence of relative selectivity on concentration does not fit the preconceived model.

One explanation for these phenomena is the formation of atrazine–atrazine complexes both in solution and on the polymer surface and two hypothesise are proposed to explain the increased affinity of the MIP for atrazine at higher concentrations. The first hypothesis (Fig. 5) being that during MIP formation the template was present at a very high concentration (∼45 mmol/l) compared to that use for the rebinding experiments. Although previous atrazine molecular imprinting studies have not reported direct evidence of atrazine–atrazine complexation during the pre-polymerisation stage unrelated NMR studies demonstrated that atrazine forms higher complexes in solution (Welhouse and Bleam, 1993; Welhouse et al., 1993). Therefore, it is not unreasonable that at the concentration of atrazine used in the preparation of the MIP the 'template' was a mixed population of free and complexed atrazine. The resultant population of imprinted sites would therefore mirror this. It is therefore proposed that when rebinding atrazine at low concentrations (when atrazine–atrazine complexation is not favoured) the solution phase atrazine population differs from the original 'template' atrazine population. This results in low template affinity and poor selectivity. Conversely at the higher rebinding concentration (which might be expected to favour atrazine–atrazine complex formation) the solution phase atrazine population more closely resembled that which was present during the imprinting process. Logically this would lead to greater binding site occupation and this would result in the observed apparent increase in affinity for atrazine. The second hypothesis (Fig. 5) does not rely on the formation of solution phase atrazine–atrazine complexes during rebinding but instead relies on the promotion of atrazine–atrazine complex formation within 'atrazine complex' templated binding sites. This is akin to a nucleation process

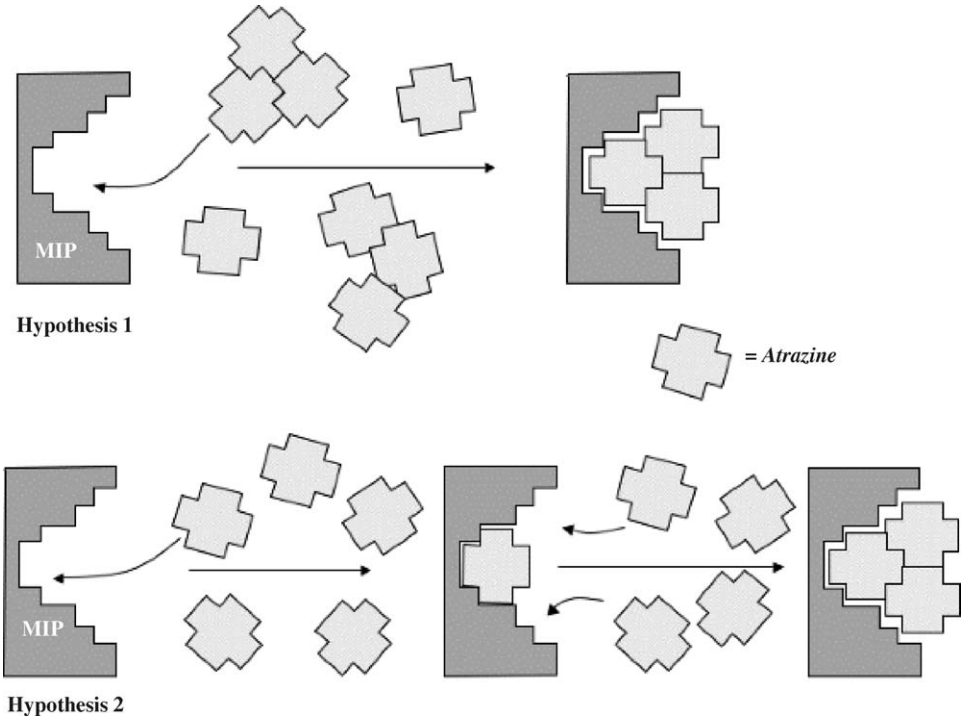

Fig. 5. Atrazine complex formation and polymer association. Hypothesis 1: solution phase atrazine complex binding within an atrazine complex templated binding site. Hypothesis 2: uncomplexed atrazine nucleating within an atrazine complex templated binding site.

and parallels the work of D'Souza et al. (1999). In this case it is proposed that at low analyte concentrations the affinity of atrazine and the cross-reactants for the large 'atrazine complex' imprinted sites is comparable. However, at higher concentrations the ability of atrazine to generate complexes *within* the imprinted sites results in increased atrazine selectivity. Either of these processses would explain the variation in distribution coefficients and the change in selectivity that was observed and, based upon this data, it would be difficult to distinguish between the two. Interestingly, variation in chromatographic non-equilibrium selectivity with analyte concentration was not observed suggesting that the kinetics of atrazine complex formation also plays a role. Such 'cooperative' selectivity, has been previously been show to occur in a (–)-nicotine imprinted polymer system (Andersson et al., 1999) where it was proposed that increased enantioselectivity at higher sample loadings resulted from the presence of higher order template complex binding sites.

4. Conclusions

The results show that the atrazine-imprinted polymer used in these studies possessed enhanced affinity and good selectivity for the template. However, selectivity and template affinity was shown to increase with concentration. This observation is generally contrary to accepted wisdom. The results indirectly suggest that atrazine–atrazine complex formation, both during polymerisation and during rebinding, influenced the performance of the MIP. Previous related studies have shown that, for atrazine-imprinted polymers, the ability of the MIP to bind atrazine relies purely on reciprocal interactions between the template and the polymer in a 1:1 (binding site:ligand) model, however, this study shows that concentration dependent atrazine–atrazine complex formation has a major effect on MIP performance.

Acknowledgement

We would like to thank the Animal Procedures Committee of the Home Office for financial support to NL.

References

Allender, C.J., Brain, K.R., Heard, C.M., 1999. Prog. Med. Chem. 36, 235–291.
Andersson, L.I., Nicholls, I.A., 2004. J. Chromatogr. B 804, 1.
Andersson, H.S., Karlsson, J.G., Piletsky, S.A., Koch-Schmidt, A.C., Mosbach, K., Nicholls, I.A., 1999. J. Chromatogr. A 848, 39–49.
Bastide, J., Chambon, J.P., Breton, F., Piletsky, S.A., Roullion, R., 2005. Anal. Chim. Acta 542, 97–103.
Bjarnason, B., Chimuka, L., Ramstrom, O., 1999. Anal. Chem. 71, 2152–2156.
Cacho, C., Turiel, E., Martin-Esteban, A., Perez-Conde, C., Camara, C., 2004. J. Chromatogr. B 802, 347–353.
Dauwe, C., Sellergren, B., 1996. J. Chromatogr. A 753, 191–200.
D'Souza, S.M., Alexander, C., Carr, S.W., Waller, A.M., Whitcombe, M.J., Vulfson, E.N., 1999. Nature 398, 312–316.
Haupt, K., 1999. React. Funct. Polym. 41, 125–131.
Haupt, K., 2001. Analyst 126, 747–756.
Hulme, E.C., Birdsall, N.J.M., 1990. Receptor-effector coupling—A practical approach. Oxford University Press, Oxford.
Jie, Z., Xiwen, H., 1999. Anal. Chim. Acta 381, 85–91.
Matsui, J., Fujiwara, K., Takeuchi, T., 2000. Anal. Chem. 72, 1810–1813.
Matsui, J., Okada, M., Tsuruoka, M., Takeuchi, T., 1997. Anal. Commun. 34, 85–87.
Matsui, J., Miyoshi, Y., Doblhoffdier, O., Takeuchi, T., 1995. Anal. Chem. 67, 4404–4408.

Muldoon, M.T., Stanker, L.H., 1995. J. Agric. Food Chem. 43, 1424–1427.
Pacakova, V., Stulik, K., Jiskra, J., 1996. J. Chromatogr. A 754, 17–31.
Pap, T., Horvath, V., Tolokan, A., Horvai, G., Sellergren, B., 2002. J Chromatogr. A 973, 1–12.
Ramström, O., Ye, L., Krook, M., Mosbach, K., 1998. Anal. Commun. 35, 9–11.
Sellergren, B., 2001. Molecularly Imprinted Polymers—Man-made Mimics of Antibodies and their Applications in Analytical Chemistry. Elsevier, London.
Sellergren, B., 1989. Makromol. Chemie. 190, 2703–2711.
Sergeyeva, T.A., Piletsky, S.A., Brovko, A.A., Slinchenko, E.A., Sergeeva, L.M., Panasyuk, T.L., El'skaya, A.V., 1999. Analyst 124, 331–334.
Shoji, R., Takeuchi, T., Kubo, I., 2003. Anal. Chem. 75, 4882–4886.
Shea, K.J., Spivak, D.A., Sellergren, B., 1993. J. Am. Chem. Soc. 115, 3368–3369.
Siemann, M., Andersson, L.I., Mosbach, K., 1996. J. Agric. Food Chem. 44, 141–145.
Surugiu, I., Ye, L., Yilmaz, E., Dzgoev, A., Danielsson, B., Mosbach, K., Haupt, K., 2000. Analyst 125, 13–16.
Surugiu, I., Danielsson, B., Ye, L., Mosbach, K., Haupt, K., 2001a. Anal. Chem. 73, 487–491.
Surugiu, I., Svitel, J., Ye, L., Haupt, K., Danielsson, B., 2001b. Anal. Chem. 73, 4388–4392.
Takeuchi, T., Fukuma, D., Matsui, J., 1999. Anal. Chem. 71, 285–290.
Vlatakis, G., Andersson, L.I., Muller, R., Mosbach, K., 1993. Nature 361, 645–647.
Welhouse, G., Bleam, W.F., 1993. Environ. Sci. Technol. 27, 494–500.
Welhouse, G., Barak, P., Bleam, W.F., 1993. J. Phys. Chem. 97, 11583–11589.
Ye, L., Cormack, P.A.G., Mosbach, K., 1999. Anal. Commun. 36, 35–38.
Zhu, Q.Z., Haupt, K., Knopp, D., Niessner, R., 2002. Anal. Chim. Acta 468, 217.