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Molecular recognition in imprinted polymers: thermodynamic investigation of analyte binding using microcalorimetry

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Abstract

This study aimed at elucidating the interaction mechanism between an imprinted polymer and its template in aqueous environment with thermodynamic aspects. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was chosen as a model template to imprint a co-polymer of 4-vinylpyridine (4-VP) and ethyleneglycol dimethacrylate. Equilibrium binding isotherm analysis and isothermal titration microcalorimetry were used to quantify the contribution of enthalpy and entropy to the binding process, identify the nature of the interactions involved and confirm the existence of binding pockets with shape-complementarity to the template. For the binding process of 2,4-D to the imprinted polymer, we postulate three subprocesses: (1) dehydration of the binding pocket and of the 2,4-D, (2) adsorption of 2,4-D, and (3) rearrangement of the water molecules from the dehydration process. We found that binding in aqueous environment was due to the cumulative effect of π-stacking and electrostatic interactions between the template and the functional monomers. At pH<6, entropy is the dominating driving force, while at pH>6 where the highest difference in binding between the imprinted and a non-imprinted reference polymer was observed, the enthalpy change accounts for most of the binding free energy. The developed microcalorimetric method sheds light on the binding mechanism of analyte molecules with imprinted polymers, in particular if the polymers are used in aqueous solvents.

Keywords: Molecular imprinting; Microcalorimetry; Isothermal titration calorimetry; Binding mechanism; 2,4-Dichlorophenoxyacetic acid

1. Introduction

Molecular recognition plays a profound role in biology and is the basis of many processes of life. Researchers have attempted to create mimics of biological recognition systems, such as receptor–effector, enzyme–substrate, or antigen–antibody pairs. One example is molecularly imprinted polymers (MIPs), synthetic macromolecular receptors that have a high selectivity for a certain target molecule for the purposes of analysis or purification [1–3].

Molecular imprinting is a process that in theory forms microcavities in a synthetic polymer that are sterically and functionally complementary to a target molecule. This is achieved by synthesizing the polymer in the presence of the target molecule, which acts as a molecular template. Reversible covalent bonds or non-covalent interactions between the template and polymerizable functional monomers

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are allowed to form, which are subsequently stabilized by polymerization with a high degree of crosslinking [4,5]. MIPs can be used in various applications based on selective ligand binding, such as affinity separation, assays, sensors, and chemical synthesis and catalysis [6–9].

The imprinting process is commonly believed to result in the formation of shape-complementary microcavities with defined spacial arrangement of functional groups [10,11]. Whereas specific rebinding of the template has been confirmed by hundreds of reports, the interaction mechanisms underlying the observed phenomena have been studied only in a few cases. In particular, if an MIP that has been synthesized in the presence of an organic solvent is later used in an aqueous environment, the contribution of the different forces involved in binding can change dramatically, which has to be taken into consideration in order to assure specific recognition and selective binding. From a thermodynamic point of view, the presence or absence of microcavities is also an important factor for binding, which could be confirmed by microcalorimetry through a quantification of binding enthalpy and entropy measurements. Thereby, calorimetric measurements will reflect the global binding event, comprising specific interactions of the target molecules with the imprinted sites, but also partition effects of hydrophobic analytes into the hydrophobic polymer phase, and analyte stacking.

The present work aimed at investigating the binding mechanism of a model analyte, 2,4-dichlorophenoxyacetic acid (2,4-D) to an MIP in an aqueous solvent system, in terms of binding thermodynamics.

2. Experimental

2.1. Materials

The polymer molecularly imprinted with 2,4-D, and a non-imprinted control polymer, were synthesized as described in a previous publication [5]. 2,4-D was obtained from Merck (Darmstadt, Germany). Tween 20 (polyoxyethylene-20-sorbitan monolaurate was from Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical grade.

2.2. Equilibrium binding isotherm measurements

Batch analyses of the binding isotherms were done at various pH levels (3, 6, 9.5). Formate buffer was used for pH 3, phosphate buffer for pH 6, and carbonate buffer for pH 9.5. All buffers were modified with 0.1% Tween 20 to prevent adsorption of the hydrophobic analyte to test tubes, and pipette tips, and to obtain a better suspension of the polymer particles and better access of the aqueous solvent to the pores [5]. The equilibrium binding analysis was using the procedure developed by Chen et al. [12]. The 2,4-D solution (0.5 ml), prepared in the equilibrium buffer at various concentrations, was added to 0.5 ml of a homogenous suspension of the MIP in a 1.0-ml microcentrifuge tube. The 2,4-D–MIP suspensions in the microcentrifuge tubes were equilibrated by incubating at 10 rpm and 298.15 K for 6 h and then spun at 6000 rpm for 3 min. The concentrations of 2,4-D remaining in free solution were determined by UV spectrometry at 280 nm, and the equilibrium concentrations of the bound 2,4-D on the MIP were calculated from the material balance.

2.3. Binding enthalpy measurement by isothermal titration calorimetry (ITC)

ITC was performed using the thermal activity monitor, which is a heat conduction type microcalorimeter (Thermometric, Sweden) controlled by Digitam software. For ITC measurements, a microreaction system was used comprising a 4-ml stainless steel ampoule, which was filled with a suspension of imprinted or control polymer particles. When thermal equilibrium between the ampoule and the heat sink was reached, a 2,4-D solution was titrated into the polymer suspension by injecting 25-μl aliquots under constant stirring at 120 rpm, through a Hamilton syringe, at time intervals of 30 min. The output signal was collected as power versus time and integrated. The binding enthalpy was quantified by the amount of 2,4-D bound from the binding isotherm data. The apparent heat from the titration has to be corrected by the dilution heat of 2,4-D and the polymer (determined separately) to obtain the net heat of interaction. The binding enthalpy of the process (ΔH_{ads}) thus can be calculated by the equation:

$$Q_{ads} = Vq^*\Delta H_{ads}$$

where Q_{ads} is the net heat attributed to the adsorption between 2,4-D and the adsorbents, V is the volume of the polymer suspension in the ampoule (ml) and q^* (mol/ml) is the amount of bound 2,4-D obtained from the binding isotherms.

3. Results and discussion

3.1. Binding isotherms

Equilibrium binding studies were conducted with the 2,4-D-imprinted polymer (MIP) and control polymer (CON). If the imprinted polymer has cavities complementary to the 2,4-D molecules, the steric specificity of the interaction between 2,4-D and these cavities should be the major difference between the MIP and CON. The binding process of 2,4-D to the polymers can conceivably be divided into the following three subprocesses: (1) dehydration of the binding sites in the polymer, and of the 2,4-D molecule, (2) binding of 2,4-D to the microcavity (MIP) or to randomly distributed functional groups (CON), (3) rearrangement of the water molecules from the dehydration process. In the view of energy changes accompanied with the subprocesses, process 1 requires energy and the system enthalpy increases as well as the system entropy due to the release of bound water molecules. Upon binding (process 2) the system enthalpy should decrease, as most binding events are exothermic. The amount of heat released is dependent on the type of interaction, and provides information about the binding mechanism. Among the interaction forces, electrostatic attraction provides the highest energy release in the order of tens of kJ/mol. π-Stacking between the aromatic ring of 2,4-D and the pyridine group in the polymer can only contribute up to 5–10 kJ/mol to the system free energy change. This stacking contributes more via the system entropy than the enthalpy, according to molecular modeling calculations [13]. The change of the system entropy is also due to the dehydration and rearrangement of water molecules, and to the steric fitting of 2,4-D molecules into the microcavities of the MIP.

In light of the above, the binding mechanism of the formation of the 2,4-D–MIP complex can be elucidated by measuring the binding enthalpy, as described in our previous reports [14–23]. For the binding of 2,4-D to MIP, if the molecular memory of MIP is the major driving force, then the decrease in the system's free energy is due to the entropy term, although enthalpy will contribute too. With CON, the binding should be mainly due to electrostatic attraction, depending on pH, and π-stacking; in this case the system is driven by the enthalpy change.

The binding isotherms of 2,4-D binding to MIP and CON at different pH are presented in Fig. 1 (data for pH 5 and 8 are not shown). The Langmuir isotherm equation was used to fit the data, and the maximum binding capacities were thus calculated (Fig. 2). The results are similar to the ones obtained with radioligand binding assays [5]. Binding of 2,4-D to MIP in the pH range studied is always higher than to CON. At pH 3, the 2,4-D molecule is nearly uncharged, while the pyridine functional groups in the polymer are positively charged. Therefore, an ionic interaction cannot be formed and π-stacking and binding site complementarity play a major role in the binding [5]. Upon closer inspection of the isotherm, multi-step isotherms can be suspected for both MIP and CON (Fig. 1). The maximum concentration of binding sites, with respect to the amount of template used [5], is $2.32 \cdot 10^{-7}$ mol/ml of MIP. A possible explanation for multi-layer adsorption is that 2,4-D binds not only to the microcavities

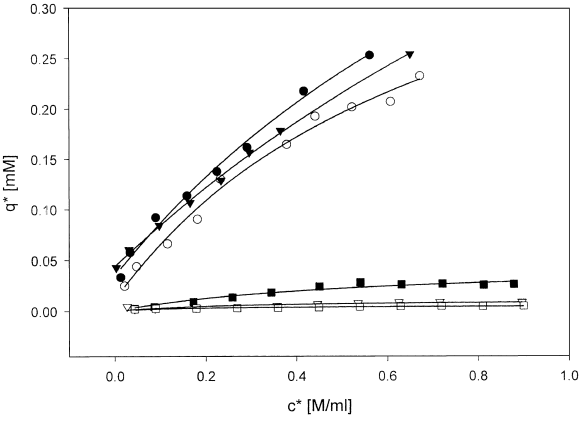

Fig. 1. The binding isotherms of 2,4-D binding to MIP and CON at different pH values. ●, MIP at pH 3; ○ CON at pH 3; ▼, MIP at pH 6; ▽ CON at pH 6; ■, MIP at pH 9.5; □, CON at pH 9.

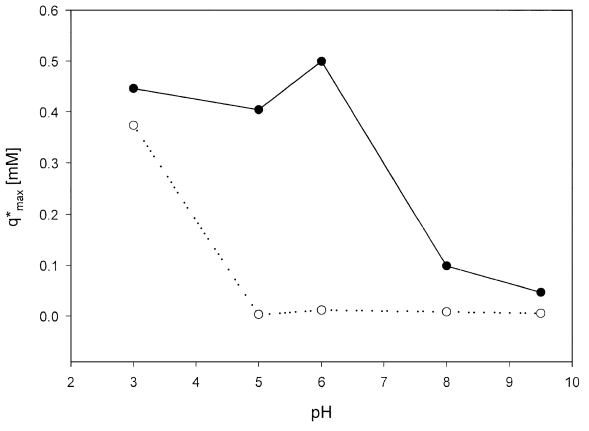

Fig. 2. The maximum binding capacities of 2,4-D binding to MIP (●—●) and CON (○—○) at different pH values.

of MIP but also to the randomly distributed pyridines of MIP and CON. Additionally, the bound 2,4-D induces the binding with 2,4-D molecules in the solution, and this is promoted as 2,4-D is uncharged at pH 3. At pH 6, multilayer adsorption becomes less probable due to the electrostatic repulsion between negatively charged 2,4-D molecules. The binding to MIP is the same as at pH 3. Pyridine is less positively charged than at pH 3, but 2,4-D now carries a negative charge and electrostatic attraction between 2,4-D and pyridine can occur. Binding of 2,4-D to CON drops noticeably at pH 6 compared to pH 3 (Fig. 2), which can be attributed to the lack of the complementary binding site of CON. In conclusion, the microcavities with steric complementarity to 2,4-D, generated by molecular imprinting, seem to be responsible for the difference in binding to MIP and CON.

3.2. Binding enthalpy measurements

As discussed above, the Langmuir isotherm may not well describe the whole binding range between 2,4-D and MIP or CON. For the purpose of obtaining binding affinity and the binding free energy calculation, the initial slope of the binding at the lower adsorbate is only needed. Therefore, the best fit of a Langmuir isotherm was chosen conceivable for the comparison of the relative values biding thermodynamics calculation. Although the existence in MIPs of microcavities sterically complementary with the template molecule is generally believed, a thermodynamic proof is still absent. In the present investigation attempted to measure the binding enthalpy directly, and used these values together with the information from the binding isotherms to calculate the free energy and the entropy of the binding process. Table 1 lists the free energy (ΔG_{ads}), enthalpy (ΔH_{ads}) and entropy (ΔS_{ads}) of the binding processes at various pH values. At pH 3, for MIP and CON, ΔS_{ads} contributes most to the binding (negative values of ΔG_{ads}) and compensates the unfavorable binding enthalpy (positive value of ΔH_{ads}). The positive enthalpy value may be due to the dehydration process (process 1) and the binding energy released upon formation of the complex is mainly from the complementary fitting and π-stacking (process 1), which contribute more to entropy than to enthalpy, as discussed above [13]. The measured enthalpy values are comparable with the calculated data for the dehydration of benzene, and for π-stacking [13]. The data obtained at pH 6 show distinct differences in the binding mechanism of 2,4-D to MIP and CON. 2,4-D binding to the MIP is driven by entropy and that to CON by enthalpy. These results support the binding mechanism proposed in the binding isotherm section, since the binding to MIP involves steric complementary and is thus entropy dominated, while binding to CON is driven by the energy released due to the electrostatic attraction. At pH 9.5, the electrostatic repulsion between 2,4-D molecules becomes dominant and is responsible for the higher positive enthalpy value,

Table 1
The thermodynamic parameters of 2,4-D binding to MIP and CON at different pH values

Thermodynamic parameter	pH 3		pH 6		pH 9.5	
	MIP	CON	MIP	CON	MIP	CON
ΔG_{ads} (kJ/mole)	−4.35	−2.78	−3.11	−5.38	−3.27	−6.26
ΔH_{ads} (kJ/mole)	3.25	2.85	0.85	−9.52	8.14	10.25
$T\Delta S_{ads}$ (kJ/mole)	7.6	5.63	3.96	−4.14	11.41	16.51

Table 2
The contribution of different factors to the interaction mechanism of 2,4-D binding to MIP and CON

Interaction forces	pH 3		pH 6		pH 9.5	
	MIP	CON	MIP	CON	MIP	CON
Structure specific interaction	+		+		+	
Electrostatic attraction (2,4-D–4-VP)			+	+		
Electrostatic repulsion (2,4-D–2,4-D)			−	−	+	+
π-stacking (2,4-D–4-VP)	+	+	−	−	+	+
π-stacking (2,4-D–2,4-D)	+	+	−	−	−	−

Note: +: favourable, −: unfavourable.

besides binding site dehydration (process 1). The values obtained in this investigation are comparable with the literature [18], and provide a rational thermodynamic explanation for the molecular interaction between 2,4-D and the imprinted and control polymer. For a better illustration, the contribution of the different factors to the interaction is represented in Table 2.

4. Conclusion

Binding isotherms of 2,4-dichlorophenoxyacetic acid binding to an imprinted polymer were determined in equilibrium binding assays, and binding enthalpies were measured directly by isothermal titration calorimetry. Based on these data, the binding mechanism was postulated from a thermodynamic point of view. Specifically, the binding behavior at pH 3, 6 and 9.5 were discussed in detail with the possible involvement and strength of the electrostatic, π-stacking, and steric complementary interactions. The accompanied enthalpy and entropy contribution with these interactions were also stated. Conclusively, ITC provides useful thermodynamic analysis of binding between a template molecule a corresponding imprinted polymer.

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