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Synthetic Creatinine Receptor: Imprinting of a Lewis Acidic Zinc(II)cyclen Binding Site to Shape Its Molecular Recognition Selectivity

Michael Subat,[†] Andrew S. Borovik,[‡] and Burkhard König*,[†]

Contribution from the Institut für Organische Chemie, Universität Regensburg, D-93040 Regensburg, Germany, and Department of Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, 2010 Malott Hall, Lawrence, Kansas 66045-7582

Received October 10, 2003; E-mail: Burkhard.Koenig@chemie.uni-regensburg.de (B.K.); aborovik@ku.edu (A.S.B.)

Abstract: Molecularly imprinted polymers (MIPs) from polymerizable Lewis acidic zinc(II)cyclen complexes and ethylene glycol dimethyl acrylate have been prepared. For the imprinting process the template molecule creatinine is reversibly coordinated to the zinc atom. The high strength of this interaction allows analyte binding to the MIP from aqueous solution with high affinity. Its pH dependence is used for controlled guest release with nearly quantitative analyte recovery rate. The binding capacity and selectivity profile of the MIP remains constant through several pH controlled binding and release cycles. MIPs missing a suitable metal binding site showed no significant affinity for thymine or creatinine. Flavin adsorbs nonspecifically to all polymers. The imprinting process reverses the binding selectivity of zinc(II)cyclen for creatinine and thymine from 1:34 in homogeneous solution to 3.5:1 in the MIP. Scatchard plot analysis of creatinine binding isotherms reveals uniform binding of the imprint, with fits indicating a one-site model; however, similar analysis for thymine indicate high and low affinity sites. This corresponds to unrestricted coordination sites freely accessible for thymine, e.g., at the polymer surface, and misshaped imprinted sites, which still can accommodate thymine. More than 50% of all binding sites exclusively bind creatinine and are not accessible to thymine. The binding properties of a copolymer of polymerizable zinc(II)cyclen and ethylene glycol dimethyl acrylate missing the creatinine template, which match the binding selectivity of the complex in solution, confirm that the origin of altered selectivities is the imprinting process. With binding ability at physiological pH, the MIPs are applicable for tasks in medicinal diagnostics or biotechnology. Imprinted zinc(II)cyclen complexes provide, like a metalloenzyme binding motif, high binding affinity by reversible coordination while the surrounding macromolecule determines binding selectivity.

Introduction

The technique of molecular imprinting allows the formation of specific recognition sites in synthetic polymers through the use of templates or imprint molecules.[1,2] The template molecule organizes functional and cross-linking polymerizable monomers during the polymerization and is extracted from the insoluble network, leaving behind domains of complementary size, shape, and functional group orientation. Organic molecules and metal ions[3] have been widely employed as templates, and catalysts[4] have been imprinted to improve their selectivity, but the number of examples using noncatalytic coordination complexes to shape imprinted polymer binding sites is still limited. However, substrate binding, which relies on reversible metal−ligand interactions, is less susceptible to cleavage or interception by water or other protic solvents.[5] This allows the preparation of

[†] Universität Regensburg.
[‡] The University of Kansas.

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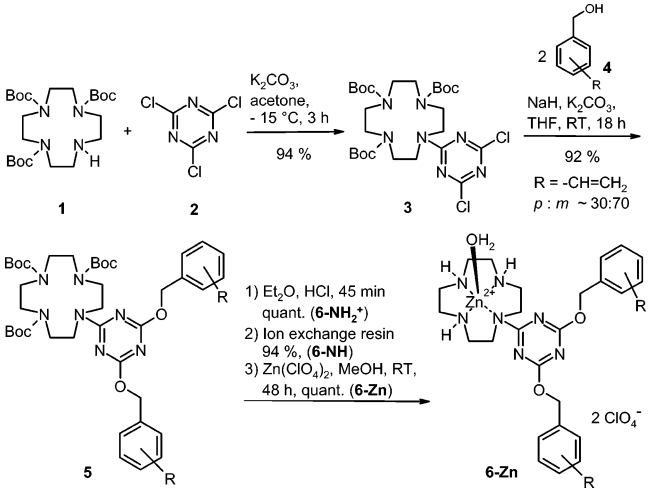
synthetic receptors for recognition in an aqueous environment. Shea et al.[6] recently reported synthetic peptide receptors based on imprinted Ni(II)−nitrilotriacetic acid complexes, which have affinity to N-terminal histidine (His) residues. The molecularly imprinted polymers (MIPs) bind His-containing small peptides and show different capacities depending on the peptide residues beside His. Takeuchi et al.[7] imprinted zinc porphyrin based recognition centers,[8] but binding is in this case restricted to organic solvents.[9,10] We now report the imprinting of zinc(II)-cyclen complexes yielding MIPs with affinity for imides in aqueous solution. The zinc(II)cyclen binding site is well studied. Kimura et al. have investigated the reversible coordination of anions and neutral molecules to the complex in aqueous solution.[11] High anion affinity was found for phosphate and phosphate ester anions, and deprotonated imide structures, which displace the weakly coordinated axial water molecule of the zinc complex. This leads to the development of synthetic receptors for the nucleobase thymine, barbiturates, and vitamine B2 (riboflavin), which function at physiological pH. In many cases the binding motif was structurally characterized by X-ray structure analysis.

The observed pH-dependent binding affinity of imides to zinc-(II)cyclen is determined by the pK_a value of the imide N−H, which has to be deprotonated for coordination to a metal ion. Imide pK_a's are usually around 10 (thymine 10.1; riboflavin 9.9), which leads to rather similar binding strength[12] that cannot be distinguished by a zinc(II)cyclen binding site. The design of an artificial receptor based on zinc(II)cyclen with the ability of preferential binding to one imide requires additional interaction sites. The technique of molecular imprinting seems suitable to create a binding cavity around the metal ion coordination site, which should determine binding selectivities. We report here the synthesis of a polymerizable zinc(II)cyclen monomer, the preparation of MIPs with riboflavin and creatinine templates, and the investigation of their binding properties.

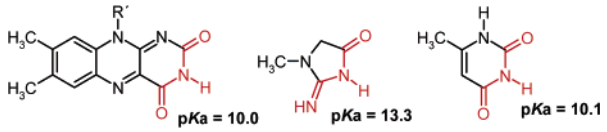
Results and Discussion

Syntheses. The synthesis of polymerizable zinc(II)cyclen monomers (Scheme 1) starts from 3-fold Boc-protected cyclen **1**, which is reacted at −15 °C with trichlorotriazene (**2**) to yield monosubstitution product **3**. The two remaining chloro substituents of the triazene unit are substituted by commercially

Scheme 1. Synthesis of Polymerizable Zinc(II)cyclen Monomers



Scheme 2. Structures of Imides for Recognition by Zinc(II)cyclen[a]



[a] R′ = $CH_2CH(OAc)CH(OAc)CH(OAc)CH_2(OAc)$.

available vinyl benzyl alcohol **4**. The benzyl alcohol **4** is a mixture of the para and meta isomers in a ratio of approximately 30:70. The 2-fold substitution product **5** is obtained in good yield[13] and treated with diethyl ether saturated with HCl to remove the Boc protecting groups. Other deprotecting methods, such as treatment with TFA, lead to a partial cleavage of the benzyl ether groups. The resulting ammonium chloride salt **6-NH_2^+** is neutralized to the free base **6-NH** on a basic ion exchange resin. The ligand can now be converted into the zinc-(II) complex **6-Zn** by treatment with zinc(II) perchlorate in methanol. The complexation is quantitative and divinyl complex **6-Zn** is stable if stored cool under nitrogen in the dark. For comparison the cobalt(II) complex **6-Co** was prepared analogously. Co(II)cyclen complexes do not show interaction with imides in homogeneous solutions.

Tetraacetylriboflavin[14] (**7**) (Scheme 2) was chosen as one potential template for an imprinting process, because it has an imide N−H pK_a value of 10.0, and therefore its binding affinity to zinc(II)cyclen is similar to thymine (**9**), which can be used as a competing analyte with different steric demand. Creatinine (**8**)[15,16] was selected as a much more challenging target for binding. We recently discovered that the HN═C−NH−C═O

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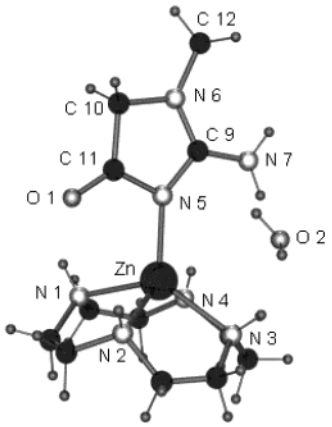


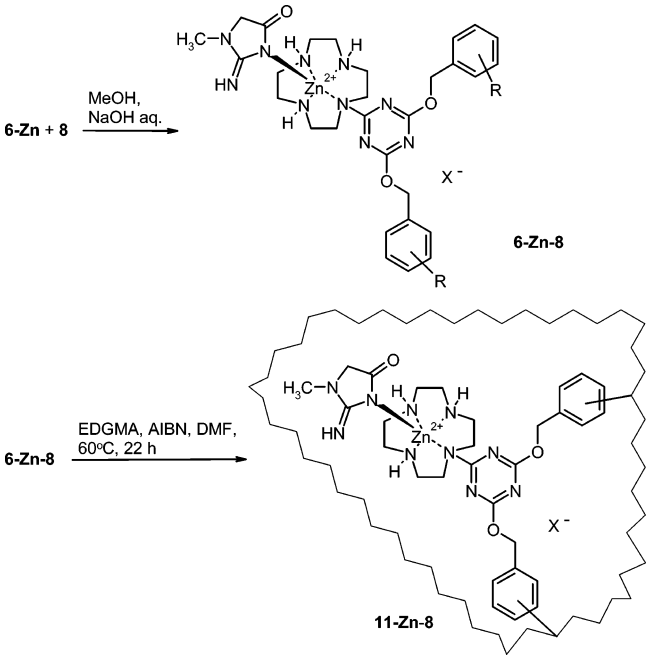
Figure 1. Molecular structure of creatinine coordinated to zinc(II)cyclen in the crystalline phase. Two perchlorate ions are omitted for clarity.

moiety, which is isoelectronic to an imide, is also recognized by zinc(II)cyclen complexes.[17] The binding motif of a zinc nitrogen coordination is identical to other imides as confirmed by an X-ray structure analysis of a cocrystal (see Figure 1 and Supporting Information). The bond distance N5−Zn of 1.996 Å is similar to the value observed in zinc(II)cyclen−azidodeoxythymidine complexes of 2.053 Å.[18] The plane of the creatinine heterocycle is dissecting the cyclen ring between N1, N2, and N3, N4, respectively. Hydrogen bonds between O1 and N1 or N2 are likely according to the distances. The water molecule found in the unit cell (O2) seems to form hydrogen bonds to N3 and N7.

The reduced electron-withdrawing character of the imine compared to a carbonyl group results in a less acidic N−H proton with a pK_a of 13.3 as determined by potentiometric titration (see Supporting Information). This results in a smaller apparent binding affinity of creatinine to zinc(II)cyclen at pH 9 of 1:34 if compared with thymine. We anticipated that the complex could have a reversal in the binding affinity for these two compounds when prepared specifically for creatinine by the MIP technique.

To ensure that the template molecule and metal complex binding site were in optimal stoichiometry and arrangement for the imprinting process, stoichiometric salts were prepared. Tetraacetylriboflavin (**7**) or creatinine (**8**), respectively, and complex **6-Zn** were dissolved in methanol and aqueous sodium hydroxide. The ESI-MS analysis of the obtained salts revealed the formation of coordination compounds **6-Zn-7** and **6-Zn-8** with 1:1 stoichiometry. (Scheme 3) Mixtures of ethylene glycol dimethyl acrylate (EDGMA), with AIBN as initiator, complex **6-Zn-template**, and DMF as solvent were used for MIP preparation. An approximate ratio of 3:1 of DMF to ethylene glycol dimethyl acrylate proved to be optimal for the polymerization process. While complexes **6-Zn-8** and **6-Co** readily dissolved in these mixtures to give suitable concentrations of 5 mol %, complex **6-Zn-7** did not dissolve in substantial quantities. Several other solvents (DMF, CH_3CN, DMSO, THF, toluene, CH_2Cl_2, methanol) with various amounts of water were tried, but the solubility of **6-Zn-7** remained low. Attempts to prepare MIP from **6-Zn-7** with increased amounts of solvent

Scheme 3. Preparation of Stoichiometric Template Zinc(II)cyclen−Creatinine Monomer Salt and Copolymerization of **6-Zn-8** with Ethylene Glycol Dimethyl Acrylate[a]



[a] R = *m*-/*p*-CH=CH_2; X^- = ClO_4^-, Cl^-, or OH.

were not successful due to incomplete polymerization process giving no solids. Therefore MIPs were prepared from **6-Zn-8**, **6-Zn**, and **6-Co**. A blank polymer (**10-blank**) without addition of a metal complex was made for comparison of properties. All polymers were grained with a bullet mill and exhaustively extracted in a Soxhlet apparatus to remove all low molecular material and nonreacted monomer.

IR spectra of all polymers were measured to detect residual amounts of the creatinine template after washing. The creatinine characteristic vibrations at 3251 cm^{-1} (N−H) and 1685 cm^{-1} (amide) are not observed, which indicates that no significant amount of template remained in the polymer (see Supporting Information for IR spectra).

Properties. The cyclen complexes are the only source of nitrogen atoms in the polymers.[19] Elemental analyses of the polymer were therefore used to estimate the amount of incorporated monomers and the corresponding theoretical maximum number of binding sites. Table 1 summarizes the results. The rate of incorporation of the metal complex monomer units into the polymer is not quantitative and is very sensitive to the polymerization conditions. Without optimizing the reaction conditions, a maximum incorporation rate of 60% was achieved.[20]

The acidic conditions used to remove the creatinine template may cause decomplexation of zinc(II)cyclen, which would lead to a difference in the amount of ligand and active binding sites. To ensure that all accessible cyclen ligands, which were incorporated during polymerization, were loaded with zinc ions, a control experiment was performed. Polymer **11-Zn-8-B** was refluxed in alkaline aqueous solution in the presence of excess

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(19) If nitrogen atoms from other sources, e.g., the radical initiator, are incorporated into the polymer during preparation, the theoretical maximum number of binding sites would be smaller than calculated. The more important value of accessible binding sites was determined from binding isotherms.

Table 1. Nitrogen Content and Corresponding Theoretical Number of Maximum Binding Sites per Gram of Polymer[a]

entry	polymer	nitrogen content [%] measd	nitrogen content [%] calcd[b]	incorporated amount of monomer [%]	max no. of binding sites in 1 g of polymer [μmol]
1	11-Zn-8-A	0.90	1.83	49.2	91.8
2	11-Zn-8-B	0.43	1.62	26.1	43.9
3	11-Co	1.15	1.87	61.5	117.0
4	11-Zn	0.83	1.72	48.3	84.7

[a] Two batches of **11-Zn-8-A** and **-B** were prepared in separate experiments with different amounts of **6-Zn-8**, cross-linker, and solvent. [b] This is the theoretical value calculated for 100 % incorporation of cyclen monomer.

of $Zn(ClO_4)_2$. The creatinine binding capacity of the polymer before and after $Zn(ClO_4)_2$ treatment was determined under identical conditions and was found to be the same (28.8 μmol/g after zinc reloading; 29.1 μmol/g before) within experimental error. This shows that all incorporated, accessible cyclen ligands are present as their zinc(II) complexes and no zinc ions were lost upon template removal. Only zinc(II)cyclen complexes act as binding sites. Zinc ions that may bind to other parts of the polymer do not serve as binding sites.

Binding Studies. The binding ability of the polymers to creatinine, thymine, and tetraacetylriboflavin in aqueous buffered solution was investigated in detail. The polymer was shaken in buffered aqueous solutions ($c = 5 \times 10^{-4}$ mol/L, Tris, pH 9) with the respective concentration of thymine, creatinine, tetraacetylriboflavin, or a thymine/creatinine mixture at room temperature for 12 h. Concentration of free and bound analytes were determined by an HPLC assay and tracer technique (see Supporting Information) to ensure high accuracy and allow competitive binding studies. The results of the binding assays with a single analyte are summarized in Table 2.

A possible nonspecific absorption was tested with polymer **11-Co**, for which no binding of imides is expected (Table 1, entry 1). Only negligible adsorption of creatinine and thymine are found within the error limits of the analytical method, but flavin is bound in large amounts. The binding of flavin to the polymeric material is irreversible, and attempts to release it from the polymer failed. Polymers **10-blank**, **11-Zn-8-B**, and **11-Co** were left with aqueous riboflavin tetraacetate solution ($c = 5 \times 10^{-5}$ mol/L) buffered at pH 9 and pH 7.1 for 12 h. At pH 7.1 the significant decrease in flavin UV absorption (447 nm) showed that flavin is bound even in the absence of metal complex binding sites. At pH 9 changes in the UV spectrum indicate decomposition in addition to adsorption (see Supporting Information for spectra).

(20) The sensitivity of the metal complex monomer incorporation rate to the reaction conditions is illustrated with polymers **11-Zn-8-A** and **-B**. By a slight increase of the amount of cross-linker (EDGMA + 13%) and the use of more solvent for polymerization, the rate of metal complex incorporation decreases from 50% (**11-Zn-8-A**) to 26% (**11-Zn-8-B**). The rate of incorporation seems to be concentration dependent, yielding higher rates of incorporation at higher concentrations, but the poor solubility of **6-Zn-7** does not allow increasing its concentration further. Another possible rationale for the observed incomplete incorporation may come from the metal complex monomer structure, which bears two flexible vinyl units. During polymerization an intramolecular reaction followed by radical transfer may happen, which will exclude the monomer from incorporation into the polymer matrix. Collman et al. recently described the copolymerization of vinyl Fe−porphyrins; see ref 9. While a porphyrin monomer with one polymerizable group was incorporated in more than 90%, a second monomer with three flexible vinyl groups was embedded into the polymer in only ~60%. Functional monomers used for other MIP preparation have one polymerizable unit or two geometrically well-separated groups (see refs 3m, 3n, and 8).

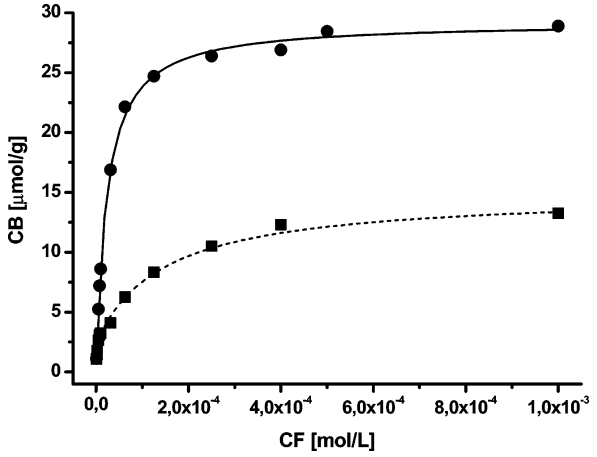

Figure 2. Binding isotherms of creatinine (●, —) and thymine (◆ and ■, ···) binding to **11-Zn-8-B** in aqueous buffer, pH 9. Parameters of nonlinear curve fitting to a one set of sites binding model for creatinine (—): $K = 4 \times 10^4$ L/mol; $B_{max} = 29$ μmol/g; $R = 0.9994$. Parameters of nonlinear curve fitting to a two sets of sites binding model for thymine (···): $K_1 = 8 \times 10^5$ L/mol, $B_{max} = 3$ μmol/g; $K_2 = 7 \times 10^3$ L/mol, $B_{max} = 12$ μmol/g; $R = 0.9967$. MIP **11-Zn-8** with lower overall binding capacity was used for isotherm determination.

MIP **11-Zn-8-A** binds 55.1 μmol/g creatinine from the aqueous buffer. The control polymer **11-Zn** (Table 3, entry 3), which was analogously prepared, but no creatinine was imprinted, shows only very weak binding on the order of the nonspecific absorption. The binding of creatinine to **11-Zn-8-A** is reversible with a recovery rate of 97% (Table 1, entry 2). The same MIP can be reused for creatinine binding, without significant changes in binding or release capacity (Table 1, entries 3 and 4). The comparison of creatinine binding from solution with the maximum number of binding sites calculated from incorporated **6-Zn-8** reveals that 60% (for **11-Zn-8-A**) and 66% (for **11-Zn-8-B**) of the binding sites are accessible.[21] The binding of thymine from aqueous solution with equal analyte concentration was determined to be 26 μmol/g, again with nearly quantitative release of the bound guest. These data already suggest an altered binding selectivity in favor of creatinine for the imprinted metal complex binding sites, if compared to the binding processes in homogeneous solution. At pH 9 the relative binding affinity of creatinine vs thymine to monomeric zinc(II)cyclen in solution is 1:34 as derived from potentiometric titration (see Supporting Information).

Binding isotherms of **11-Zn-8-B** (Figure 2) for creatinine and thymine were measured to determine affinity constants. The isotherm for creatinine fits well to a one site binding model giving an affinity constant $K = 4 \times 10^4$ L/mol and a maximal loading capacity $B_{max} = 29$ μmol/g. The isotherm for thymine binding is described by a two sets of sites binding model, which provides two affinity constants and loading capacities: $K_1 = 8 \times 10^5$ L/mol, $B_{max} = 3$ μmol/g; $K_2 = 7 \times 10^3$ L/mol, $B_{max} = 12$ μmol/g. The Scatchard plot analysis (Figure 3) of the binding data illustrates the binding site type distribution of the MIP even more clearly. For the imprinted molecule creatinine one type of binding sites is observed. The structurally similar thymine, which has in homogeneous solution a much higher affinity to zinc(II)cyclen than creatinine, encounters two different binding situations: (1) The first is a small number of sites with high

(21) This value exceeds reported numbers of accessible binding sites of respectively 20% [6] and 7−10%.[7]

Table 2. Summary of Binding and Release of Creatinine, Thymine, and Flavin from Aqueous Buffer to Polymer **11-Zn-8-A**, **11-Zn-8-B**, and **11-Co** as Blank Control[a]

		creatinine (**8**, $c = 5 \times 10^{-4}$ mol/L)[b]			thymine (**9**, $c = 5 \times 10^{-4}$ mol/L)[b]			flavin **7** ($c = 5 \times 10^{-5}$ mol/L)[b]		
entry	polymer	bound [μmol/g polymer]	released [μmol/g polymer]	rate of recovery [%]	bound [μmol/g polymer]	released [μmol/g polymer]	rate of recovery [%]	bound [μmol/g polymer]	released [μmol/g polymer]	rate of recovery [%]
1	**11-Co**	0.3			0.5			88.4		
2	**11-Zn-8-A**	55.1	53.2	97	26.1	25.1	97	94.3		
3	**11-Zn-8-A** (second cycle)	54.1	52.5	97						
4	**11-Zn-8-A** (third cycle)	52.8	51.7	98						
5	**11-Zn-8-B**[c]	28.8	27.7	96	13.3	13.0	98			
6	**11-Zn-8-B**[d]	29.1								

[a] Error limits for all values are ± 0.25 μmol/g. [b] Concentration of guest molecule in aqueous buffer. [c] Two batches of **11-Zn-8** (**A** and **B**) were prepared in separate experiments with different amounts of **6-Zn-8**. [d] Treated with excess $Zn(ClO_4)_2$; see text.

Table 3. Summary of Competitive Binding and Release of Creatinine and Thymine from Aqueous Buffer to Polymer **11-Zn-8-A**, **11-Zn-8-B**, and Control Polymer (**11-Zn**)[a]

		creatinine (**8**, $c = 5 \times 10^{-4}$ mol/L)[b]			thymine (**9**, $c = 5 \times 10^{-4}$ mol/L)[b]		
entry	polymer	bound [μmol/g polymer]	released [μmol/g polymer]	rate of recovery [%]	bound [μmol/g polymer]	released [μmol/g polymer]	rate of recovery [%]
1	**11-Zn-8-A**	44.7	40.1	90	14.4	13.5	94
2	**11-Zn-8-A** (second cycle)	41.2	39.1	95	12.1	11.0	91
3	**11-Zn**	0.22	0.21[c]	96	17.0	16.2	95
4	**11-Zn-8-B** (pH 7.1)	12.1	11.9	98	7.8	7.6	97

[a] Error limits for all values are ± 0.25 μmol/g. [b] Concentration of guest molecule in aqueous buffer. [c] General error limit does not apply. Sample was concentrated before analytical determination.

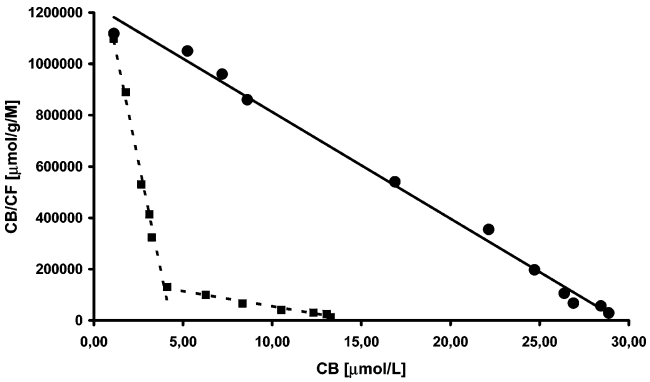

Figure 3. Scatchard plot analyses of creatinine (●) and thymine (■) and thymine binding to **11-Zn-8-B** in aqueous buffer. Parameters of linear fit for creatinine: $K = 4 \times 10^4$ L/mol; $B_{max} = 29$ μmol/g, $R = 0.994$. Parameters of linear fit for thymine: (a) CB < 4 mmol/L, $K = 4 \times 10^5$ L/mol, $B_{max} = 4$ μmol/g, $R = 0.989$; (b) CB > 4 mmol/L, $K = 1 \times 10^4$ L/mol, $B_{max} = 15$ μmol/g, $R = 0.980$.

affinity similar to the solution value. The cavities of these sites must be a little larger than necessary for creatinine, so that they can accommodate thymine. These sites presumably include the guest molecule incompletely, a situation likely at the surface of the polymer. (2) A second type of binding sites is accessible to thymine up to 15 μmol/g. These sites are not ideally complementary to creatinine in shape, so that thymine can still enter. However, the reduced affinity suggests some hindrance. An additional type of binding sites with a capacity of ca. 15 μmol/g is only accessible for creatinine. This indicates that half the binding sites have a shape complementary to the creatinine structure, which allows a distinction between the quite similar structures of thymine and creatinine. Recent investigations report that the Freundlich isotherm gives a good mathematical approximation of the binding characteristics of noncovalently MIPs.[22] Our data could not be described using the Freundlich isotherm equation, but fit well to a Langmuir isotherm (see Supporting Information). The Freundlich isotherm assumes a model of dependent binding sites, while in the Langmuir model binding sites are independent.[23] This indicates that in the here described imprinting process individual coordinative binding sites are obtained.[24]

A competition experiment (Table 3, entries 1 and 2) revealed the preferred binding of creatinine over thymine in a ratio of approximately 3.5:1. Again, the polymer can be reused and recovery rates of both released guest molecules are high. The imprinting effect is nicely demonstrated with polymer **11-Zn** (Table 3, entry 3), which was analogously prepared, but no creatinine was imprinted. The relative binding affinity of the polymer, 1:77 in favor for thymine, resembles approximately the ratio found in homogeneous solution for zinc(II)cyclen of 1:34.[25] This confirms that the altered selectivity has its origin in the imprinting process. A binding and release study at pH 7.1 (Table 3, entry 4) showed, as expected from the pH-dependent affinity of the metal complex binding site, a reduced binding capacity. The binding ratio for creatinine and thymine drops to 1.6:1. Nevertheless, the experiment confirms that binding of biologically important guest molecules at physiological pH is possible with metal binding site MIPs.

(22) (a) Umpleby, R. J.; Baxter, S. C.; Bode, M.; Berch, J. K.; Shah, R. N.; Shimizu, K. D. *Anal. Chim. Acta* **2001**, *435*, 35−42. (b) Kim, H.; Spivak, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11269−11275.
(23) Atkins, P. W. *Physikalische Chemie*, 2nd ed.; VCH-Wiley: New York, 1990; pp 797−801.
(24) An additional argument for this hypothesis comes from the fit of the binding isotherm to a one set of sites model, which fails to describe MIP in many cases.
(25) Binding of creatinine to polymer **11-Zn** is very weak. Therefore, the ratio of binding affinities which is derived from polymer binding experiments must have a much larger error than the ratio derived from two solution studies.

Conclusions

Polymerizable monomers with Lewis acidic zinc(II)cyclen complexes and their stoichiometrically defined salts with template molecules, such as creatinine or flavin, have been prepared and characterized. Copolymerization of zinc(II)cyclen−creatinine (**11-Zn-8**) and ethylene glycol dimethyl acrylate yield MIPs with binding affinity to the imprinted analyte from aqueous solution. The material is stable through several pH controlled binding and release cycles. High binding affinity for imides as analytes is provided by the Lewis acidic metal complex, while selectivity is shaped by the imprinting process as shown by control polymers missing the metal complex or having no binding ability. Scatchard plot analysis of the binding isotherms reveals a uniform binding situation for the imprinted creatinine, while thymine encounters two different sites with unrestricted access and with some hindrance, respectively.

The fully reversible coordination of analytes to the stable complex zinc(II)cyclen as immobilized binding site providing high and controllable affinity in aqueous environment is particularly useful if combined with the selectivity shaping process of molecular imprinting. Many applications may be envisaged, because imide structures are found in important molecules of biological origin for which materials with specific affinity are desirable. The well-documented binding ability of zinc(II)cyclen complexes to anions, such as phosphates, including phosphorylated amino acids or peptides, and carboxylates, indicates likely extensions to other interesting imprinting targets.

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Supporting Information Available: Binding isotherms for creatinine and thymine fitted to Langmuir equation, details of X-ray structure determination of zinc(II)cyclen−creatinine complex, potentiometric titrations of zinc(II)cyclen, creatinine, and thymine, and experimental details of HPLC analyses, IR spectra of polymers, UV binding assay of flavin to polymers, syntheses, and binding studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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