Quantitative Determination of Heavy Metal Contaminant Complexation by the Carbohydrate Polymer Chitin

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Chitin is a cost-efficient and nontoxic biopolymer with potential for use in heavy metal chelation from industrial wastewater. In this study we report the binding strength of chitin and the common water contaminants mercury, copper, iron, nickel, chromium, lead, zinc, cadmium, silver, and cobalt. We have found that the strongest binding takes place with mercury and weakest with cobalt with binding constants of $1.16 \cdot 10^5$ M^{-1} and $3.96 \cdot 10^3$ M^{-1}, respectively. We observed that the formal charge state of the heavy metal inversely affects the binding strength. The divalent metal cation−chitin interactions are all enthalpically driven binding reactions. These results serve to benchmark industrial wastewater treatment by chitin chelation.

Introduction

Chitin has been reported to be the second most abundant natural polysaccharide in nature and is commonly found in crab and shrimp shells.[1−11] Given this ubiquity, chitin is commercially accessible and inexpensive in its pure form.[1,10,12,13] Chitin has been used in the pharmaceutical, environmental, cosmetic, and agricultural industries for applications that include wound dressings, contacts, lotions, package film coatings, pesticides, and wastewater treatment.[1] The biopolymer has great potential in drug delivery and tissue engineering applications.[2,9] Chitin is composed of linear repeating β-1,4-linked *N*-acetyl-D-glucosamine monomers. A related more reactive polymer called chitosan contains the same monomer units but with free amines on the glucosamine backbone.[1,2,7,8] Chitin is known to be less reactive toward metal chelation than chitosan; on the other hand, it is more stable than chitosan, which could make it a better choice to remove metal cations.[14] Interestingly though, despite its many applications, the binding affinity of chitin to common metal cations found in biological systems or as heavy metal contaminants in industrial effluents has never been quantified. Quantitative data would provide not only the basis for better computational force fields related to carbohydrate−metal interactions but also a better understanding of the molecular basis for these interactions. Herein we report the first such quantification using isothermal titration calorimetry to dissect the thermodynamics of binding of chitin to a range of metal cations.

Removal of heavy metals from contaminated solutions can be carried out by different methods, such as precipitation, oxidation/reduction, ion exchange, electrochemical methods, filtration, coagulation, adsorption, osmosis, membrane operations, or evaporation.[10−13,15−18] Biosorption is a complementary, inexpensive, and easy method in such applications.[5,10,12,16,17,19] To this end, chitin has been known to chelate heavy metal ions for wastewater treatment purposes (see Scheme 1).[1,4−7,11,18,20] For instance, removal of cadmium and lead from contaminated effluents by chitin chelation has been reported.[12] In another study, vanadate and chromium cations have been adsorbed by chitin.[21] Adsorption of chromium, cadmium, copper, lead, and zinc by chitin has also been shown.[22] A separate study also showed that lead as well as iron cations are adsorbed on chitin.[5] Cadmium, chromium, mercury, zinc, copper, lead, iron, silver, molybdenum, and nickel are major pollutants in industrial wastewater and are of particular interest for removal given the serious problems these metals can cause in the environment.[10,22] Environmental exposure to metals is problematic as humans cannot process heavy metals, and metal accumulation in specific organs can cause serious health problems.[23−26]

Heavy metal chelation of chitin has been the subject of several studies.[6,7,11,18,20] Surprisingly, there have not yet been investigations that quantify the strength of metal binding to chitin. Knowledge of kinetic and thermodynamic binding data for chitin/heavy metal interactions could help with the removal of cations, such as Hg, Cd, Co, Cu, Ni, Zn, Pb, Cr, Ag, and Fe, from industrial wastewater in a very efficient and selective fashion. If the exact amount of biomaterial needed for chelation of heavy metals is known, then the least necessary amount to be able to get complete removal of the metal cations from industrial effluents in the least expensive manner can be ascertained. This approach could also be used for process design. For instance, the process of zinc and cadmium removal from industrial effluents at battery production plants can be designed better by knowing exactly how much adsorbent is theoretically required. Another application of our data would be for computational modeling of such biological systems for wastewater treatment as there is currently no available quantitative data for modeling studies. To obtain such quantitative data, isothermal titration microcalorimetry (ITC) is a useful technique that can measure the interactions between two binding partners. A ligand solution is titrated into a macromolecule solution, and heat absorbed or released from this chemical reaction is measured.[27−31] This computer-controlled technique measures the enthalpy of binding directly, can provide entropy and free energy of binding values as well as binding constants, and does not require labeling of components.

Experimental Section

Lead nitrate, zinc nitrate, cadmium nitrate, cobalt nitrate, nickel nitrate, copper sulfate, iron sulfate, chromium chloride, magnesium chloride, calcium chloride, and manganese chloride were purchased from Fisher Scientific (Hanover Park, IL). Chitin, mercury chloride, and silver nitrate were obtained from Sigma-Aldrich (St. Louis, MO). All reagents were used as they were received; no further purifications were utilized. A nanopure Barnstead E-pure water purification system (18.1 MΩ) was used to obtain deionized water for this study.

A VP-ITC isothermal titration microcalorimeter (Northampton, MA) was used for the binding experiments. All metal solutions were prepared in deionized water at 5 mM concentrations. A 0.05 mM chitin solution is prepared in water and stirred

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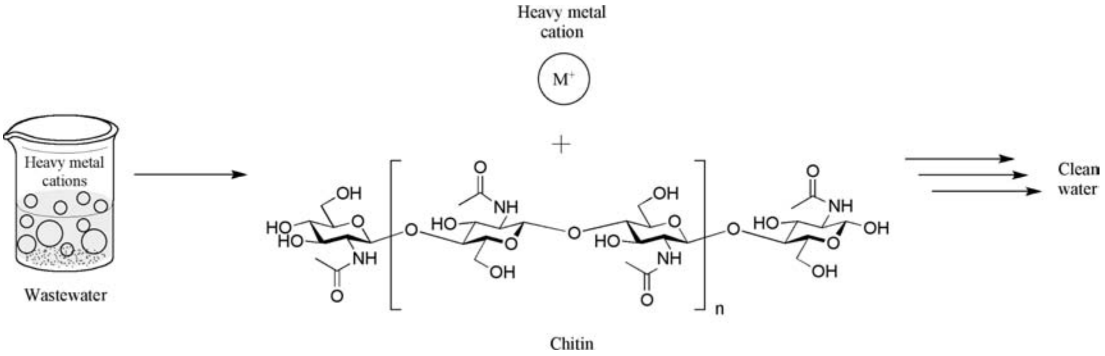
Scheme 1. Biosorption of Heavy Metals by Chitin



with a magnetic bar for 3 h to obtain a homogeneous solution. All solutions are degassed immediately before the ITC experiments. Metal solutions (5 mM) were added to the instrument syringe (301 μL), and 10 μL titrations were carried out into the chitin solution in the reaction cell (1.4288 mL) at 25 °C. Subtraction of a blank experiment for each run, which does identical titrations into the reaction cell without the chitin in the solution, eliminated heat of dilution effects. Origin (7.0, OriginLab Corp., Northampton, MA) was used to analyze and fit the ITC data to obtain the kinetic and thermodynamic binding parameters: K (binding constant), enthalpy of binding (ΔH), and entropy of binding (ΔS). The change in the differential heat during an experimental run is integrated over time by the software to determine the enthalpy of reaction. Successive iterations allow fitting of the binding parameters until they converge to a value. The software then calculates the entropy of binding after determining the binding constant and enthalpy of binding directly from the binding thermograms. From this information, the free energy of binding and then ΔS values are calculated.

Results and Discussion

To quantify the strength of binding of various metals to the carbohydrate polymer chitin, 14 different metal cations were titrated into chitin in individual experiments using ITC. Binding constants, enthalpies of binding, and entropies of binding values were then extracted from the thermograms produced from each titration after subtraction of the heat of dilution effects. Free energies of each reaction could then be calculated using the enthalpy and entropy results.

Binding thermograms for mercury(II) and copper(II) are presented in Figures 1 and 2, respectively. The mercury(II)−chitin interaction gave a binding constant of $1.16 \cdot 10^5$ M^{-1} with a large amount of heat released. In contrast, copper binding released much less heat to provide about a 4-fold smaller binding constant for copper(II) of $2.91 \cdot 10^4$ M^{-1}. Although Hg has a smaller ionic radius,[32] it is a soft cation and binds to *N*-containing ligands more tightly than does copper.

The chitin−iron(II) thermogram (Figure 3) showed a 1.3× decrease in the binding constant compared to copper(II). The formal charge state of iron in this experiment is 2+. We have also repeated the same experiment for iron with a formal charge of 3+ (Figure 4). This data showed an about 2-fold decrease in the binding of iron, thereby suggesting that the formal charge state of the heavy metal is inversely proportional to the binding strength to chitin. There is approximately a 6-fold difference in the chitin−nickel(II) (Figure 5) and

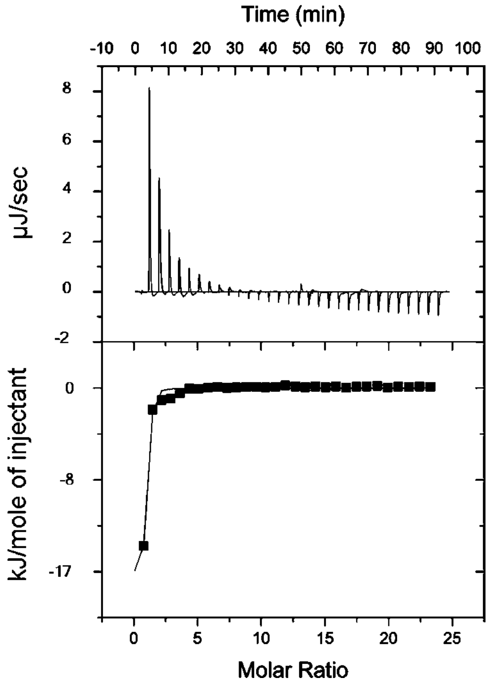


Figure 1. Binding thermogram and isotherm for the chitin−mercury(II) interaction are given at the top and bottom, respectively.

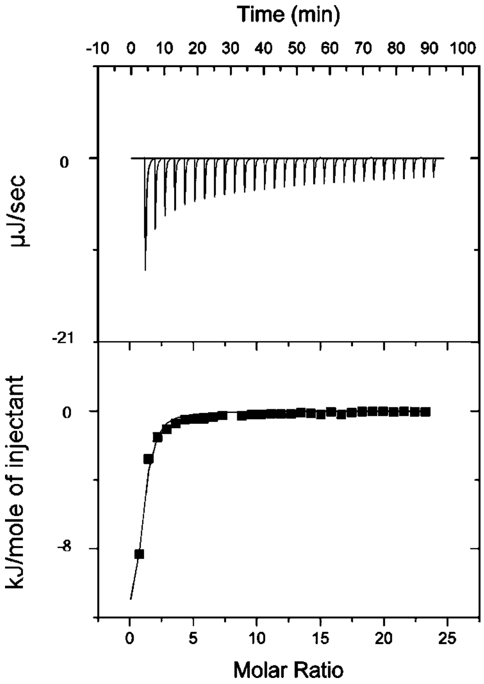


Figure 2. Binding thermogram and isotherm for the chitin−copper(II) interaction are given at the top and bottom, respectively.

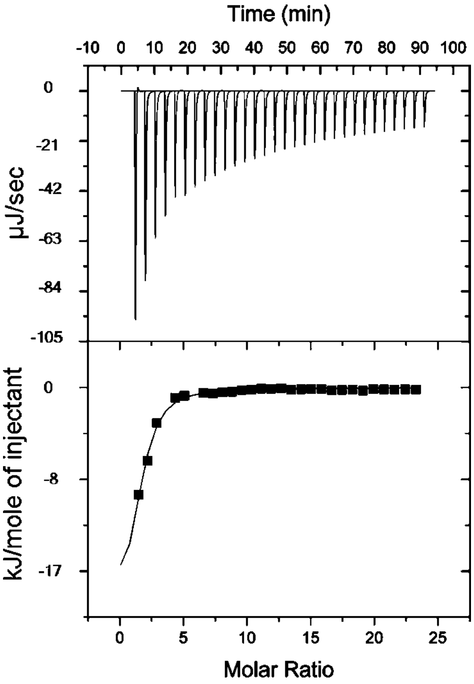


Figure 3. Binding thermogram and isotherm for the chitin−iron(II) interaction are given at the top and bottom, respectively.

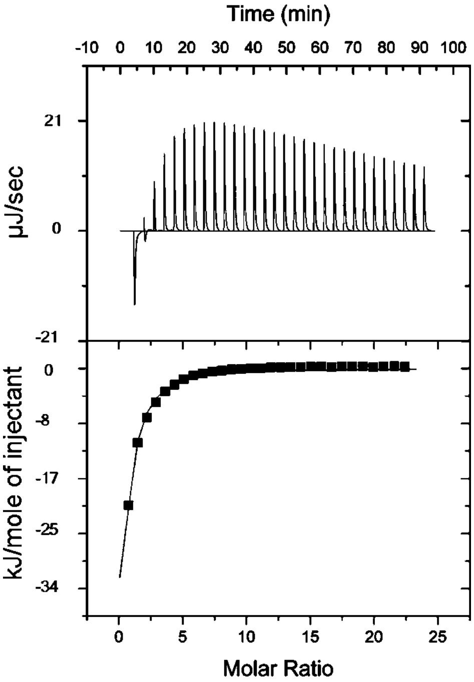


Figure 4. Binding thermogram and isotherm for the chitin−iron(III) interaction are given at the top and bottom, respectively.

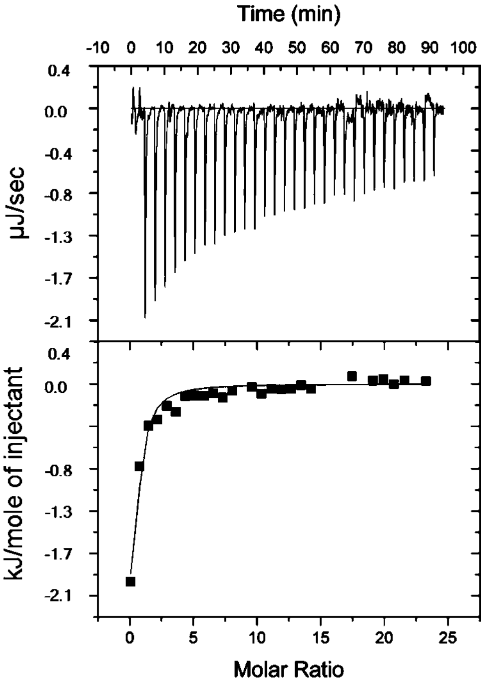


Figure 5. Binding thermogram and isotherm for the chitin−nickel(II) interaction are given at the top and bottom, respectively.

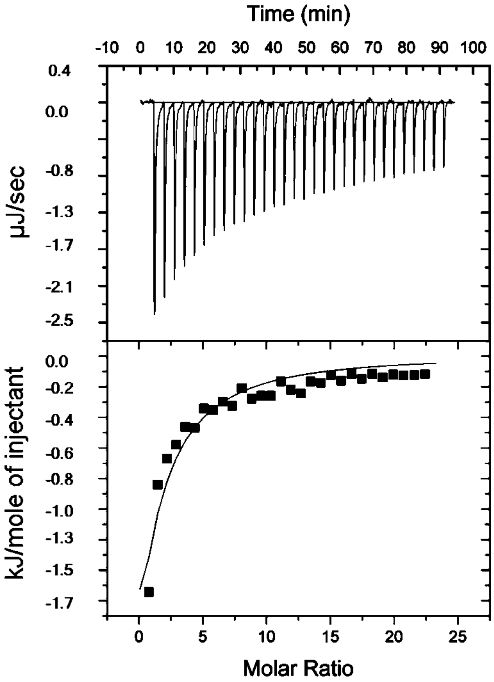


Figure 6. Binding thermogram and isotherm for the chitin−cobalt(II) interaction are given at the top and bottom, respectively.

chitin−cobalt(II) interaction (Figure 6) in terms of binding constants. Cobalt(II) gave the weakest interaction toward chitin with a binding constant of $3.96 \cdot 10^3\ \mathrm{M}^{-1}$. No detectable binding of magnesium(II) with chitin (Figure 7) was found by ITC. We did not observe any detectable binding with manganese and calcium either. This trend is consistent with previously reported studies on the related polymer chitosan in which binding to that carbohydrate polymer followed the pattern: Cu(II) > Fe(II) > Zn(II) > Cd(II).[33]

The overall order of binding affinities toward chitin was determined to be as follows: Hg(II) > Cu(II) > Ni(II) > Fe(II) > Cr(III) > Fe(III) > Pb(II) > Zn(II) > Cd(II) > Ag(I) > Co(II). This binding trend is similar to those of chitin−cellulose composite fiber−metal cation interactions which were determined by a spectrometric method [Hg(II) > Cu(II) > Pb(II) > Ni(II) > Cd(II)].[34] In addition, the sequence of metal complex stabilities given by Irving and Williams relates to our studies.[35] Bar graphs for binding constants are given for comparison purposes in Figure 8. Also, a short list of the thermodynamic parameters is given in Table 1. There is about a 30-fold difference in binding interactions toward mercury(II) and cobalt(II).

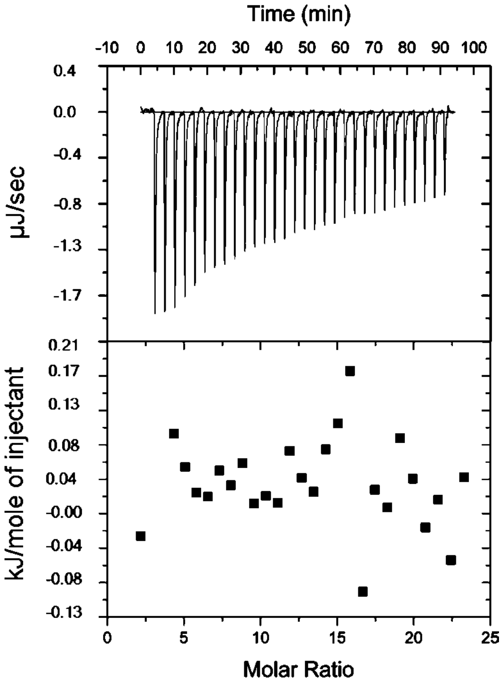


Figure 7. Binding thermogram and isotherm for the chitin−magnesium(II) interaction are given at the top and bottom, respectively.

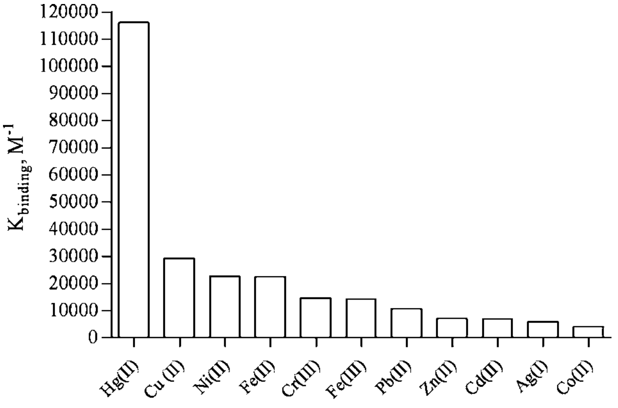


Figure 8. Comparison of binding constants for the chitin−metal cation interactions (all interactions are included).

All metal cation−chitin interactions showed negative enthalpy of reaction values at 25 °C in our study, implying that these interactions are all enthalpically favored. We obtained negative changes in the entropy of binding and negative changes in the free energy of reaction values in our experiments. All of the studied metal chitin interactions are enthalpically driven reactions at 25 °C. Free energy of reaction values have been calculated using the enthalpy and entropy values from the ITC experiments by eq 1 given below:

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

All free energy of reactions resulted in negative numbers, indicating energetically favored interactions for the heavy metal cations used in this study and chitin under our experimental conditions.

The Origin 7.0 software was used to evaluate the ITC data to obtain the thermodynamic parameters of the reaction systems studied. Both one-binding site and two-binding site models are utilized to determine the binding parameters. The two-site binding model did not provide a good fit of the data; on the other hand, a one-site binding model worked well for fitting of the ITC data and was therefore applied to evaluate the data throughout. The 100 iterations technique was chosen to minimize the χ square value for our entire data set.

Table 1. Thermodynamic Binding Parameters for Chitin−Metal Cation Interaction

Metal cation	$K\cdot10^{-3}$, M^{-1}	ΔH, kJ/mol	ΔG, kJ/mol
Hg(II)	116±12	-174±2.1	-29.3
Cu(II)	29.1±1.6	-142±2.9	-25.6
Ni(II)	22.5±3.0	-37.7±3.8	-24.9
Fe(II)	22.4±3.5	-439±15.9	-23.9
Cr(III)	14.5±1.4	-249±12.1	-23.6
Fe(III)	14.1±0.9	-498±15.5	-22.6
Pb(II)	10.6±0.8	-136±5.9	-23.1
Zn(II)	6.90±0.5	-87.0±3.8	-22.0
Cd(II)	6.81±0.7	-41.6±2.5	-21.9
Ag(I)	5.77±0.4	-233±5.9	-21.0
Co(II)	3.96±0.5	-82.8±5.9	-20.7

High K_b
↓
Low K_b

In summary, ITC experiments have been carried out for the first time to determine kinetic and thermodynamic parameters for chitin−metal cation chelation reactions. Mercury(II) is found to exhibit the strongest affinity toward chitin. Copper(II) is the next strongest cation followed by nickel(II) and chromium(III). Cobalt(II) is found to show the weakest measurable affinity for chitin chelation. Binding of all these cations to chitin takes place with negative enthalpy and free energy values. Binding of all of the heavy metal cations to chitin is determined to be enthalpically driven with negative change in entropy values at 25 °C. From this data, chelation of copper by chitin can be more effectively carried out in comparison to nickel, iron, or lead. Calcium, magnesium, and manganese interactions against chitin are determined to be so weak that they cannot accurately be measured by the ITC method. Clearly, a free amine as found in chitosan is not necessary for binding to a range of metal cations. The difference in magnitude of binding of chitin with different metal cations is likely the result of a combination of several factors, including the geometry of the metal complexes, ionic radii of the metal cations, valency of the metal, and hard−soft acid−base considerations. Available electrons from nitrogen and oxygen atoms on the sugar polymer chain have the potential to induce interactions toward positively charged metal cations. However, the exact molecular mode of binding is still unclear. Further studies are needed to determine whether carbohydrate polymers might exhibit multivalent binding to increase affinities to metals significantly over values expected for monosaccharide−metal binding.[36−40]

Glossary

Abbreviations

G	free energy of binding, $kJ\cdot mol^{-1}$
H	enthalpy of binding, $kJ\cdot mol^{-1}$
K	binding constant, M^{-1}
N	stoichiometry of binding
S	entropy of binding, $J\cdot mol^{-1}\cdot K^{-1}$
T	temperature, K

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Supporting Information Available:

Additional ITC binding curves and complete table for thermodynamic parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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