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Short communication

The use of isothermal titration calorimetry to determine the thermodynamics of metal ion binding to low-cost sorbents

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ABSTRACT

The thermodynamics of Al^{3+}, Cr^{3+}, and Pb^{2+} binding to the abundant biopolymer chitin have been determined using isothermal titration calorimetry (ITC) and compared to what is observed for binding to activated carbon. The use of ITC enables the detection of two distinct binding sites on chitin for all three metal ions. For the relative strong binding sites, free energy changes ranges from −37.6 kJ/mol to −41.8 kJ/mol while the same values are from −30.1 kJ/mol to −31.8 kJ/mol for the relative weak binding sites. All binding reactions to chitin are entropically driven. Interactions of the metal ions to activated carbon are best fitted as a single-site binding with relative weak binding with free energy changes from −26.3 kJ/mol to −26.8 kJ/mol.

1. Introduction

Metal contamination in water is a serious problem because they are not biodegradable and tend to accumulate in living organisms [1]. Examples include Al^{3+}, Cr^{3+}, and Pb^{2+} that can contaminate water and have shown to have toxic effects [2–5]. The use of low-cost for metal-ion removal has been extensively studied. Especially, the use of chitin derivatives has attracted considerable interest since chitin is an abundant biopolymer in nature [1,6–14]. Chitin, an insoluble linear polysaccharide consisting of repeated units of β-1,4-*N*-acetylglucosamine, is common as a structural polymer in crustaceans, arthropods, fungi, and parasitic nematodes. Naturally occurring chitin normally has a fraction of deacetylated sugar units where the acetamido group on carbon 2 is replaced with an amino group [15]. This has consequences for the ability of the chitin polymer to bind metal ions as discussed in the references above and in the work described here. The use of isothermal titration calorimetry (ITC) has become the gold standard for the thermodynamic investigation of binding interactions. A total of 623 articles are cited in an ITC literature review of 2007 written by Bjelic and Jelesarov showing the value on the use of ITC [16]. When ITC is used to determine thermodynamic parameters for a binding interaction, typically a ligand is titrated with a receptor. The ITC measures the heats of the interactions of each injection and calculates the enthalpy change since this takes place at constant pressure. By plotting the enthalpy changes for each injection against the amount of ligand vs. receptor, an equilibrium binding association constant (K_a) and the stoichiometry (n) can be modeled from the shape of the curve [17]. In this work, the binding interactions of Al^{3+}, Cr^{3+}, and Pb^{2+} to a chitin polymer that has 92% acetylated units and 8% deacetylated units as determined by NMR spectroscopy [15] is studied using ITC and the thermodynamic signatures are interpreted with respect to the degree of acetylation of the chitin polymer. The values are compared to those obtained for activated carbon, a commonly used sorbent that is high-cost relative to chitin.

2. Experimental

2.1. Chemicals

The chemicals used in the study were purchased from France Chitin (β-chitin 180 μM, Marseille, France, Sigma–Aldrich ($PbNO_3$, St. Louis, MO, U.S.A.)), and Merck ($CrCl_3{\cdot}6H_2O$, and $AlCl_3{\cdot}6H_2O$, Whitehouse Station, NJ, U.S.A.). The degree of acetylation for the β-chitin was determined to be 0.92 using the method of Einbu and Vårum [15].

2.2. Isothermal titration calorimetry experiments

ITC experiments were performed with a VP-ITC system from Microcal, Inc. (Northampton, MA) [17]. Solutions were thoroughly degassed prior to experiments to avoid air bubbles in the calorime-

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Table 1
Thermodynamic parameters of Al^{3+}, Cr^{3+}, and Pb^{2+} to chitin and activated carbon at $t = 25\,^{\circ}C$ as determined by isothermal titration calorimetry.

Metal ion	K_a (M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/K mol)	n (μmol/g)
β-Chitin					
Al^{3+}	$(4.0 \pm 2.4) \times 10^6$	−37.6	17.6 ± 3.6	184	18
	$(2.3 \pm 0.5) \times 10^5$	−30.5	75.3 ± 5.0	355	20
Cr^{3+}	$(6.0 \pm 1.4) \times 10^6$	−38.5	16.3 ± 2.9	184	28
	$(3.6 \pm 2.8) \times 10^5$	−31.8	8.2 ± 2.1	134	31
Pb^{2+}	$(2.0 \pm 1.4) \times 10^7$	−41.8	−10.9 ± 1.5	105	7
	$(2.0 \pm 0.7) \times 10^5$	−30.1	2.1 ± 0.9	109	31
Activated carbon					
Al^{3+}	$(5.0 \pm 0.9) \times 10^4$	−26.8	38.5 ± 3.4	217	2
Cr^{3+}	$(6.5 \pm 1.5) \times 10^4$	−27.6	−24.3 ± 3.2	12	3
Pb^{2+}	$(4.4 \pm 1.2) \times 10^4$	−26.3	−14.2 ± 2.2	42	2

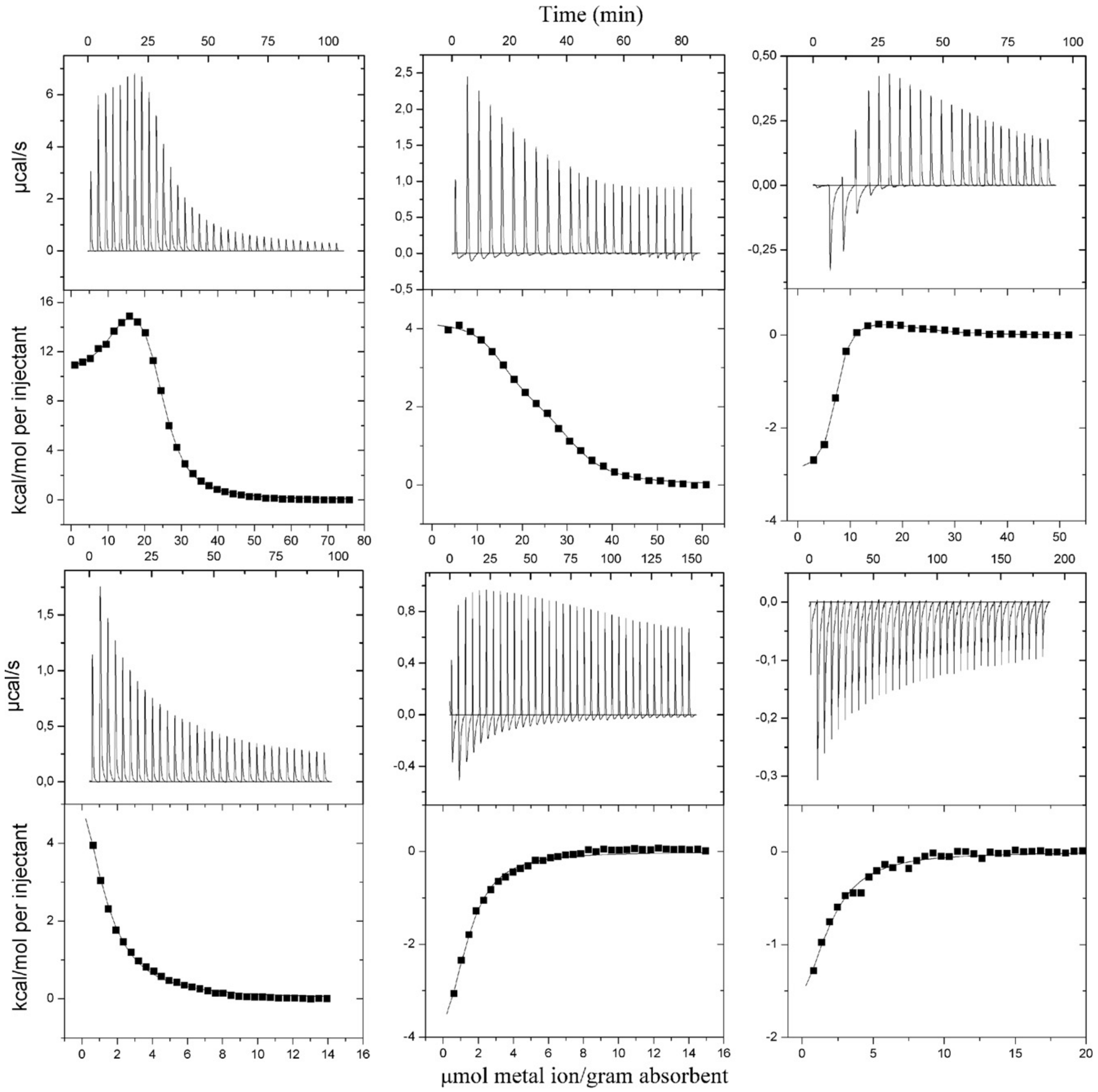

Fig. 1. Thermograms (top) and binding isotherms (bottom) for the titration of Al^{3+} (left), Cr^{3+} (middle), and Pb^{2+} (right) against β-chitin with $F_A = 0.92$ (top three) and activated carbon (bottom three) at $t = 25\,^{\circ}C$.

ter. In a typical titration, suspensions of 2.0–10 mg/mL of chitin or activated carbon that had been in water for at least 24 h were placed in the reaction cell with a volume of 1.42 mL, and 3.0 mM Al^{3+}, Cr^{3+}, or Pb^{2+} were placed in the ITC syringe. Aliquots of 4 μL were injected into the reaction cell at 180–360 s intervals at $t = 25\,^{\circ}C$ with a stirring speed of 260 rpm. The titrations were normally complete after 25–40 injections. At least three independent titrations were performed for each binding reaction.

2.3. Analysis of calorimetric data

ITC data were collected automatically using the Microcal Origin v.7.0 software accompanying the VP-ITC system [17]. All data were corrected for heat of dilution by subtracting the heat remaining after saturation of binding sites on the adsorbent prior to further data analysis. Data were fitted using a non-linear least-squares algorithm using a single-site or two-site binding model employed by the Origin software that accompanies the VP-ITC system. Fitting of theoretical data to experimental yielded the stoichiometry (n) as amount of metal ion per gram adsorbent (n/g), equilibrium binding association constant (K_a), and the reaction enthalpy change (ΔH_r°) of the reaction. The changes in the reaction free energy (ΔG_r°) and entropy (ΔS_r°) were calculated using Eq. (1). Errors in ΔH_r° and K_a are obtained as standard deviations of at least three experiments.

$$\Delta G_r^{\circ} = -RT \ln K_a = \Delta H_r^{\circ} - T\,\Delta S_r^{\circ} \quad (1)$$

3. Results and discussion

3.1. Binding of metal ions to β-chitin

The thermograms (Fig. 1) show clearly that Al^{3+}, Cr^{3+}, and Pb^{2+} bind to two distinct binding sites on the chitin polymer, one strong ($\Delta G = -37.6$ kJ/mol for Al^{3+}, -38.5 kJ/mol for Cr^{3+}, and -41.8 kJ/mol for Pb^{2+}) and one relative weak binding site ($\Delta G = -30.5$ kJ/mol for Al^{3+}, -31.8 kJ/mol for Cr^{3+}, and -30.1 kJ/mol for Pb^{2+}) (Table 1). The dissection of metal ion binding thermodynamics to two distinct sites using ITC have previously been described for the interactions of Hg^{2+}, Pb^{2+}, and Cd^{2+} with cyclooctapeptides [18]. Furthermore, all binding interactions are entropy driven with enthalpic pentalties with the exception of Pb^{2+} binding to the strong binding site (Table 1). The large and positive entropy change is most likely due to expulsion of water. It has been estimated that the loss of a single water molecule from a metal center to bulk water corresponds to 8 cal/K mol [19]. Metal ion binding to chitin involves the *N*-acetyl and hydroxyl functional groups [20,21]. Binding to chitosan, a partially deacetylated form of chitin, is proposed to take place either between two chitin chains where the free amino group on carbon 2 and the hydroxyl group on carbon 3 interact with the metal ion or with a single chain with the same functional groups in addition to two water molecules [8]. Since 8% of the sugar monomers on the chitin used have free amino groups, it is possible that the two thermodynamic signatures observed are due to binding to *N*-acetyl/hydroxyl groups and free amine/hydroxyl groups.

3.2. Binding of metal ions to activated carbon

Binding of the metal ions to activated carbon are best fitted as a single-site binding. All interactions are relatively weak ($\Delta G = -26.8$ kJ/mol for Al^{3+}, -27.6 kJ/mol for Cr^{3+}, and -26.3 kJ/mol for Pb^{2+}, Table 1). There are individual differences on the entropic and enthalpic contributions to the binding interactions. Al^{3+} is strongly entropically driven with an enthalpic pentalty ($\Delta S = 217$ J/K mol and $\Delta H = 38.5 \pm 3.4$ kJ/mol), Cr^{3+} is mainly enthalpically driven with a small positive entropi change ($\Delta S = 12$ J/K mol and $\Delta H = -24.3 \pm 3.2$ kJ/mol), while the origin of Pb^{2+} binding is equally divided by entropy and enthalpy changes ($\Delta S = 42$ J/K mol and $\Delta H = -14.2 \pm 2.2$ kJ/mol).

4. Conclusion

The use of ITC allows for the determination of the thermodynamics of Al^{3+}, Cr^{3+} and Pb^{2+} binding to two distinct binding sites on chitin. The differences in the thermodynamic signatures may be attributed to binding of the metal ions to different functional groups on the abundant biopolymer chitin. Binding of the metal ions to activated carbon, a relative high-cost sorbent, takes place at a single-binding site that is relatively weak.

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