Enhancement of the Metal-Binding Properties of Chitosan through Synthetic Addition of Sulfur- and Nitrogen-Containing Compounds

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SYNOPSIS

Several new chitosan derivatives were synthesized with the intent of forming polymers that could be used in hazardous waste remediation as toxic metal-binding agents in aqueous environments. The ability of these derivatives to bind Cu^{2+}, Pb^{2+}, Cd^{2+}, and Fe^{2+} was tested and compared to chitosan. Four of the new compounds, the products of the reaction of chitosan with mercaptosuccinic acid, thiirane, pyridoxal hydrochloride, and succinamide, show promising results as binding agents for the above metal ions. The compound with mercaptosuccinic acid bound twice as much Cd^{2+}, five times as much Pb^{2+}, and virtually no Fe^{2+} when compared to chitosan. The compound with thiirane bound three times as much Pb^{2+}, whereas the pyridoxal hydrochloride derivative bound 30% more Cu^{2+} and twice the Pb^{2+}. The succinamide derivative gave results comparable to chitosan, but with decreased solubility at low pH. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Chitosan, a polysaccharide derived from chitin, consists largely of glucosamine units and a smaller percentage of *N*-acetyl-glucosamine residues (Fig. 1). This polymer has been the object of continued study for several decades, and recent review articles outline much of the broad ranging research on this polymer to date.[1,2] In particular, its metal-binding properties have attracted attention as a potential hazardous waste remediation material, since chitosan has a demonstrated ability to bind transition metals and uranium while essentially ignoring Group I and II metals.[3] This metal-binding property has led to consideration of chitosan as a low-cost material for use in, for example, waste water treatment plants, water containing radioactive elements, and streams contaminated by accumulations of toxic metals released by industrial activities.

Although chitosan binds transition metals, it does so to varying degrees. A high percentage of copper, for example, is bound, while the percentage of cadmium and lead is somewhat lower.[4] A less desirable characteristic of chitosan is its affinity for iron. This nontoxic metal can be found in natural or waste waters in substantially higher concentrations than the toxic metal of interest where it could interfere with the binding of the toxic metals by chitosan.

The purpose of the work described in this paper was to prepare derivatives of chitosan with enhanced transition metal-binding ability while attempting to decrease its binding affinity for iron. The derivatives must also remain insoluble at pH values where their use is anticipated. Mine waste waters, for example, with pH 2–3 are not uncommon.

The rationale directing the present work is based on the binding sites for metal ions in the polymer. These sites and coordination numbers appear to vary from metal to metal, but in the case of Cu^{2+}, it is known that the nitrogen of the amine group at C-2 is involved in binding and perhaps the hydroxyl group on C-3 or C-6 as well.[4,5] The amine group and the two hydroxyl groups in the glucosamine residue are the chemically active sites as is, to a lesser extent, the amide group of the *N*-acetyl-glucosamine residue. Preservation or enhancement of the amine site appeared to be critical for continued successful metal binding, whereas the hydroxyl groups were considered as possible cross-linking sites for increasing the

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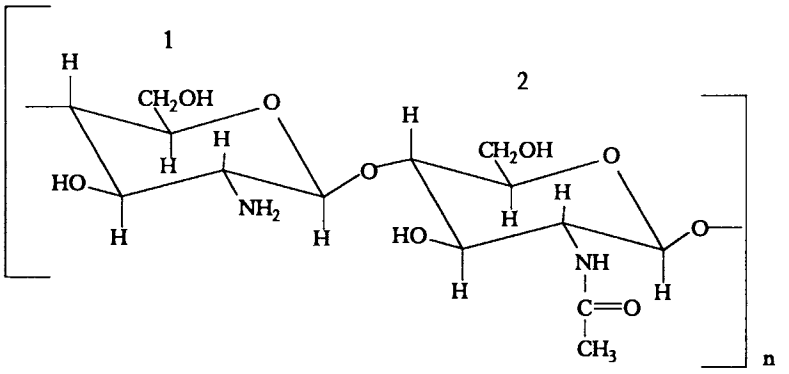

Figure 1 Chitosan structure. The polymer is composed of approximately (1) 75 mol % β-D-glucosamine and (2) 25 mol % *N*-acetyl-β-D-glucosamine with *n* approximately 1000.

insolubility of chitosan or as groups that could be derivatized to provide new metal-binding sites. The synthesis of several chitosan derivatives and their metal-binding properties will be presented in this paper.

EXPERIMENTAL

Chitosan was purchased from CTC Organics, Atlanta, Georgia, and prepared for use by dissolving in dilute acetic acid and precipitating with potassium hydroxide. The solid was washed with water until neutral, rinsed with methanol, and allowed to air-dry. This step was considered necessary to remove impurities and process chemicals possibly remaining after industrial preparation. The chitosan sample was analyzed to determine percent deacetylation using standard methods and was found to contain 75% glucosamine residues.[6,7] Other reagents were purchased from Aldrich Chemical Co., Milwaukee, WI, and were used without further purification.

Elemental analyses of metal-ion solutions were made using a Varian SpectrAA-20 atomic absorption spectrophotometer. Infrared spectra were scanned with a Perkin-Elmer 1600 Series FTIR spectrophotometer using either a KBr pellet or a thin film formed by dissolving the chitosan derivative in 10% acetic acid and allowing the liquid to evaporate. A Hitachi 110A spectrophotometer was used to scan UV-VIS spectra, while NMR spectra were collected on an IBM NR/300 FT-NMR spectrometer.

Preparation of Mercaptosuccinic Acid–Chitosan (CHTS-MS)

Mercaptosuccinic acid (4.5 g, 0.030 mol) was dissolved in 200 mL acetone and 0.082 mL concentrated sulfuric acid. Chitosan (6.0 g, 0.026 mol glucosamine residue) was added and the mixture stirred for 2 days. The violet product was collected on a filter funnel, washed with acetone to remove excess acid, rinsed with methanol, and air-dried. This compound was less soluble at low pH than was chitosan.

Preparation of Thiirane–Chitosan (CHTS-TH)

Chitosan (5.0 g, 0.022 mol glucosamine residue) was suspended in 250 mL ethanol. Thiirane (2.0 mL, 0.034 mol) was added to the constantly stirred mixture at the rate of 0.50 mL twice per day for 2 days to avoid polymerization of the thiirane. The reaction was allowed to proceed for three additional days, after which the derivative was collected by centrifugation, washed several times with ethanol and methanol, and then air-dried. This compound also appeared to be less soluble than was chitosan under acidic conditions.

Preparation of Pyridoxal–Chitosan (CHTS-PYR)

Chitosan (6.0 g, 0.026 mol glucosamine residue) was dissolved in 600 mL 10% acetic acid with heat and stirring. Pyridoxal hydrochloride (7.5 g, 0.037 mol) was dissolved in 50 mL cold water and then added over a period of 1 h to the stirred chitosan solution, which became yellow as the reaction proceeded. The reaction vessel was kept in an ice/water bath to prevent decomposition of the pyridoxal hydrochloride. After 4 h, the pH was adjusted to 4.0 with sodium hydroxide and 95% sodium cyanoborohydride (3.5 g, 0.053 mol) was added to reduce the imine. The solution was made basic with sodium hydroxide and stirred to achieve complete precipitation. The solid, more soluble than chitosan at low pH, was

washed with water until neutral, rinsed with methanol, and air-dried.

Preparation of Succinamide–Chitosan (CHTS–SA)

Chitosan (6.0 g, 0.026 mol glucosamine residue) was dissolved in 10% acetic acid as above. Succinamide (8.6 g, 0.074 mol) was added to the solution with stirring and heat. The reaction was allowed to continue for 3 days, after which the solution was made basic with sodium hydroxide and stirred to ensure complete precipitation. The precipitate was washed with water, methanol, and air-dried. This compound was also less soluble at low pH relative to chitosan.

Preparation of Unproductive Derivatives

Other derivatives whose metal-binding results were unfavorable with respect to chitosan include a compound with azetidinone formed under acidic conditions, as well as compounds with adipic and thioctic acid in inert solvents. Cysteine and tryptophan were each separately reacted with chitosan using dicyclohexylcarbodiimide in a standard amino acid addition reaction.[8] Several thiophene compounds reacted with chitosan including 2-thiopheneacetyl chloride in pyridine, 2-thiophenecarboxaldehyde in 0.1*M* HCl, and 4-(2-thienyl)butyric acid in acetone. Propionamide formed an addition compound with chitosan in 10% acetic acid, while succinyl chloride reacted with chitosan in pyridine. Evidence of reaction with chitosan in the above cases was provided by FTIR spectra.

Metal-ion Adsorption

Four metal ions, Cu^{2+}, Cd^{2+}, Pb^{2+}, and Fe^{2+}, were chosen to test the binding ability of the chitosan derivatives. This selection was based on the high affinity of chitosan for Cu^{2+}, which allows sensitive binding assays to be performed, and the environmental toxicity of Cd^{2+} and Pb^{2+}, which makes their removal from aqueous systems desirable. Lastly, Fe^{2+} was chosen because of its abundance and possible interference in the adsorption of target metal ions. These metals were used as sulfates except in the case of Pb^{2+} where $PbCl_2$ was chosen for its solubility.

A 50 ppm solution of the desired metal ion was prepared; 0.100 g chitosan derivative was added to 100.0 mL of this solution and then stirred for 2 h at 25°C. A control solution was treated in the same manner without stirring or the addition of the chitosan derivative. The pH of the solutions was adjusted to 5.0 before the addition of the derivative to be tested and maintained at that value using a pH controller. The amount of a given metal bound by chitosan is known to be pH-dependent with a greater amount bound at higher pH.[9,10] The pH used in these binding experiments reflects a value where chitosan shows significant binding capacity yet which is acidic enough to be representative of actual environmental situations where the derivatives may be employed. This metal-binding test was performed in triplicate on chitosan and each derivative.

The necessity of maintaining a constant pH is dictated by the rise in pH seen when chitosan is added to water at pH 5.0 in the absence of metal ions. This behavior varied somewhat among different brands of chitosan, but was seen in all three samples tested by us. Most of this effect occurs within the first 30 min, with pH largely stable after 90 min. The cause of this pH rise is unknown but may be due to the presence of an impurity from the industrial process or swelling of the chitosan causing rupture of internal hydrogen bonds and protonation of the amine groups with water as the source of the proton.

RESULTS AND DISCUSSION

The structure of the chitosan derivatives discussed here was examined through elemental analysis and NMR and FTIR spectroscopy. Elemental analysis of sulfur and the assumption that at the concentration of the reactants no more than one molecule would react with each residue in chitosan indicate a degree of substitution of 0.27 for CHTS–TH and 0.29 for CHTS–MS.

The FTIR spectra of CHTS-MS and CHTS-TH are shown along with that of chitosan in Figure 2. The spectrum of CHTS–MS shows a new carbonyl stretch at 1720 cm^{-1} as well as a shift in one amide–amine band from 1650 to 1625 cm^{-1} and in a second amide–amine band from 1575 to 1515 cm^{-1}. This evidence supports the reaction of mercaptosuccinic acid with chitosan at both a hydroxyl group as well as at the amine site.

The FTIR spectrum of CHTS–TH shows an increase in intensity of the methylene stretch at 2878 cm^{-1} and a shift in the amide–amine vibrations similar to CHTS–MS, indicating that a reaction occurred at the chitosan amine group.

The FTIR spectrum of CHTS–SA (not shown) also demonstrates substantial changes in the amine region and an enhanced symmetric and asymmetric

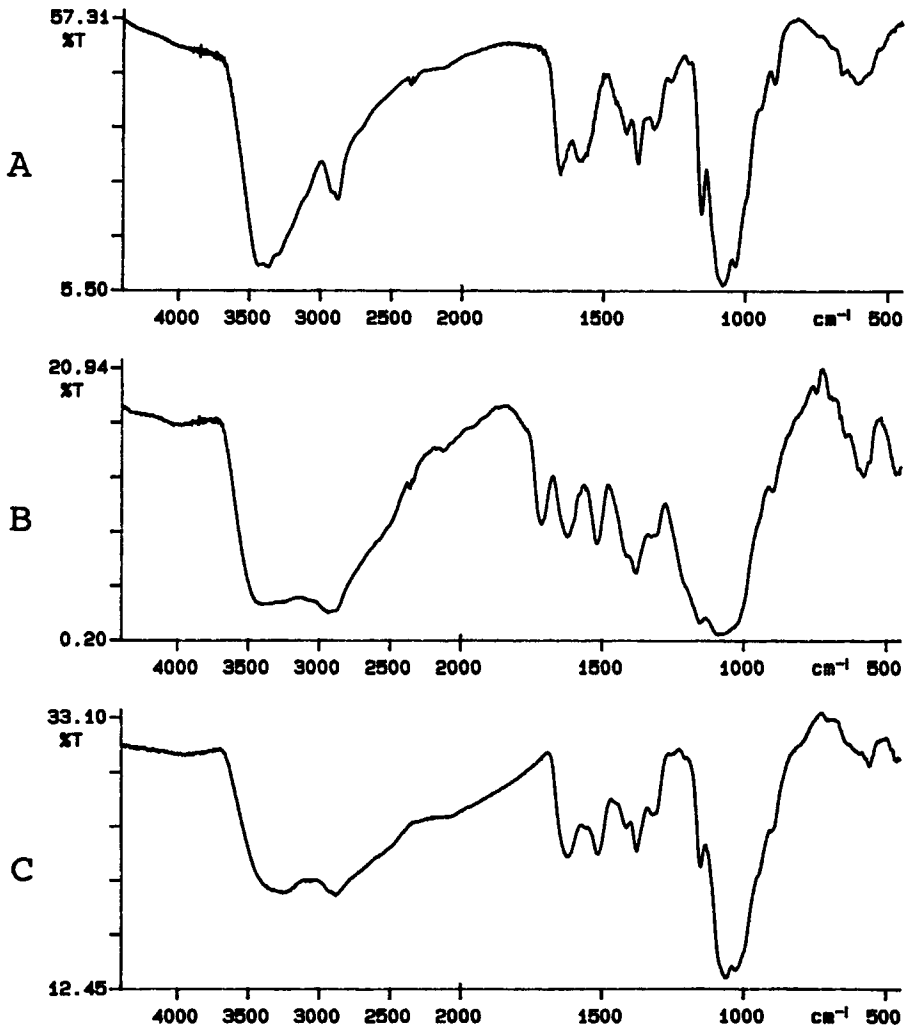

Figure 2 FTIR spectra: (A) chitosan; (B) CHTS-MS; (C) CHTS-TH.

amide NH stretch at 3340 and 3180 cm^{-1}, as would be expected from the addition of this amide to chitosan. The lack of an ester peak in the spectrum indicates that little or no reaction occurred at a chitosan hydroxyl site.

Changes are also apparent in the FTIR spectrum of CHTS-PYR (not shown) and support reaction of pyridoxal hydrochloride at the chitosan amine site. Although aldehydes can also react with alcohols under these conditions, there is no indication in the spectrum that such a reaction occurred. Additional evidence for the reaction of chitosan with pyridoxal hydrochloride was provided by the UV-VIS absorption spectrum of this compound, which differed significantly from chitosan in the 230–370 nm region.

The ^{13}C-NMR studies of chitosan, CHTS-MS, and CHTS-TH were done as solid samples since these derivatives could not easily be dissolved. Figure 3 compares the chitosan spectrum with these two derivatives. The carbonyl peak at 175 ppm shows a broadening as well as an increase in area due to the presence of additional carbonyl functionality in CHTS-MS.

The NMR spectrum of CHTS-TH shows a unique peak at 32 ppm and is presumably due to the thiirane methylene carbon bound to the thiol group. The second methylene carbon that is bound to the amine group would be expected to appear further downfield where it would be obscured by the C-2 peak of the chitosan backbone at 58 ppm.[11,12]

The metal-ion-binding results are presented in Table I as the mean of three trials. These studies show an improvement in the capacity for one or more metal ions in three of the four derivatives tested. The binding of Cu^{2+} was enhanced in CHTS-PYR, whereas CHTS-MS, CHTS-TH, and CHTS-SA bound roughly the same amount of this ion as chitosan.

Two of the derivatives showed increased binding of Cd^{2+}. CHTS-MS bound roughly twice as much

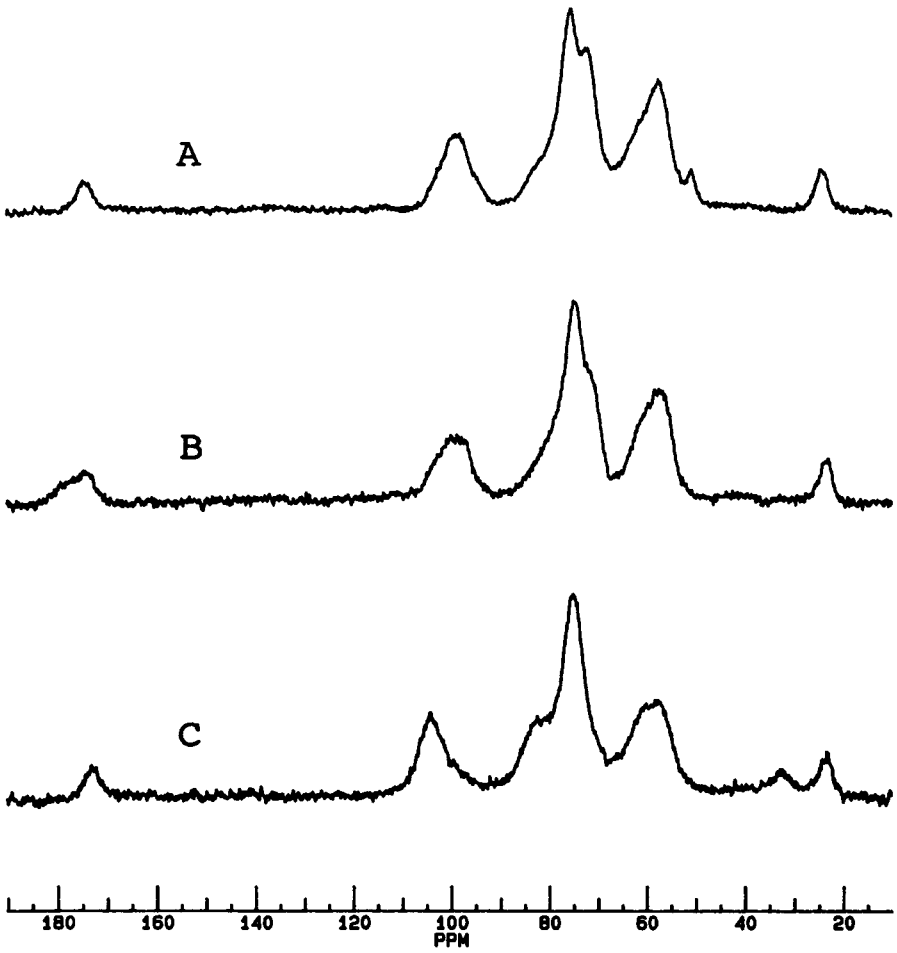

Figure 3 Solid-state ^{13}C-NMR spectra: (A) chitosan; (B) CHTS–MS; (C) CHTS–TH.

of this metal ion as chitosan, and CHTS–TH bound about 30% more.

The binding of Pb^{2+} was improved in three of the four derivatives. CHTS–MS bound five times; CHTS–TH, three times; and CHTS–PYR, roughly twice the amount of Pb^{2+} as compared to chitosan.

Since the derivatives were designed to select against Fe^{2+}, the most successful chitosan derivative was CHTS–MS, which bound essentially none of this ion. CHTS–TH bound roughly half the amount bound by chitosan, and CHTS–PYR bound somewhat more Fe^{2+}.

Two derivatives, CHTS–MS and CHTS–PYR, had metal-binding capabilities that were significantly better than those of the parent chitosan. CHTS–MS bound a substantially higher percentage of Cd^{2+} and Pb^{2+} and a lower percentage of Fe^{2+}. The Cu^{2+}-binding performance of this derivative was little altered. CHTS–PYR bound more Cu^{2+} and Pb^{2+}, somewhat more Fe^{2+} than chitosan, and essentially the same amount of Cd^{2+}.

Although the metal-binding capability of CHTS–PYR was improved over that of chitosan in terms of Cu^{2+} and Pb^{2+}, this derivative is more soluble at low pH. Since the derivative itself is potentially useful, further work involving cross-linking is planned for this compound in order to decrease its solubility.

Table I Percentage of Metal Ions Bound by 100 mg Chitosan and Chitosan Derivatives from 100 mL 50 ppm Solutions at pH 5.0, 25°C, in 2 h

	% Metal Ion Bound			
Polymer	Cu^{2+}	Cd^{2+}	Pb^{2+}	Fe^{2+}
Chitosan	54 (7)	22 (2)	10 (3)	18 (4)
CHTS–MS	43 (3)	43 (5)*	49 (2)*	0.3 (.6)*
CHTS–TH	59 (5)	28 (3)*	27 (3)*	9 (2)*
CHTS–PYR	71 (5)*	27 (3)	21 (4)*	24 (3)
CHTS–SA	60 (6)	24 (4)	10 (4)	13 (2)

Standard deviations are given within parentheses. Starred values (*) are significantly different ($P < .05$) from those for parent chitosan under identical conditions.

The derivative CHTS–SA appeared to have no significant enhancement of metal-ion binding compared to chitosan, yet it may find some use based on its greater insolubility than that of chitosan at low pH.

These derivatives will be further characterized by performing kinetic and equilibrium studies in which the effect of typical parameters, such as pH, temperature, and metal-ion concentration, will be examined.

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