Study on Chitosan tetrasaccharide monomer Recognition System Based on Molecular Imprinting Technology

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Abstract. In this paper, we use chitosan as template molecule and N-[2-(dimethylamino)ethyl]-N’-[2-hydroxy-4-vinylphenyl)] oxamide (H₃eoxdmpe) as bridge ligand and directionally synthesize N-[2-(dimethylamino)ethyl]-N’-[2-hydroxy-4-vinylphenyl)] oxamide dinuclear zinc-chitosan [Zn₂(eoxdmpe) (tetrachit)] (ClO₄) coordination monomer. We study the interaction between complex and template molecules by ultraviolet spectrophotometry and the recognition ability of complex to monosaccharide molecule quantitatively by formula. The crystal structures of N-[2-(dimethylamino)ethyl]-N’-[2-hydroxy-4-vinylphenyl)] oxamide binuclear zinc-chitosan complex are determined and analyzed by X-ray single crystal diffractive technique and the results show that the metal coordination bond is a favorable binding activity for chitosan. This study provides an ideal model for the construction of metal coordination molecularly imprinted polymers which are selectively recognized by chitosan.

1. Introduction

Chitooligosaccharides (COS) usually consists of 3 to 10 aminoglucose which is connected by beta-1,4-glycoside bond and is the product of chitin after deacetylation and degradation [1, 2]. Research results show that the biological activity of COS is closely related to its Degree Polymerization (DP) and COS with different DP has many specific biological activities and functions. After we degrade and dispose of Chitosan with technology like bioenzymatic hydrolysis, chemical degradation and physical degradation, we usually get complex mixtures consisting of COS with different DP [3]. Large-scale preparation of COS with high purity and different DP has become the technical bottleneck restricting its application and development. Finding new separation methods and technologies and realizing its large-scale preparation has been one of the difficulties in developing and utilizing COS.

Molecular Imprinting Technique (MIT) has been widely used in areas such as Chemical sensors, natural enzyme and antibody simulation, selective catalysis, chiral separation of drugs, controlled drug release, pesticide residue analysis, chromatographic stationary phase, and solid phase extraction [4, 5]. The fact that MIT can be used to prepare inexpensive and efficient separation materials has become a new research direction in the field of separation and purification of complex systems. MIT has developed continuously and researchers at home and abroad have successfully extended this technology to the research fields of glucose, sucrose and raffinose in recent years [6, 7]. It is a very important basic work in the process of molecular imprinting research to carry out molecular simulation and pre-assembly of
functional monomers based on the properties of template molecules and screen functional monomers with excellent binding properties to template molecules. At present, molecular imprinting screening of functional monomers is still in the exploratory stage. It has been reported in the literature that experimental methods such as ultraviolet-visible spectroscopy, fluorescence spectroscopy and nuclear magnetic resonance spectroscopy have been applied to carry out researches on such systems with the constantly deepening of research. Among these methods, ultraviolet-visible spectroscopy is a common screening method. According to the changes of ultraviolet-visible spectra, information on the interaction strength and composition ratio between template molecules and functional monomers can be inferred. Through these theoretical and experimental studies, it is a significant content in molecular imprinting research to screen the functional monomers according to the strength of interaction between template molecules and functional monomers and to realize systematic optimization of the structure and performance of the constructed molecular imprinting recognition system [8].

In this paper, we use chitosan as template molecule, N-[2-(dimethylamino)ethyl]-N’-[2-hydroxy-4-vinylphenyl] oxamide (H3eoxdmpe) as bridging ligand, coordinate it with zinc ion and chitosan and directionally synthesize coordination monomer N-[2-(dimethylamino) ethyl]-N’-[2-hydroxy-4-vinylphenyl] oxamide dinuclear zinc-chitosan [Zn2 (eoxdmpe) (tetrachit)] (ClO4). The construction method of chitosan oligosaccharide monomer recognition system has been explored and the system of the constructed coordination molecule imprinting recognition system optimized; on this basis, we have studied the interaction between template molecules and functional monomers both qualitatively and quantitatively with ultraviolet spectrophotometry and this builds a solid foundation for the study of synthesis, recognition mechanism and adsorption properties of coordination molecularly imprinted polymers.

2. Experiments

2.1. Reagents and Materials
2-hydroxy-4-vinyl aniline, oxaloethyl ester monoacyl chloride, N, N-dimethyl ethylenediamine, sulfoxide chloride, tetrahydrofuran, ethyl ether, petroleum ether, ethyl acetate, methanol, anhydrous ethanol, zinc sulfate, sodium hydroxide, hydrochloric acid, ammonia water and the like are all commercial analytical pure reagents. Methanol is a commercial pure reagent for chromatography.

2.2. Experimental Instruments
XEXUS-470 Fourier Transform Infrared Spectrometer (Nicolet Company, USA), SMART 1000 CCD X-ray single crystal diffractometer (Bruker Company, Germany).

2.3. Synthesis of Coordination Monomers

2.3.1. Synthetic Route. Taking chitosan, as an example, the synthetic routes of functional ligands (H3eoxdmpe) and complexes [Zn2 (eoxdmpe) (tetrachit)] (ClO4) are as follows:
2.3.2.  Synthesis Method. (1) Synthesis of intermediates N-(2-hydroxy-4-vinylphenyl) oxaloyl monoethyl ester. Under stirring, 10 mmol 2-hydroxy-4-vinylaniline is dissolved in 25mL THF and slowly drips into 25mL THF solution containing 10mmol oxaloethyl ester chloride. After the drip addition, the reaction continues at room temperature of 25 ℃ for an hour, a large number of white precipitate and then we filter and recrystallize it with ethyl acetate to obtain intermediates.

(2) Synthesis of Functional Ligand N-[2-(Dimethylaminoethyl)]-N’-(2-hydroxy-4-vinylphenyl)] Oxamide. We dissolve 5mmol N, N-dimethyl ethylenediamine in 10mL THF, slowly drip it into 20mL THF solution containing 5mmol intermediate, stir for 5 hours at indoor temperature, concentrate and precipitate a large amount of white precipitation under vacuum and wash it with ice ethanol and ether repeatedly to obtain the target product.

(3) Synthesis of functional coordination monomer N-[2-(dimethylamino)ethyl)]-N’-(2-hydroxy-4-vinylphenyl)] oxamide binuclear zinc-chitosan. Under stirring at room temperature, 10 mmol of N-[2-(dimethylamino)ethyl)]-N’-(2-hydroxy-4-vinylphenyl)] oxalamide ligand methanol solution 20 mL and 30 mmol of piperidine methanol solution 10 mL are added to flask respectively to obtain colorless clarifying solution. In this mixed system, we add 20 mmol of Zn(ClO₄)₂·6H₂O methanol solution 10 mL drop by drop and then obtain a yellowish transparent solution after 30 min reaction at room temperature. Then we add a proper amount of template molecule chitosan aqueous solution and the reflux reaction is continued for 3 h. The obtained reaction solution was concentrated at controlled temperature and pressure to obtain yellow microcrystals. The precipitation is dissolved and filtered with acetonitrile-methanol-water mixed solvent to make it evaporate at room temperature. After 7 days, yellow bulk single crystals suitable for X-ray single crystal diffraction will grow in 85% yield.

2.4. Study on the Interaction of Binuclear Zinc Complexes with Chitooligosaccharide Monomers

We study the interaction between oxalamide binuclear zinc complex and chitooligosaccharide monomer both qualitatively and quantitatively with ultraviolet spectroscopy. A suitable amount of functional ligand N-[2-(dimethylamino)ethyl)]-N’-(2-hydroxy-4-vinylphenyl)] oxamide and methanol-water (1:1) solution of Zn(ClO₄)₂·6H₂O are added to the colorimetric dish respectively, then we adjust the pH of the system by hydroxymethyl aminomethane (Tris) -HCl and scan the ultraviolet-visible spectra in the range of 200-800nm. Before the measurement, we add a 5 UL sugar reserve solution (0.2 M) into the solution of the above binuclear zinc complex system with a microinjector each time to make the concentration ratio of template molecule sugar to functional ligand binuclear zinc complex increases continuously until saturation and then observe on the variation of the absorption value of the Ultraviolet Spectra of Complexes. On the Bonding Strength of Binuclear Zinc Complexes with functional ligands to template molecule chitosan, we calculate the bonding constant Kₘ between them using the formula [Sugar]/(εᵣ−εₐ) = [Sugar]/(εᵣ−εₗ) + 1/Kₘ(εᵣ−εₗ). In the formula: [Sugar]: sugar concentration; εₐ: molar extinction coefficient; εᵣ and εₗ respectively represents molar extinction coefficients of chitosan-free oligosaccharides and chitosan oligosaccharides when fully bonded. [Sugar]/ (εᵣ−εₐ) is used to plot [Sugar] and we obtain the slope and intercept. Then the ratio of slope to intercept is the bonding constant Kₘ.
3. Results and discussion

3.1. Ultraviolet spectroscopy study on the interaction of functional ligand metal complexes with chitooligosaccharide monomers

Ultraviolet-visible absorption spectroscopy is one of the most common and effective methods to study the interaction between complexes and sugars because of its simple operation and intuitive results. Still taking chitosan as an example, we study the changes of ultraviolet-visible absorption spectra of N-[2-(dimethylaminoethyl)]-N'-(2-hydroxy-4-vinylphenyl) oxalamide zinc complex before and after adding chitosan monomer to determine its interaction with chitosan oligosaccharide monomer.

![Figure 2 Ultraviolet-Visible Absorption Spectra of Dinuclear Complexes of Chitosan and N-[2-(dimethylamino)ethyl]-N'-[2-hydroxy-4-vinylphenyl)] Zinc Oxamide at Different Concentrations](image)

As we can see in Figure 2, with the increasing concentration of chitosan in the system, the absorption peaks of N-[2-(dimethylamino)ethyl]-N'-[2-hydroxy-4-vinylphenyl)] zinc oxalamide complexes at 609nm shows a decrease in color and a redshift in intensity and that indicates the interaction between binuclear zinc complexes and chitosan molecules. Plot [Sugar] with [Sugar]/(ε_a−ε_f) and then calculating from the slope and intercept of the straight line that the bonding constant K_b of the chitosan tetrasaccharide system is 1.9×10^6 M^{-1} ; we find it in a similar way that the K_b values of chitosan and chitosan are 8.9×10^5 and 5.2×10^5 M^{-1}, respectively. The results show that the zinc complex of functional ligand has a strong binding ability to template molecule chitosan oligosaccharides, while the K_b value decreases with the increase of degree of polymerization of chitosan oligosaccharides, which might be related to the steric hindrance of chitosan oligosaccharides.

3.2. Structure of [Zn2(eoxdmpe)(tetracl)](ClO4), the Complex of N-[2-(dimethylamino)ethyl]-N'-[2-hydroxy-4-vinylphenyl)] oxamide dinuclear zinc-chitosan

To further obtain the structural information of functional monomers of binuclear complexes, we determine and analyse the crystal structure of N-[2-(dimethylamino)ethyl]-N'-[2-hydroxy-4-vinylphenyl)] oxalamide binuclear zinc-chitosan complex using X-ray single crystal diffraction. The crystal parameters and ORTEP diagrams of the complexes are shown in Table 1 and Figure 3, respectively.

**Table 1. Crystallographic data and structural analysis parameters of binuclear zinc (II) - chitosan complexes**

| Molecular formula            | Zn2C38H56N7O24Cl |
|-----------------------------|------------------|
| molecular weight            | 1063.82          |
| temperature                 | 293 K            |
| Crystal system, space group | Three oblique, P1 |
Unit cell parameter

\[ \begin{align*}
\text{Unit cell parameter} & \quad a = 11.1651(2) \, \text{Å}, \quad \alpha = 89.925(2)^\circ \\
& \quad b = 12.1562(3) \, \text{Å}, \quad \beta = 96.153(8)^\circ \\
& \quad c = 16.1563(2) \, \text{Å}, \quad \gamma = 98.163(2)^\circ \\
\text{Unit cell volume} & \quad 2192.8(7) \, \text{Å}^3 \\
\text{Z value, theoretical density} & \quad 1, \quad 1.52 \, \text{mg/cm}^3 \\
\text{Linear absorption coefficient} & \quad 0.121/\text{mm} \\
\text{Structural factor (F000)} & \quad 1072 \\
\text{Crystal size} & \quad 0.09 \times 0.22 \times 0.32 \, \text{mm} \\
\text{Scope of data collection} & \quad \theta 3.2 \, \text{to} \, 27.5^\circ \\
\text{Scope of Crystal Surface Index} & \quad 11 \leq h \leq 12, \quad 13 \leq k \leq 14, \quad 15 \leq l \leq 16 \\
\text{Total number of diffraction points, number of independent diffraction points} & \quad 45231/6218 (R_{\text{int}}=0.043) \\
\text{Independent diffraction point data/limitation/refinement parameters} & \quad 6218/2/162 \\
\text{R factor (all data)} & \quad R=0.0751, \quad \omega R^2=0.1432, \quad S=1.01 \\
\text{Maximum residual electron peak/valley} & \quad 0.22/-0.12 \, \text{eÅ}^3
\end{align*} \]

The complex belongs to 1:1 electrolyte type complex and it can be seen as being composed of binuclear ternary coordination structure units which consist of a proton-free N-[2-(dimethylaminoethyl)]-N’-(2-hydroxy-4-vinylphenyl)] oxamide (eoxdmpe3-) bridging two zinc in a trans-mode ("endogenous bridge") and a neutral tetrachit molecule acting as an "exogenous bridge" and perchlorate as counterion anion is outside the complex. In Figure 3, the crystal cell of the complex, the Zn1 atom is in the N2O3 tetragonal cone coordination environment and the environment consists of Proton-free phenolic hydroxyl O1 and N1, carbonyl O3 in oxamide ligands and N4 on amino group and O10 on hydroxyl group in chitosan molecule; among the four coordination atoms of zinc dioxide, two nitrogen atoms are from N2 and N3 of oxalamide ligand, the other two are from N7 and O18 atoms of 4 hydroxyl groups on 3 amino groups of chitosan monomer. The Zn2 is at the center of the tetrahedron which is composed of these coordination atoms, and the distance between Zn1 and Zn2 is 4.8375 (15) Å. In the structure of the complex, chitosan molecule, as a neutral tetradentate ligand, interacts with Zn1 and Zn2 atoms in the binuclear zinc complex by alternating nitrogen atoms of three amino groups and oxygen atoms of four hydroxyl groups on four saccharide rings. Therefore, chitosan acts as an "exogenous bridge" in this complex.

**Figure 3.** ORTEP Diagram of Dizinc-Chitosan Complexes (for simplification, the anions and hydrogen atoms are omitted).
4. Conclusion
The paper aims at exploring the construction of chitosan oligosaccharide functional monomer recognition system and its binding activity to template molecule chitosan oligosaccharide. For this reason, we take chitosan as the representative and have synthesized a new N-(4-styryl) oxalamide functional ligand, that reacted with chitosan oligosaccharide monomer and Zn$^{2+}$, synthesized a ternary complex of "functional ligand-Zn$^{2+}$-chitosan oligosaccharide", and then determined its exact structure by X-ray single crystal diffraction. Functional monomers of such binuclear complexes are still under reported. Considering that the type and structure of the complex and the configuration of the sugar molecule all affect the bonding ability between the complex and the sugar, we have studied the bonding properties of the zinc complex and the target chitosan oligosaccharide molecule by ultraviolet spectroscopy, and found that the complex has preferable bonding activity to the chitosan oligosaccharide molecule. This provides an ideal model for the construction of metal coordination molecularly imprinted polymers selectively recognized by chitosan oligosaccharides.

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