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Metal-chelating chitin derivatives via reaction of chitosan with nitrilotriacetic acid

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Abstract

Chitosan was cross-linked to various extents with nitrilotriacetic acid in the presence of a water-soluble carbodiimide in homogeneous solution, and was then fully *N*-acetylated. The structure of the nitrilotriacetic acid residues attached to the chitin backbone was determined by acid–base titration. It was shown that the chitin derivatives contained both iminodiacetate and iminomonoacetate residues which form 1:1-type complexes with copper ions. The chitin gels obtained swelled reversibly with water and behaved like ampholytic cross-linked resins in aqueous solutions of acids and bases. © 1996 Elsevier Science Ltd.

Keywords: Chitin; Chitosan; Nitrilotriacetic acid; Cross-linking; Copper ions

1. Introduction

Chitin, a (1 → 4)-2-acetamido-2-deoxy-β-D-glucan, is the most abundant aminopolysaccharide among the naturally occurring polysaccharides and is believed to be distributed almost as widely as cellulose [1,2]. Chitosan represents partially or fully *N*-deacetylasted chitin. During the last two decades chitin, chitosan, and their derivatives have found numerous applications in medicine, pharmacology, biotechnology, and food technology [3–5]. Among other properties of chitosan, its ability to extract heavy metal ions from aqueous media, arising from its characteristic structure, has been especially investigated [6–17]. It has been shown that the sorption capacity of chitosan, having free amino groups capable of chelating metal ions, is much higher than that of the original

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chitin, and the sorption behaviour of the latter towards heavy metal ions depends on its degree of deacetylation [7,18,19].

Moreover, although a considerable number of studies of the preparation of metal-chelating derivatives of chitosan and cross-linked chitosan gels has so far been carried out [20–32], only a few chitin-based derivatives have been prepared [22,25,33], because of its inherent intractability and very low solubility in aqueous media, in contrast with chitosan.

The work described here arose from the need to prepare a water-soluble chitin having attached residues of iminodiacetic acid, which is a bifunctional analogue of ethylenediaminetetraacetic acid and forms very stable complexes with a wide range of heavy metal ions [34,35]. These complexes being conjugated to chitooligosaccharides, specific for the target biomolecules (proteins, lectins, etc.), could find an application as radiotherapeutic and affinity reagents or sorbents [36]. This article deals with the reaction of chitosan with nitrilotriacetic acid in the presence of a water-soluble carbodiimide; the structures and some characteristic properties of the resulting products are described.

2. Materials and methods

Reagents.—Nitrilotriacetic acid, acetic anhydride, and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide hydrochloride of chemical grade were purchased from Reachim (Moscow) and used without further purification.

Chitosan with a degree of deacetylation of 72% and viscosity-average molecular mass of 300 kDa produced from Kamchatka-peninsula-shelf crab chitin was purchased from the Pacific Ocean Institute of Bioorganic Chemistry (Vladivostok).

Chitosan and chitin gel preparations.—A mixture of chitosan (1 g), nitrilotriacetic acid (0.8 g), and water (50 mL) was stirred at 60 °C during 3 h. To the resulting clear solution was added 1 M NaOH (4 mL). The stirring and heating were continued until full dissolution of a white precipitate occurred. After that the solution was cooled down to 40 °C, and the calculated amount of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide hydrochloride was added. The solution was thoroughly stirred, while heating at 40 °C, until gelation was observed to start, and then the heating was continued without stirring for 1 h. The gel which formed was allowed to cool to ambient temperature and kept for 10–12 h. The resulting gel was thoroughly filtered, and the volume of the filtrate solution was measured. The gel was crushed, then washed with an excess of aq AcOH (5% w/w) and finally with water. The resulting cross-linked chitosan gel was treated with an excess of MeOH–aq 5% AcOH–Ac_2O (4:2:1) for 3 h at room temperature. The swollen cross-linked chitin gel was washed with 0.1 M NaOH, water, 0.1 M HCl, and thoroughly with water.

Copper ions uptake.—The swollen chitin gel (~ 10 mL) was washed with an excess of 1 M aqueous ammonia, then water, and rinsed with 0.1 M $CuSO_4$ (100 mL) for 10–12 h. The resulting product was washed with water, and dried by rinsing with acetone and heating in vacuum at 60 °C. The content of copper was determined by elemental analysis of the xerogel obtained.

Degree of swelling.—The swollen gel was rinsed with an excess of appropriate solution (1 M NaOH, 1 M HCl, or water) overnight, filtered, centrifuged at 3000 *g* for 10 min, and weighed. Then it was rinsed with acetone and dried in vacuum at 60 °C, and the resulting xerogel was weighed. Degree of swelling (DS, g per g of xerogel) was calculated as follows:

$$DS = (m_s - m_d)/m_d,$$

where m_s and m_d are the masses of swollen or dried gel, respectively.

Titrations.—(1) The chitin gel (~ 10 mL) swollen with water was washed with an excess of 1 M NaOH and thoroughly with water. The washed gel was placed in a 100-mL flask and a measured amount of 0.5 M (0.5 mmol/mL) hydrochloric acid (V_{HCl}, mL) was added up to a total volume of 100 mL. After 12 h the gel was filtered and 25 mL of the filtrate were titrated with 0.5 M (0.5 mmol/mL) NaOH (V_{NaOH}, mL), using phenolphthalein as an indicator. The uptake of hydrochloric acid (T_{HCl}, mmol) was calculated as follows:

$$T_{HCl} = 0.5 \times V_{HCl} - 4 \times 0.5 \times V_{NaOH}.$$

(2) The same protocol of titration was carried out to evaluate the uptake of sulfuric acid ($T_{H_2SO_4}$, mmol) using 0.25 M (0.25 mmol/mL) sulfuric acid:

$$T_{H_2SO_4} = 0.25 \times V_{H_2SO_4} - 4 \times 0.5 \times V_{NaOH}.$$

(3) The gel used above was washed with 0.1 M NaOH, water, aq 25% (w/w) acetic acid, and thoroughly with water. Then the gel was placed in a 100-mL flask, and such an amount of 0.5 M aq NaOAc was added to the flask so that the total volume of the mixture comprised 100 mL. After 12 h the gel was filtered and 25 mL of the filtrate were titrated with 0.5 M (0.5 mmol/mL) NaOH solution (V_{NaOH}, mL). The amount of AcOH formed (T_{AcOH}, mmol) was calculated in accordance with following equation:

$$T_{AcOH} = 4 \times 0.5 \times V_{NaOH}.$$

(4) After the titrations the swollen gel was washed with 0.1 M NaOH and an excess of water, then rinsed with acetone, dried in vacuum at 60 °C, and weighed.

3. Results and discussion

The data described here were obtained during the investigation of the reaction of chitosan with nitrilotriacetic acid (NTA) in the presence of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide, a water-soluble carbodiimide (WSC). Seeking for a water-soluble product of the reaction we have found that if chitosan reacted in aqueous solution with NTA taken in the amount of one molar equivalent towards free aminosaccharide units of chitosan, in the presence of more than 0.2 molar equivalent of WSC, the gelation of the solution occurred in some minutes or less. As the reaction developed, the solution was transformed into a rigid, transparent gel. The gel then decreased in volume and some of the solvent separated because of macrosyneresis (Fig. 1) depending on the WSC/NTA ratio. An increase of the ratio enhanced the syneresis, but the latter reached

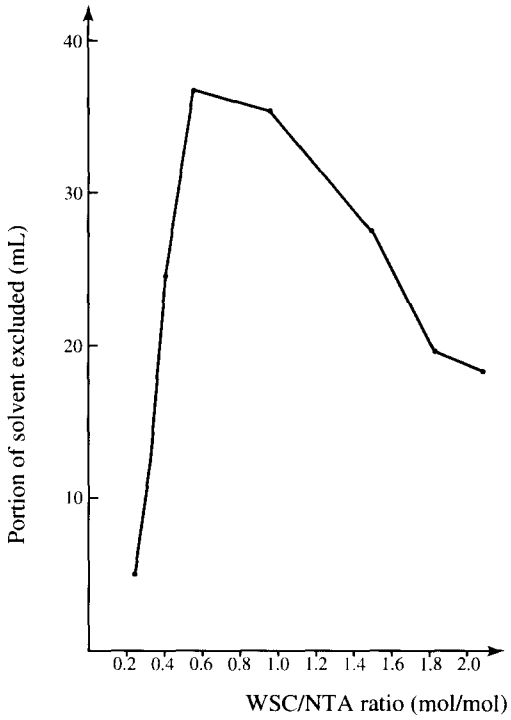


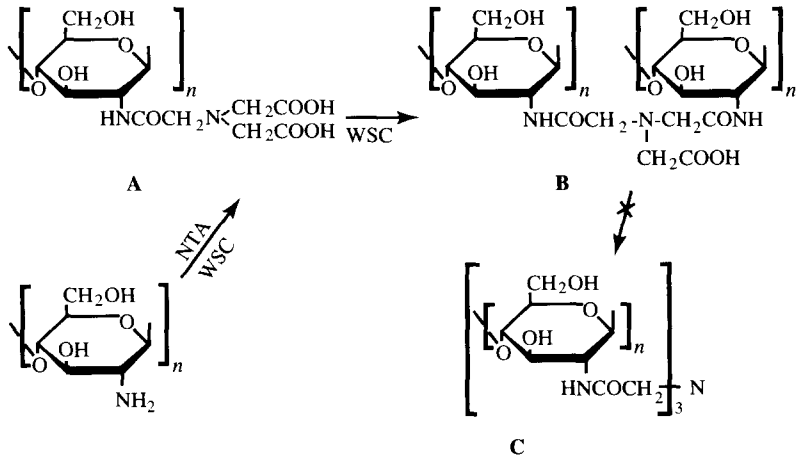
Fig. 1. Dependence of the portion of excluded solvent on WSC/NTA ratio.

a maximum at 0.5–0.6 mol of WSC per mol of NTA. A further rise in the WSC/NTA ratio reduced the syneresis.

This phenomenon seems to be closely related to the swelling behaviour of the macronet isoporous highly cross-linked polystyrene which is obtained by the cross-linking of linear polystyrene with bifunctional agents in the presence of a large amount of a thermodynamically 'good solvent' [37]. This behaviour of such a hydrophilic polymer as chitosan suggests that it might be possible to prepare macronet isoporous hydrophilic polymers having the property of swelling with both solvating and nonsolvating solvents, like macronet isoporous polystyrene. However, this supposition requires some additional experiments which will be published later.

The chitosan polymer molecule contains free amino groups and only these are affected by the cross-linking reaction with NTA under the described conditions. In contrast, NTA has three carboxyl groups which in principle are able to react with amino groups of chitosan, so that three possible derivatives of chitosan might be formed.

In order to simplify identification of the reaction products, which could contain one or two carboxyl groups, imino groups of NTA residues, and free amino groups of chitosan not affected by reaction with NTA, the amino groups were blocked by Hirano's method of exhaustive *N*-acetylation using an excess of acetic anhydride [38]. The corresponding chitin gel derivatives were thus obtained. The latter gels showed no



Scheme 1. Chemical structures of NTA-residues and chitin derivatives.

reactivity towards 2-hydroxybenzaldehyde, indicating the absence of free primary amino groups.

It seems obvious that the relation between the amounts of different NTA residues attached to the chitin molecule depends on the amount of WSC used in the reaction, so that an increase of the WSC/NTA ratio must lead to products differing in the relative amounts of **A**-, **B**-, and **C**-type residues of NTA, as shown in Scheme 1. The content of different NTA residues in the chitin gel obtained was evaluated using acid–base titration of the reaction products. The method was based on a discrepancy in the amounts of hydrochloric and sulfuric acids absorbed by the same sample which contained the cation-exchange groups in Na^+-form and the anion-exchange ones in HO^--form, as shown in the reactions (1a)–(1c) (Scheme 2).

The total consumption of sulfuric acid ($T_{H_2SO_4}$, mmol) in the reactions (1a)–(1c) was calculated in accordance with eq (1):

$$T_{H_2SO_4} = 2\mathbf{a} + 3/2\mathbf{b} + \mathbf{c}. \quad (1)$$

Titration of the attached NTA residues with hydrochloric acid was carried out based on the reactions (2a)–(2c) (Scheme 2). The total consumption of hydrochloric acid (T_{HCL}, mmol) used for titration of the same sample of chitin gel was calculated as follows:

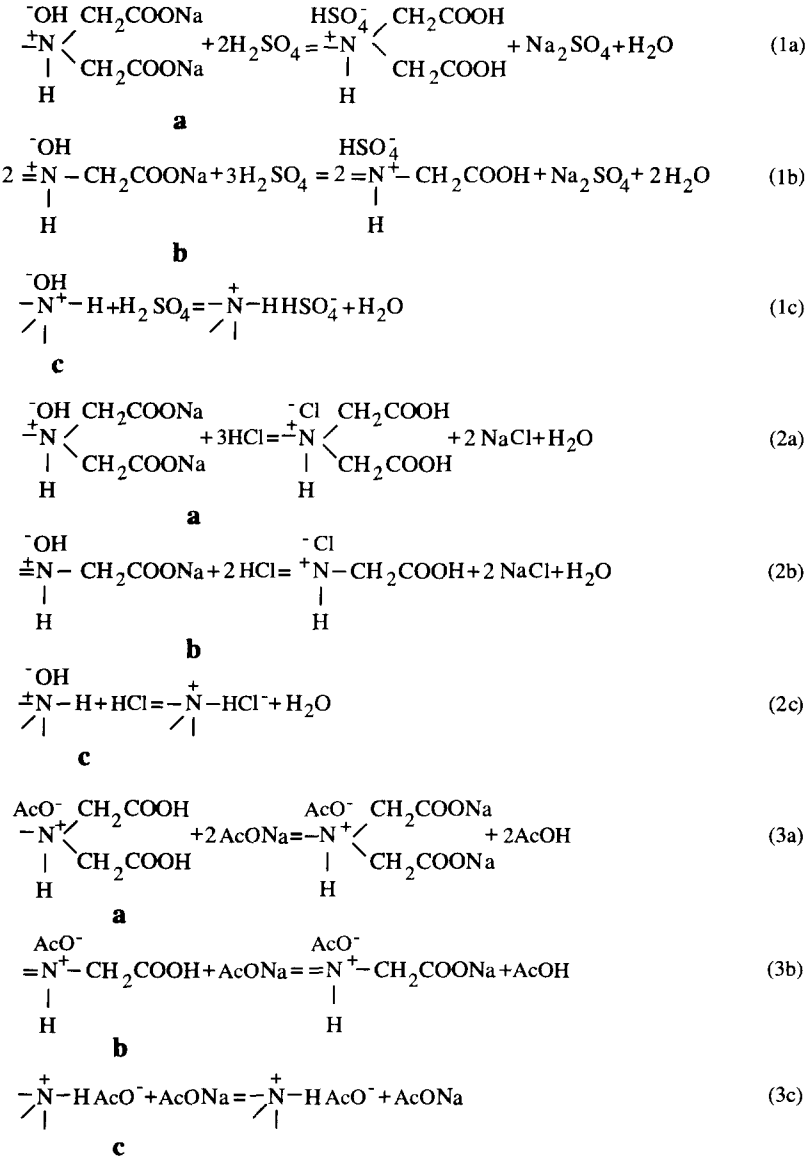
$$T_{HCl} = 3\mathbf{a} + 2\mathbf{b} + \mathbf{c}. \quad (2)$$

The amount of acetic acid (T_{AcOH}, mmol) formed in the reactions (3a)–(3c) (Scheme 2) of the same specimen with NaOAc was determined in accordance with eq (3):

$$T_{AcOH} = 2\mathbf{a} + \mathbf{b}. \quad (3)$$

The solution of the three equations allowed determination of the content of NTA residues attached to each sample of the reaction products and the structures of the chitin gel obtained (Table 1).

In accordance with the results of titration procedures, presence of **C**-residues was not



Scheme 2. Scheme of titration of the NTA-residues: **a**-, **b**-, **c**-content (mmol/g) of the corresponding **A**-, **B**-, **C**-residues.

found, suggesting that NTA had not reacted as a tri-functional cross-linking agent, probably because of large steric hindrance caused by the three polymer chains.

On the basis of the data obtained, the degree of cross-linking of each reaction product (DC, %), as a bridge fraction content (%) versus the total number of network structural elements, was calculated using an equation suggested previously [39].

Table 1
Composition of the reaction products and their capacity towards copper ions

WSC/NTA ratio (mol/mol)	Content of NTA residues (mmol/g)		Copper content		Degree of cross-linking (%)
	A-residues	**B**-residues	(%)	(mmol/g)	
0.25	0.60	0.15	4.75	0.74	3.3
0.50	0.55	0.40	5.81	0.91	9.5
1.00	0.35	1.10	8.86	1.38	31.5
1.80	0.10	1.35	9.17	1.43	41.4
2.10	0.10	1.45	9.81	1.53	46.2

In the first step of the reaction when chitosan reacted with NTA in the presence of WSC, the activated NTA participated in the reaction as a monofunctional reagent, so that iminodiacetate residues were formed. If the reaction developed in the presence of an enhanced amount of WSC, some carboxyl groups of iminodiacetate residues were activated, and then they reacted with amino groups of chitosan and cross-linked the polymer chains. If one or more molar equivalent of WSC per mol of NTA was used (Table 1), the most highly cross-linked gels were found to form.

As the cross-linking reaction developed, the swelling properties of NTA–chitin gels altered (Fig. 2). These gels swelled in aqueous media and behaved like ampholytic

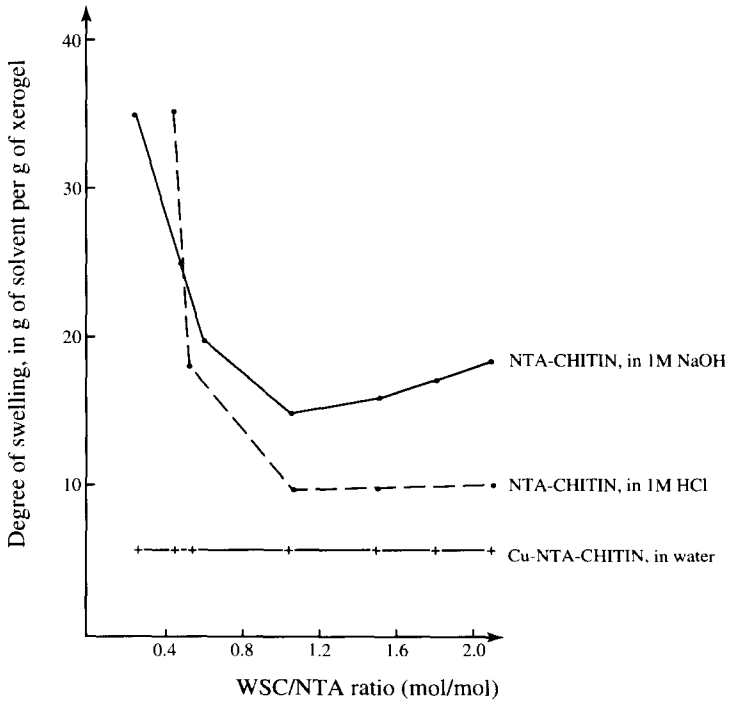


Fig. 2. Swelling properties of NTA–chitin (•) and Cu–NTA–chitin (+).

ion-exchange resins in aqueous solutions of acid and bases. From copper(II) sulfate solution, the modified chitin gels take up copper ions (Table 1), but the latter seem to cause an extra cross-linking of the polymer network so that the degree of swelling of Cu–NTA–chitin gels was significantly less than that of the former modified chitin gel. The complexing capacity of the modified chitin gel reached its maximum at about 1.4 mmol Cu(II)-ions per gram of xerogel which is unlikely to be enhanced to a large extent because of the development of cross-linking. It should be noted that fully *N*-acetylated (under the conditions described) chitosan (i.e., chitin) does not adsorb any copper ions as determined by elemental analysis.

One important conclusion can be made from a comparison of the amount of copper ions bound and the content of NTA residues attached, namely that only 1:1-type copper–ligand complexes are formed during the copper ion uptake by the polymer network.

Thus, the reaction of chitosan with NTA in the presence of a large amount of WSC leads to the formation of cross-linked chitosan derivatives bearing both iminodiacetate and iminomonoacetate residues, able to take up copper ions. The products of the reaction may possibly be useful as chromatography supports for lectin separations, enzyme immobilization, and water treatment.

Acknowledgements

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