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Carbohydrate Research 342 (2007) 1329–1332
Carbohydrate RESEARCH

Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan

Zhanyong Guo,[a,b] Ronge Xing,[a] Song Liu,[a,b] Zhimei Zhong,[a,b] Xia Ji,[a,b] Lin Wang[a,b] and Pengcheng Li[a,*]

[a]*Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*
[b]*Graduate University of the Chinese Academy of Sciences, Beijing 100039, China*

Received 4 December 2006; received in revised form 6 April 2007; accepted 15 April 2007
Available online 24 April 2007

Abstract—Schiff bases of chitosan, N-substituted chitosan, and quaternized chitosan were synthesized and their antifungal properties were analyzed against *Botrytis cinerea* Pers. (*B. cinerea* pers.) and *Colletotrichum lagenarium* (Pass) Ell.et halst (*C. lagenarium* (Pass) Ell.et halst) based on the method of D. Jasso de Rodríguez and co-workers. The results showed that quaternized chitosan had better inhibitory properties than chitosan, Schiff bases of chitosan, and N-substituted chitosan.

Keywords: Schiff bases of chitosan; N-Substituted chitosan; Quaternized chitosan; Antifungal activity

1. Introduction

Chitosan is one of the most abundant naturally occurring amino-polysaccharides and it has attracted attention because of its unique physiochemical characteristics and biological activities.[1–3] Among various bioactive properties of chitosan, its antifungal activity has received considerable interest due to problems associated with fungicidal agents.[4–6] El Ghaouth and co-workers have reported that chitosan could inhibit the growth of *Alternaria alternate*, *Botrytis cinerea*, *Colletrotichum gloeosporioides*, and *Rhizopus stolonifer* and that the inhibitory index was affected by the concentration of chitosan.[7] The growth of fungi such as *F. oxysporum*, *R. stolonifer*, *Penicillium digitatum*, and *C. gloeosporioides* can be inhibited completely by chitosan at a concentration of 3%.[8,9] With regard to the antifungal activity of chitosan, researchers have focused on the effects of molecular weight and the degree of deacetylation. However, comparatively less work has been reported on the antifungal activities of chitosan derivatives.

It is proposed that the antifungal activity of chitosan is due to its polycationic properties.[10] In the different chitosan derivatives, there are different amido groups such as primary amines ($R–NH_2$) in chitosan (after protonation at physiological pH), imines (–C=N) in Schiff bases of chitosan, secondary amines ($R_2–NH$) in N-substituted chitosan and quaternary ammonium (R_3N^+) in quaternized chitosan. Quaternized chitosan can be synthesized from chitosan step-by-step according to the above-mentioned order. For these four kinds of chitosan derivatives, only the antifungal activity of chitosan has been investigated earlier. To investigate the antifungal activity of the other kinds of chitosan derivatives, we synthesized Schiff bases of chitosan, N-substituted chitosan, and quaternized chitosan and measured their antifungal activities against *Botrytis cinerea* Pers. (*B. cinerea* Pers) and *Colletotrichum lagenarium* (Pass) Ell.et halst (*C. lagenarium* (Pass) Ell.et halst).

2. Materials and methods

2.1. Materials

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). The degree of deacetylation

* Corresponding author. Tel.: +86 532 82898707; fax: +86 532 82968951; e-mail addresses: qdioqdio@yahoo.com.cn; pcli@ms.qdio.ac.cn

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doi:10.1016/j.carres.2007.04.006

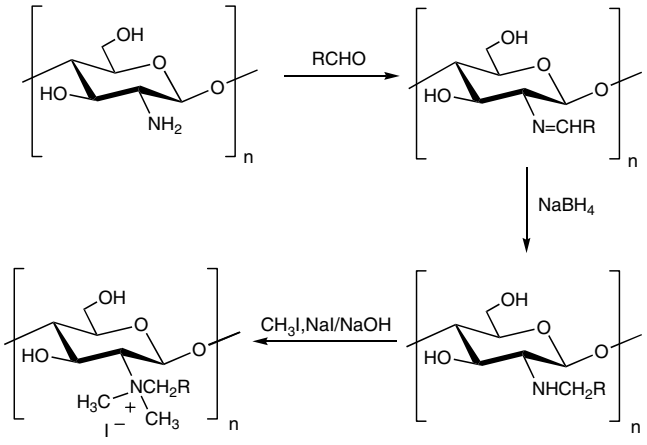
was 97% and the viscosity average-molecular weight was 7.6×10^3. Benzaldehyde, salicylaldehyde, sodium iodide (anhyd), *N*-methyl-2-pyrrolidone (NMP), sodium borohydride ($NaBH_4$), and methyl iodide (CH_3I) were purchased from Sigma–Aldrich Chemical Co. Elemental analyses (C, H, N) were performed on a Carlo-Erba 1106 elemental analyzer. FTIR spectra were measured on a Nicolet Magne-Avatar 360 on samples prepared as KBr disks. Other reagents were of analytical grade and were used without further purification.

2.2. Synthesis of chitosan derivatives

2.2.1. Synthesis of Schiff bases of chitosan (As, Bs). Schiff bases of chitosan (As and Bs) were synthesized according to Scheme 1. Chitosan (3 g) was dissolved in 100 mL H_2O at room temperature, and 0.05 mol aldehyde (benzaldehyde or salicylaldehyde) was added with stirring. After 4 h, the products were precipitated using excess acetone and filtered. The unreacted aldehydes were extracted in a Soxhlet apparatus with ethanol and ether for 2 days. The Schiff bases of chitosan were dried at 60 °C for 24 h.

2.2.2. Synthesis of N-substituted chitosan (An, Bn). N-Substituted chitosan and quaternized chitosan were synthesized according to a previous method.[11,12] To prepare the N-substituted derivatives (An, Bn), chitosan (3 g) was dissolved in 100 mL H_2O at room temperature, and 0.05 mol aldehyde (benzaldehyde or salicylaldehyde) was added with stirring. After 2 h, 10% $NaBH_4$ (0.15 mol) was added and the reaction was continued for 2 h. The products were precipitated in acetone and filtered and the N-substituted chitosan derivatives (An, Bn) were dried at 60 °C for 24 h.

2.2.3. Synthesis of quaternized chitosan (Aq, Bq). To prepare the quaternized chitosan derivatives, N-substituted chitosan (1 g) was dispersed in 50 mL NMP for 12 h at room temperature. Then, 0.12 mL NaOH (1M), 1.5 g NaI and 4 mL CH_3I were added to this mixture, and each reaction was carried out with stirring at 50 °C for 20 h. The products were precipitated by the addition of excess acetone and filtered. The quaternized chitosan derivatives (Aq, Bq) were dried at 60 °C for 24 h (Scheme 1).



Scheme 1. Synthetic pathway for preparation of the chitosan derivatives; (A) R = phenyl; (B) R = 2-hydroxylphenyl.

2.3. Antifungal essays

Antifungal assays were performed based on the method of Jasso de Rodríguez and co-workers.[13] Different concentrations of chitosan, Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan derivatives (100, 500, and 1000 ppm) were, respectively, added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27 °C after transferring the mycelium of fungi. When the mycelium of fungi reached the edges of the control plate (without added samples), the antifungal index was calculated as follows:

$$\text{Antifungal index } (\%) = (1 - D_a/D_b) \times 100$$

where D_a is the diameter of the growth zone in the test plates and D_b is the diameter of the growth zone in the control plate.

Each experiment was performed three times, and the data were averaged. The Scheme method was used to evaluate the differences in antifungal index in antifungal tests. Results with $P < 0.05$ were considered statistically significant.[14]

3. Results and discussion

3.1. Structure and physicochemical characteristics of chitosan derivatives

The elemental analysis results, yield, and the grafted degree of Schiff bases of chitosan, N-substitution chitosan and quaternization chitosan are shown in Table 1.

Table 1. The elemental analysis results, yield, and the grafted degree of chitosan derivatives

Compounds	Yield[a] (%)	Elemental analysis (%)			Grafted degree of chitosan derivatives (%)
		C	N	H	
As	68.0	59.95	6.08	6.14	78.5
Bs	71.3	57.22	5.64	5.80	81.7
An	65.3	60.88	5.71	6.78	90.8
Bn	68.5	57.33	5.49	6.40	87.5
Aq	36.2	44.88	3.71	5.46	86.3
Bq	40.6	43.55	3.65	5.36	80.5

As, Bs: Schiff bases of chitosan as shown in Scheme 1.
An, Bn: N-substituted chitosan as shown in Scheme 1.
Aq, Bq: quaternized chitosan as shown in Scheme 1.
[a] Yield is the ratio of chitosan derivatives and added chitosan.

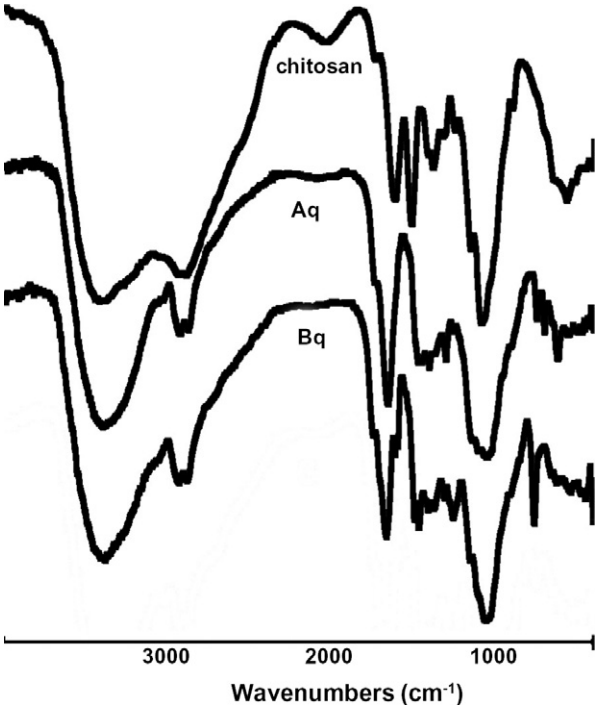


Figure 1. The IR spectra data of chitosan and quaternized chitosan derivatives.

The IR spectral data of the quaternized chitosan derivatives are shown in Figure 1. The IR spectrum of chitosan shows peaks assigned to the saccharide structure at 895 and 1155 cm^{-1}. The characteristic peaks for the amine (N–H) vibration deformation appear at 1618 cm^{-1} for chitosan. After quaternization, new peaks appear at about 1660 cm^{-1}, which were assigned to the quaternary ammonium salt.[11] There are peaks at about 1415–1430 cm^{-1}, which were assigned to the characteristic absorption of N–CH_3.[11] Moreover, the spectra for Aq and Bq have peaks at about 1400, 1470, 1500, and 1580 cm^{-1} corresponding to the phenyl groups.[15] These results demonstrate that the quaternized chitosan derivatives were obtained.

3.2. Antifungal activity

The antifungal activity of chitosan, Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan against *B. cinerea* Pers. and *C. lagenarium* (Pass) Ell.et halst are shown in Figures 2 and 3. As can be seen in Figure 2, chitosan inhibits the growth of *B. cinerea* Pers and the inhibitory index is 45.4% at 1000 ppm. Compared with the antifungal activity of chitosan, the Schiff bases of chitosan (As, Bs) and the N-substituted chitosan derivatives (An, Bn) have a slight activity against *B. cinerea* Pers., and the inhibitory indices are 26.8%, 33.5%, 39.3%, and 32.3% at 1000 ppm, respectively. However, quaternized chitosan derivatives (Aq, Bq) have better

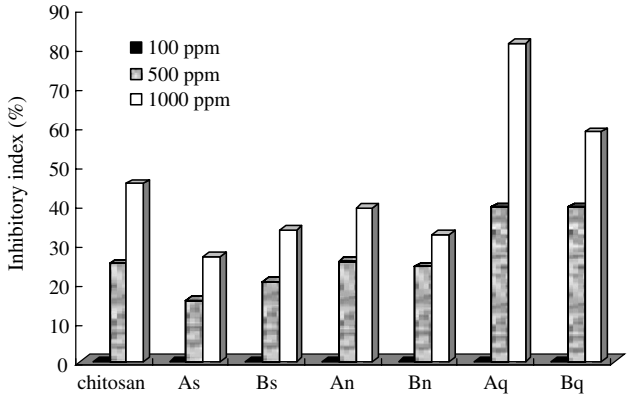


Figure 2. The antifungal activity of chitosan and chitosan derivatives against *B. cinerea* Pers.

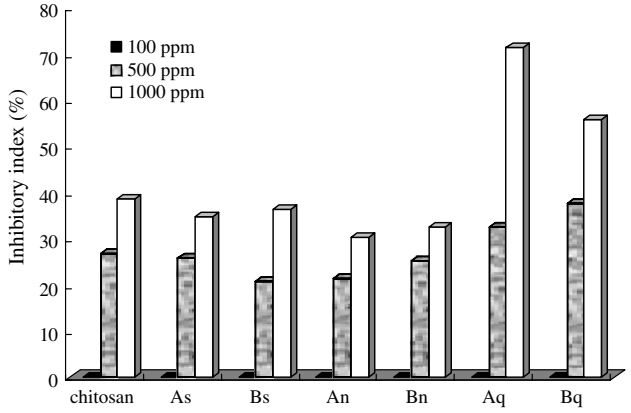


Figure 3. The antifungal activity of chitosan and chitosan derivatives against *C. lagenarium* (Pass) Ell.et halst.

antifungal activity against *B. cinerea* Per. than chitosan, Schiff bases of chitosan, and N-substituted chitosan derivatives, and the inhibitory indices of Aq and Bq are 81.2% and 58.6% at 1000 ppm, respectively.

As shown in Figure 3, the inhibitory index of chitosan against *C. lagenarium* (Pass) Ell.et halst is 38.5% at 1000 ppm. Similar to the antifungal activity against *B. cinerea* Pers., the quaternized chitosan derivatives have better activity than those of chitosan, Schiff bases of chitosan, and N-substituted chitosan derivatives. The inhibitory indices were 71.5% for Aq and 55.8% for Bq at 1000 ppm.

The conceivable mechanism for the antimicrobial action of chitosan is that the ammonium groups interact with the anionic groups on the microbial cell surface, which forms a layer around the cell and prevents nutrients from entering.[4] Bearing this in mind, it is not difficult to conclude that this activity should be affected by the ammonium ion density on chitosan. Similarly, Jia reported that quaternary ammonium derivatives of chitosan had better antibacterial activities against *Escherichia coli* than chitosan, which was attributed to the high charge density of quaternized chitosan.[11] We propose

that the antifungal activity of the quaternized chitosan derivatives could also be caused by the cation groups in these quaternized derivatives. As shown in Scheme 1, the cationic charge of quaternized chitosan is higher than that of chitosan, Schiff bases of chitosan, and N-substituted chitosan and thus has the best antifungal activity.

4. Conclusions

There are growing environmental problems caused by fungicides, especially chemical products. It is imperative under this situation to explore new chemical fungicides, which not only can control the pathogenic diseases of crop but which are also biodegradable and environmentally friendly.

As one of the most abundant natural biopolymers, chitosan has shown its nontoxic nature. It has been proven that the potential antifungal activity of chitosan, and the application of chitosan can increase the yield of crops at about 10–30%.[16] As a biodegradable aminopolysaccharide, chitosan and its derivatives may be further explored in the field of agriculture.

Acknowledgement

Qingdao Government Scientific and Technological Program financially supported this work: Bidding Subject (2005-9).

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