Polymer Bulletin 60, 449–455 (2008)
DOI 10.1007/s00289-007-0877-3

Polymer Bulletin

Influence of trimethylsilylation and detrimethylsilylation on the molecular weight of chitin: Evaluation of viscometry and gel permeation chromatography for molecular weight determination

Kazuhiro Sugita[1], Jin Yang[1], Manabu Shimojoh[2], and Keisuke Kurita[1] (✉)

[1]Department of Materials and Life Science, Faculty of Science and Technology, Seikei University, Musashino-shi, Tokyo 180-8633, Japan
[2]Research and Development Department, Toyo Suisan Kaisha, Ltd., Kohnan, Minato-ku, Tokyo 108-8501, Japan
E-mail: kurita@st.seikei.ac.jp

Received: 21 September 2007 / Revised version: 22 November 2007 / Accepted: 27 November 2007
Published online: 7 December 2007 –

Summary

In view of the high potential of trimethylsilylated chitin as a reaction precursor, the influence of trimethylsilylation and detrimethylsilylation on the molecular weight characteristics has been studied. Chitin was trimethylsilylated, and the product was detrimethylsilylated to regenerate chitin. The molecular weights of the original and regenerated chitins were determined by viscometry and GPC. The viscosity measurements, with either an Ubbelohde or a rotational viscometer, gave highly reproducible values, which were quite similar to each other. The molecular weight of the regenerated chitin was found to be a little over a half that of the original chitin despite after the silylation-desilylation reactions. GPC also supported a similarly low extent of main chain scission. These results indicate that the silylation-desilylation reactions are reproducible without a significant damage to the chitin main chain, and both viscometry and GPC have proved reliable in elucidating the molecular weight characteristics. Trimethylsilylation of chitin can thus be conveniently used as a key intermediate for further controlled modification reactions to prepare well-defined derivatives.

Introduction

Chitin and cellulose are essential structural polysaccharides found mainly in lower animals and plants, respectively, and are the most abundant organic substances on earth. Though cellulose is used in various fields, chitin remains an extremely underutilized biopolymer primarily because of the inherent intractable nature ascribable to the strong intermolecular forces. Recent studies on chitin and the deacetylated form, chitosan, show that these amino polysaccharides exhibit quite unique properties including non-toxicity, film- and fiber-formation, adsorption of metal ions, coagulation of suspensions, and distinctive biological activities such as biocompatibility, antimicrobial activity, immunoadjuvant and antitumor activity,

hemostatic activity, acceleration of wound-healing, hypolipidemic activity, activation of inflammatory cells, and promotion of plant growth [1].
These features suggest the high potential of chitin in developing advanced functional materials through structural modifications. Various modification reactions have thus been attempted [2], but the preparation of well-defined derivatives is generally difficult owing to the lack in solubility.
Introduction of some bulky groups are useful as protective or reactivity enhancing groups such as tosyl [3], phthaloyl [4], and triphenylmethyl groups [4,5]. In the course of these reactions, chitin and chitosan molecules would inevitably be degraded to various extents, and it is critical to elucidate the molecular weight for discussing the structure-property relationships. However, the stability of the polysaccharide main chains against the modification reactions has not been studied.
Trimethylsilylation has been found convenient as another tool to render solubility in common organic solvents as well as substantial reactivity as reported previously [6]. To further expand the scope of the utility of trimethylsilylated chitin as an organosoluble precursor for a wide variety of structural modifications, it is indispensable to elucidate the molecular weight change by reliable methods during the trimethylsilylation and subsequent detrimethylsilylation. In this paper, we report the silylation-desilylation of chitin and molecular weight measurements of chitin to discuss the influence of these reactions on the molecular weight characteristic.

Experimental

General

IR spectra were recorded on a Shimadzu FTIR-8900 instrument by the KBr method. ^{1}H NMR spectra were taken with a JEOL JNM-LA400D in actone-d_6 at ambient temperature. Elemental analysis was performed with a Perkin Elmer 2400 II. The degree of deacetylation (dd) was determined by conductometric titration with a DKK·TOA conductivity meter CM-20J. All the chemicals were of reagent grade and used without further purification. Solvents were purified in usual manners and stored over molecular sieves.

Chitin

β-Chitin was isolated from squid pens [7], and the dd was 0.11 as determined by conductometric titration. It was pulverized to 0.5 mm mesh with an ultra-centrifugal mill Retsch ZM-1, and treated with acetic anhydride in methanol at room temperature to selectively acetylated the free amino groups by the reported method [8]. The dd value of the product was 0.0.

Trimethylsilylation

The above-obtained chitin was treated with a mixture of hexamethyldisilazane and chlorotrimethylsilane in pyridine, and the product was isolated in water as described elsewhere [6]. The structure of the product was confirmed by IR, NMR, and elemental analysis. All the data supported the degree of substitution (ds) for the trimethylsilyl group to be 2.0. IR (KBr): ν 3328 (N-H), 2958 (C-H), 1664 (amide I), 1540 (amide II), 1251 (Si-CH_3), 1150-1000 (pyranose), and 842 cm^{-1} (Si-CH_3).

Anal. Calcd for $C_{14}H_{29}NO_5Si_2 \cdot 0.2H_2O$: C, 47.88; H, 8.44; N, 3.99. Found: C, 47.97; H, 8.59; N, 4.00.

Detrimethylsilylation

Trimethylsilylated chitin (ds 2.0, 0.300 g) was added to 100 mL of 10% aqueous acetic acid, and the mixture was stirred at room temperature for 1 h. The product was filtered, washed with deionized water thoroughly, and dried. The IR spectrum was identical with that of the original chitin. The yield of regenerated chitin was 0.167 g (95%). IR (KBr): ν 3450-3270 (OH), 1659 (amide I), and 1558 cm^{-1} (amide II).

Viscosity measurements

To 950 mL of *N*,*N*-dimethylacetamide (DMAc) was added 50 g of dried lithium chloride, and the mixture was stirred at 50°C to give a 5% DMAc/LiCl solution. Chitin (50 mg) was dissolved in 40 mL of DMAc/LiCl at 50°C, and the solution was made 50 mL in a volumetric flask to give a 0.1% solution. Based on this solution, 0.05, 0.025, 0.02, and 0.0125% solutions were prepared and equilibrated in a refrigerator for one week prior to viscosity measurements.
The viscosities were measured with an Ubbelohde viscometer (Shibata, 2613-001) and a rotational viscometer (Tokyo Keiki, Visconic ED) at 25°C to determine the intrinsic viscosities, from which the molecular weights were calculated using a Mark-Houwink equation:

$$[\eta] = K \cdot M\mathrm{v}^{a}$$

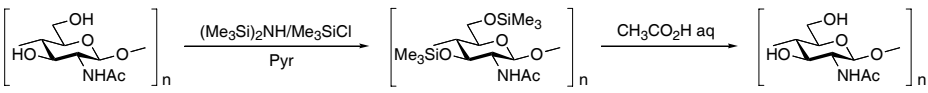
where $K = 2.1 \times 10^{-4}$ and $a = 0.88$ for chitin in 5% DMAc/LiCl [9].

GPC

GPC was performed with 0.5% chitin solutions in 5% DMAc/LiCl using a Shimadzu LC-10AVP/LC-10ADVP instrument equipped with an RI detector (RID-10A), degasser (DGU-12A), column oven (CTO-10AVP), and Shodex columns (GPC KD-G + GPC KD-806M) at 40°C. The molecular weights were calibrated with pullulan standards.

Results and discussion

Trimethylsilylation of chitin and the subsequent detrimethylsilylation were studied first to establish reproducibility of the reactions (Scheme 1), using fully *N*-acetylated structurally uniform chitin to avoid ambiguity in analyzing the data. Compared to α-chitin, β-chitin can be trimethylsilylated facilely [6], and hence the influence of trimethylsilylation and detrimethylsilylation on the molecular weight was examined with β-chitin.



Scheme 1. Trimethylsilylation of chitin and detrimethylsilylation

Trimethylsilylation

The reaction of β-chitin with hexamethyldisilazane/chlorotrimethylsilane in pyridine gave a fully substituted derivative that was isolated as a cotton-like white fluffy material. The structure was confirmed by IR and NMR spectroscopies and elemental analysis, and the ds was 2.0 for the silyl group as calculated from NMR and elemental analysis data. The reaction was quite reproducible under controlled conditions, and the yields were in the range 80-88% starting from 0.5 g of chitin in the three separate preparations. The resulting three kinds of trimethylsilylated chitin samples were used in the subsequent desilylation reaction.

Detrimethylsilylation

The trimethylsilyl groups of chitin were labile under acidic conditions and easily removed in 10% aqueous acetic acid at room temperature to regenerate chitin. The typical absorption bands due to trimethylsilyl at 1251 and 842 cm^{-1} disappeared completely, and an OH band was observed at around 3450-3270 cm^{-1}. The reactions were reproducible and quantitative judging from the results for the three kinds of trimethylsilylated chitins, and the recovery yields were 92-95%.

Molecular weight measurements

Both the original and regenerated chitins were soluble in 5% DMAc/LiCl, but the original chitin went into solution more easily than the regenerated one; the former gave a clear solution in 3 h at 50°C to give a 0.1% solution, while the latter in 24 h. This is probably because the regenerated chitin was no more a fluffy material.

1. With an Ubbelohde viscometer

Viscosities of the above prepared chitin solutions were determined with an Ubbelohde viscometer, and the intrinsic viscosities [η] were calculated by extrapolating the reduced and inherent viscosities (η_{red} and η_{inh}) to the zero concentration. Figure 1 shows typical examples of the measurements for one of the three regenerated chitins. As evidenced in this figure, the intrinsic viscosities derived from η_{red} and η_{inh} were almost the same. The measurements for each sample were done two times, and the three kinds of regenerated chitins thus gave six data for each η_{red} and η_{inh}. The values were quite close to each other and averaged.

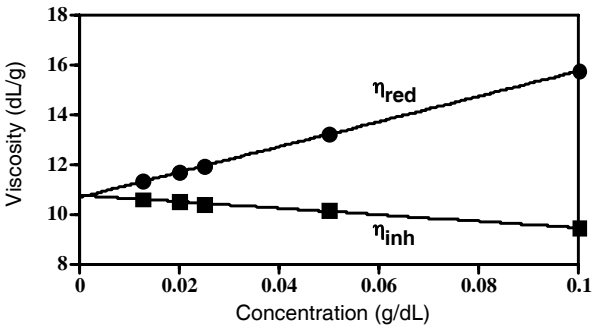


Fig 1. Viscosity measurements of a regenerated chitin with an Ubbelohde viscometer.

The viscosity-average molecular weights (Mv) were calculated using a Mark-Houwink equation, and as summarized in Table 1, the molecular weight of regenerated chitin was a little more than a half that of the original chitin. This indicates that although a sequence of trimethylsilylation and detrimethylsilylation reactions may have cleaved the chitin main chain, the extent is not significant under these reaction conditions.

Table 1. Molecular weight measurements with an Ubbelohde viscometer

		$[\eta]^a$	$10^{-4}M\mathrm{v}^b$
Original chitin	(based on η_{red})	16.74[c]	37.15[c]
	(based on η_{inh})	16.78[c]	37.24[c]
Regenerated chitin	(based on η_{red})	10.30 ± 0.35[d]	21.40 ± 0.82[d]
	(based on η_{inh})	10.47 ± 0.29[d]	21.80 ± 0.69[d]

[a]Intrinsic viscosity (dL/g).
[b]Viscosity-average molecular weight (g/mol) calculated from $[\eta] = 2.1\times10^{-4}\cdot M\mathrm{v}^{0.88}$.
[c]Average of two runs.
[d]Average and standard deviation values of six data obtained by two runs for each of the three separate samples.

2. With a rotational viscometer

The viscosities were also determined with a rotational viscometer, and as shown by typical examples in Figure 2 for a regenerated chitin, the intrinsic viscosities could be obtained from η_{red} and η_{inh} similarly. The viscosity data were again very reproducible for both the original and regenerated chitins. The Mv values were calculated from the Mark-Houwink equation, and the results are summarized in Table 2. The values are quite close to those obtained with an Ubbelohde viscometer.

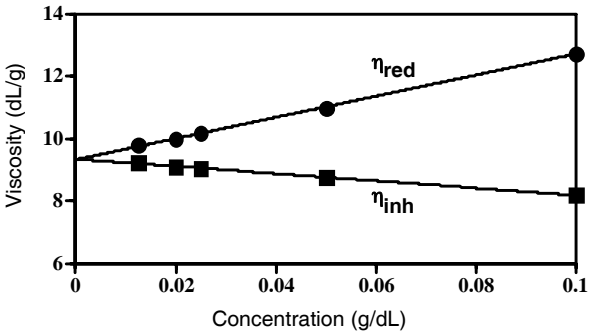


Fig 2. Viscosity measurements of a regenerated chitin with a rotational viscometer.

3. With a gel permeation chromatograph

Chitin solutions were subjected to GPC, pullulans being used as standards. As expected, three kinds of regenerated chitins gave similar GPC profiles (Figure 3), indicating the reproducible process of trimethylsilylation and detrimethylsilylation, and the molecular weight data are listed in Table 3. The number-average and weight-average molecular weights (Mn and Mw) of the regenerated chitins were again about half values of those of the original chitin. The polydispersity index (Mw/Mn) values of

the regenerated chitins were almost identical with that of the original chitin. These data strongly suggest that the degradation occurred only slightly during the silylation and desilylation process, as implied by the viscosity measurements.

Table 2. Molecular weight measurements with a rotational viscometer

		$[\eta]^a$	$10^{-4}Mv^b$
Original chitin	(based on η_{red})	16.17[c]	35.51[c]
	(based on η_{inh})	16.16[c]	35.96[c]
Regenerated chitin	(based on η_{red})	9.60 ± 0.38[d]	19.75 ± 0.90[d]
	(based on η_{inh})	9.61 ± 0.40[d]	19.77 ± 0.94[d]

[a]Intrinsic viscosity (dL/g).
[b]Viscosity-average molecular weight (g/mol) calculated from $[\eta] = 2.1\times10^{-4}\cdot Mv^{0.88}$.
[c]Average of two runs.
[d]Average and standard deviation values of six data obtained by two runs for each of the three separate samples.

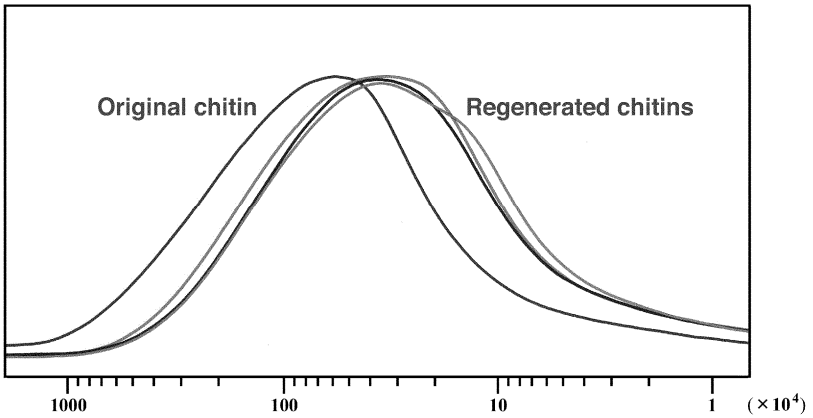


Fig 3. GPC profiles of the original chitin and three kinds of regenerated chitins.

Table 3. Molecular weight measurements by GPC

	$10^{-4}Mn^a$	$10^{-4}Mw^b$	Mw/Mn
Original chitin	22.00	112.80	5.12
Regenerated chitin	11.67 ± 0.87[c]	59.93 ± 2.67[c]	5.15 ± 0.17[c]

[a]Number-average molecular weight (g/mol).
[b]Weight-average molecular weight (g/mol).
[c]Average and standard deviation values for the three separate samples.

Conclusions

Both the trimethylsilylation and detrimethylsilylation proceeded quite reproducibly and quantitatively despite the generally recognized difficulty in modification reactions of chitin. The viscosity measurements with an Ubbelohde viscometer and a rotational viscometer were quite dependable, and moreover, almost similar values were obtained by these two methods. The latter method, however, requires less than 2 mL of solution

compared to about 15 mL for the former, and can be conveniently applied to even a small amount of sample. GPC was also available with a proper column for polar solvents such as DMAc/LiCl. All these methods were confirmed quite reliable and convenient to determine the molecular weight characteristics of chitin.

The molecular weights of the regenerated chitins were revealed to be a little over a half of those of the original chitin independent of the measuring tools, indicating that the trimethylsilylation and detrimethylsilylation are possible without a significant cleavage of the chitin main chain under appropriate conditions. Trimethylsilylation and detrimethylsilylation have thus proved to be clean reactions, and trimethylsilylated chitin will be useful as a key intermediate for various controlled modification reactions to prepare structurally well-defined derivatives, possibly leading to the advanced materials based on this unutilized but important biomass resource.

Acknowledgements. This work was partially supported by "High-Tech Research Center" Project for Private Universities: matching fund subsidy from MEXT, 2004-2008.

References

1. Uragami T, Kurita K, Fukamizo T (eds) (2001) Chitin and chitosan in life science. Kodansha Scientific, Tokyo; Uragami T, Tokura S (eds) (2006) Material science of chitin and chitosan. Kodansha Scientific, Tokyo; Kurita K (2006) Marine Biotechnol 8:203; Domard A, Guibal E, Vårum KM (eds) (2007) Advances in chitin science Vol. 9. 10th ICCC-EUCHIS'06, Montpellier
2. Roberts GAF (1992) Chitin Chemistry Macmillan, London; Kurita K (1997) Chitin and chitosan derivatives. In: Arshady R (ed) Desk reference of functional polymers: Syntheses and applications. American Chemical Society, Washington, D. C (pp. 239–259); Kurita K (2001) Prog Polym Sci 26:1921; ; Kurita K (2006) Introduction of biologically active branches through controlled modification reactions of chitin and chitosan. In: Uragami T, Tokura S (eds) Material science of chitin and chitosan. Kodansha Scientific, Tokyo
3. Kurita K, Inoue S, Nishimura S (1991) J Polym Sci Part A Polym Chem 29:937; Kurita K, Yoshino H, Yokota K, Ando M, Inoue S, Ishii S, Nishimura S (1992) Macromolecules 25:3786; Zou Y, Khor E (2005) Biomacromolecules 6:80
4. Nishimura S, Kohgo O, Kurita K, Kuzuhara H (1991) Macromolecules 24:243; Kurita K, Kojima T, Nishiyama Y, Shimojoh M (2000) Macromolecules 33:4711; Kurita K, Ikeda H, Yoshida Y, Shimojoh M, Harata M (2002) Biomacromolecules 3:1
5. Nishiyama Y, Yoshikawa T, Ohara N, Kurita K, Hojo K, Kamada H, Tsutsumi Y, Mayumi T, Kawasaki K (2000) J Chem Soc Perkin Trans 1 1161; Ouchi T, Nishizawa H, Ohya Y (1998) Polymer 39:5171; Holappa J, Nevalainen T, Savolainen J, Soininen P, Elomaa M, Safin R, Suvanto S, Pakkanen T, Másson M, Loftsson T, Järvinen T (2004) Macromolecules 37:2784
6. Kurita K, Hirakawa M, Nishiyama Y (1999) Chem Lett 771; Kurita K, Sugita K, Kodaira N, Hirakawa M, Yang J (2004) Biomacromolecules 6:1414; Kurita K, Hirakawa M, Kikuchi S, Yamanaka H, Yang J (2004) Carbohydr Polym 56:333
7. Kurita K (1997) β-Chitin and the reactivity characteristics. In: Goosen MFA (ed) Applications of chitin and chitosan. Technomic Publishing, Lancaster, PA (pp 79-87); Kurita K (1997) Preparation of squid β-chitin. In: Muzzarelli RAA, Peter MG (eds) Chitin handbook. Atec Edizioni, Grottammare (pp 491-493)
8. Kurita K, Ishii S, Tomita K, Nishimura S, Shimoda K (1994) J Polym Sci Part A Polym Chem 32:1027
9. Terbojevich M, Cosani A, Muzzarelli RAA (1996) Carbohydr Polym 29:63