Polymer Journal, Vol. 22, No. 5, pp. 429—434 (1990)

N-Hydroxyacylation of Chitosan with Lactones

Keisuke KURITA, Shin-Ichiro NISHIMURA, and Takanobu TAKEDA

Department of Industrial Chemistry, Faculty of Engineering, Seikei University, Tokyo 180, Japan

(Received October 17, 1989)

ABSTRACT: Although γ-butyrolactone is not reactive enough toward the amino groups of chitosan, the β-form has been found to give *N*-hydroxyacylated derivatives. The conditions for the reactions were studied in detail, and efficient procedures have been established. The reaction was highly dependent on the kind of solvent and confirmed to proceed only in dimethyl sulfoxide among ordinary solvents either in dispersion of powdery chitosan or in a highly swollen state of chitosan. The degree of substitution could easily be controlled by reaction temperature and time, and reached 0.65—0.67 after about 48 h at 100°C. The resulting derivatives showed much improved hygroscopicity, and those prepared in the swollen state were superior to those prepared in solid dispersion.

KEY WORDS Chitosan / Chemical Modification / Lactones / Ring-Opening Addition / Hydroxyacylation / Hygroscopicity /

The importance of chemical modifications for advanced utilization of chitin, a biomass resource almost as aboundant as cellulose, is widely understood and much attention has been paid to various modes of modification reactions.[1] Poor tractability of chitin has, however, causes trouble in many respects. Chitosan, a deacetylated form of chitin, and water-soluble chitin, a partially deacetylated form,[2] are both regarded as chitin moieties having high possibilities to cope with difficulties associated with tractability; they are much better starting materials in terms of solubility and reactivity.

Acylation is the most typical and extensively studied modification reaction, and carboxylic acids, acid anhydrides, and acyl chlorides have been used.[3] Modification with lactones is another possible way of acylation and of interest in view of the simple and efficient preparation of acyl derivatives having additional hydroxyl groups, though lactones are supposed to exhibit generally limited reactivity. The introduction of this type of hydroxyacyl groups, would effectively increase hydrophilicity owing to the hydrophilic structure of the groups and destruction of tight arrangement of the rigid polysaccharide molecules. Such derivatives are thus anticipated to find technical applications in various fields as water absorbents, chelating agents, chromatographic gels, biomedical materials, and so on. As a versatile means of *N*-hydroxyacylation of chitosan, we have examined the possibility and scope of the ring-opening addition reaction of lactones and elucidated the influence of substitution on the properties.

EXPERIMENTAL

General

Chitosan with a degree of deacetylation of 95% was prepared by alkaline deacetylation of shrimp shell chitin and pulverized to 100-mesh pass.[2] γ-Butyrolactone, ($\pm$)-β-butyrolactone, and solvents were purified by distillation. The extent of hydroxyacylation was measured primarily by conductometric titration with a

Toa conductivity meter CM-40S. IR spectra were recorded with a JEOL IRA-1. Solvent compositions were determined by gas chromatography with a Shimadzu GC-14A equipped with a Porapak Q (50—80) and a Thermon 1000 (5% Sunpak A) columns using 1-butanol as the internal reference. X-Ray diffraction diagrams were obtained on a Rigaku RAD-AB by the powder method with the use of nickel-filtered Cu-K_α radiation. Elemental analysis was performed with a Yanaco CHNcorder MT-3.

Acylation of Pulverized Chitosan Dispersed in Dimethyl Sulfoxide

A dispersion of 0.200 g of chitosan in 15 ml of dimethyl sulfoxide (DMSO) was heated at 100°C for 6.5 h, and a solution of 1.0 ml of β-butyrolactone in 9 ml of DMSO was added with stirring. The mixture was heated at 100°C for 48 h, and after cooling, poured into 150 ml of acetone. The product was collected by filtration and extracted with acetone in a Soxhlet extractor for 8 h. It was then treated with 5% aqueous sodium hydrogen carbonate at room temperature for 24 h, washed with water, and dried to give 0.253 g of *N*-3-hydroxybutanoylated chitosan.

To part of the powdered sample, 0.060 g, were added 5.0 ml of 1/10 *N* hydrochloric acid and 60 ml of deionized water, and the mixture was stirred at room temperature for 1 h. It was titrated with 1/30 *N* sodium hydroxide and then back-titrated with 1/30 *N* hydrochloric acid with a conductivity meter. The back-titration was more reproducible and reliable than the direct one to determine the amount of remaining amino groups. The degree of *N*-hydroxyacylation was calculated to be 0.66 per pyranose unit. The degree of substitution is thus determined most accurately by conductometric titration among several possible methods, but it is also supported by elemental analysis; the C/N ratio for this sample was 7.48 (calculated value = 7.49 for a degree of substitution of 0.66).

Acylation of Chitosan in Swollen State in DMSO

A solution of 0.200 g of chitosan in 10 ml of 1% acetic acid was diluted with 10 ml of methanol and poured into 150 ml of DMSO with stirring to give a highly swollen chitosan precipitate. The mixture was centrifuged, the supernatant was discarded, and the precipitate was washed with DMSO. It was treated with 40 ml of 5% sodium carbonate at room temperature for 3 h, and washed with deionized water and then with DMSO five times each by centrifugation. It was added to 50 ml of DMSO and 130 ml of benzene, and about a half the amount of the benzene was distilled off to remove remaining water in the mixture azeotropically. The chitosan precipitate was centrifuged, and the solvent containing benzene was replaced by dry DMSO. After six replacements, 1.0 ml of β-butyrolactone in 24 ml of DMSO was added, and the mixture was heated at 100°C for 48 h with stirring. The product was isolated in 150 ml of acetone, washed with acetone in a Soxhlet extractor for 8 h, and then treated with alkali as described in the preceding section to give 0.107 g of hydroxyacylated chitosan. The degree of acylation was 0.65 as determined by conductometric titration. The C/N ratio of elemental analysis was 7.45 (calculated value = 7.46 for a degree of substitution of 0.65).

Pyridine can be used in place of DMSO, and the reactions were carried out similarly.

Hygroscopicity

Samples pulverized to 100-mesh pass and thoroughly dried with phosphorus pentoxide were put in air saturated with water vapor at room temperature (*ca.* 25°C). Weight increase due to water vapor absorption was measured at appropriate time intervals.

RESULTS AND DISCUSSION

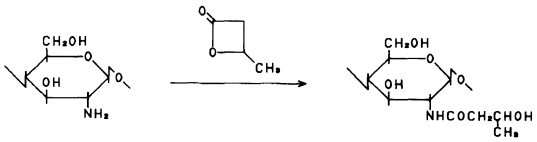
Modifications of chitosan with lactones were reported recently with α-glucoheptonic acid

γ-lactone[4] and β-propiolactone[5]; the former gave acylated derivatives in aqueous acetic acid/methanol whereas the latter resulted in alkylation by attacking the β-carbon in acetonitrile/water. Chitosan was thus subjected first to the reaction with a typical five-membered lactone, γ-butyrolactone, to examine the reactivity of the amino groups toward lactones. However, treatment in solution or in dispersion resulted in no substitution under various conditions. The acylation was then attempted though not successfully with more reactive β-butyrolactone in solution in aqueous acetic acid/methanol, or in a swollen state in aqueous acetic acid/methanol/pyridine. These results indicate that the amino groups of chitosan are not as accessible as those of ordinary aliphatic amines, and a more thorough examination of reaction conditions was necessary.

Effects of Solvent

Prior to conducting the reaction at elevated temperatures in solvents, suitability of some solvents was elucidated by heating dispersions of powdery chitosan in various solvents with high dielectric constants at 100°C for 16 h. Among them, hexamethylphosphoramide and dichloroacetic acid gave sticky masses which caused difficulty in isolation. When the dispersion in formamide or *N*,*N*-dimethylacetamide was heated, some side reactions were implied to occur from the IR spectra. *N*,*N*-Dimethylformamide (DMF), DMSO, pyridine, and acetonitrile caused no side-reaction with chitosan, and thus dispersions in these solvents were treated with β-butyrolactone at 100°C for 16 h. In pyridine and acetonitrile, however, chitosan was recovered quantitatively.

DMSO and DMF gave the expected *N*-3-hydroxybutanoylated products, but the degree of substitution (*ds*) in the latter solvent was as low as 0.02 compared to 0.6 in the former one. The reaction was also attempted in aqueous solution or ether as suggested by the literature,[5,6] but no derivatization was observed. These results indicate that DMSO is by far the most appropriate solvent among ordinary solvents.



Reaction in Dispersion of Pulverized Chitosan

The above results indicate that the hydroxyacylation proceeds in dispersion in DMSO, and various factors affecting the reaction were studied in detail.

Time Course of the Substitution

The substitution reaction was followed by heating the dispersed chitosan and β-butyrolactone in DMSO at 100°C for given times. The reaction was rather reproducible under these conditions as evident from the *ds* value and yield. The *ds* increased with reaction time, but almost leveled off after 48 h at about 0.65. The progress of the reaction is shown in Figure 1.

Effects of Temperature

The reaction was carried out at various temperatures for 48 h. The heating temperature apparently affected the *N*-hydroxyacylation; substitution took place slowly at low tempera-

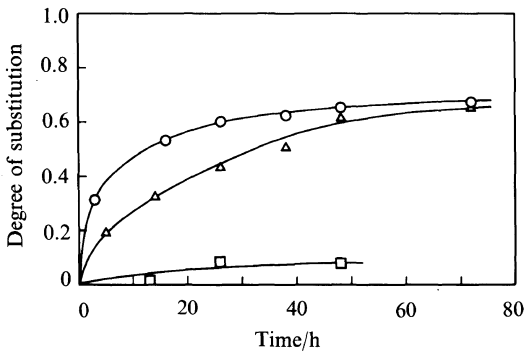


Figure 1. Progresses of *N*-hydroxyacylation of chitosan with β-butyrolactone at 100°C. (○), dispersed chitosan powder in DMSO; (△), highly swollen chitosan precipitate in DMSO; (□), highly swollen chitosan precipitate in pyridine.

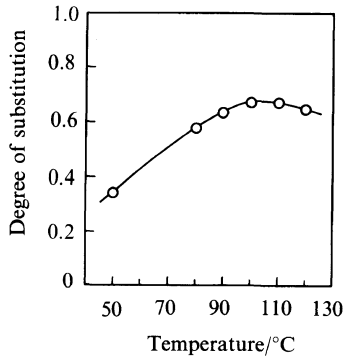


Figure 2. Effect of reaction temperature on the *N*-hydroxyacylation of chitosan powder dispersed in DMSO (reaction time, 48 h).

tures, but became rapid at elevated temperatures. The *ds* reached a maximum at 100°C and then decreased to some extent at higher temperatures as shown in Figure 2. The decrease is ascribable to the decomposition of the lactone by a trace amount of water as confirmed by a control reaction in the absence of chitosan.

Swelling of Chitosan

The influence of swelling time on the reaction was examined by heating powdered chitosan in DMSO prior to the reaction with the expectation of facilitation of reaction as in the case of carboxymethylation.[7] The dispersions were heated at 100°C for varying times, but the appearance of chitosan changed scarcely with prolonged heating. The dispersions thus preheated for 0 to 31 h were allowed to react with β-butyrolactone at 100°C for 48 h. The *ds* values were, however, in a narrow range (0.65—0.68), and no appreciable effect was observed. This implies that the swelling extent of chitosan in DMSO is low due to strong hydrogen bonding, and furthermore, long time treatment is not necessary to swell at 100°C.

The yields of the products prepared in this way were high, and generally 0.24—0.26 g starting from 0.2 g of chitosan.

Reaction in Highly Swollen State

Since the reactions described above were conducted with dispersed chitosan powder which might swell to only a limited extent, hydroxyacylation is supposed to take place from the surface of the particles. In view of achieving efficient substitution and also high hydrophilicity of the products, reactions under homogeneous or almost homogeneous conditions leading to random substitution along the main chain are considered more promising as in the case of the formation of water-soluble chitin by partial *N*-acetylation of chitosan.[8] The reaction behavior in a highly swollen state in DMSO was thus examined.

Preparation of Swollen Chitosan and Effects of Solvent Compositions

A highly swollen chitosan precipitate was prepared by pouring a chitosan solution in aqueous acetic acid/methanol into DMSO. The mixture, however, contained protic solvents such as water, acetic acid, and methanol that severely interfere with the reaction. The mixed solvent was thus replaced by DMSO repeatedly. The resulting chitosan precipitate was subjected to reaction with β-butyrolactone at 100°C, but no reaction occurred. The addition of triethylamine to regenerate free amino groups from the salt with acetic acid failed to facilitate the reaction, and moreover, the color of recovered chitosan become very dark.

The swollen precipitate in DMSO was then treated with aqueous sodium carbonate instead of triethylamine to assure the regeneration of free amino groups. It was washed with water to neutral and then DMSO thoroughly. A small amount of water remaining in the solvent was finally removed by azeotropic distillation with benzene. Solvent replacement by DMSO was subsequently repeated to remove the remaining benzene.

The change of solvent composition during this process was followed by gas chromatography to confirm removal of protic solvents. The chromatograms showed that the solvent

was completely free from acetic acid and methanol after distillation of benzene. The water content was also reduced markedly at this stage, and was less than 0.6%. The benzene content decreased by repeating the final solvent replacement by DMSO and was as low as 0.2% after six replacements. These results confirm that the solvent was almost exclusively DMSO where a highly swollen chitosan precipitate was dispersed.

Hydroxyacylation of a Highly Swollen Precipitate

The hydroxyacylation of the highly swollen chitosan with β-butyrolactone was carried out at 100°C. As expected, the reaction proceeded smoothly under these conditions, indicating the necessity of removing protic solvents and treatment with aqueous alkali. The *ds* increased with time, and the curve in Figure 1 shows the progress in comparison with that of the dispersed powder. Although the reaction in a swollen state appeared a little more sluggish than that of dispersed powder, almost the same level of *ds* values, 0.65—0.67, was achieved in the reactions for about 48 h. The yields were mostly in the range 0.10—0.14 g starting from 0.2 g of chitosan, which were lower than those in solid dispersion because of the mechanical losses during the repeated solvent replacement process.

Similar high swelling was possible in pyridine in place of DMSO. The reaction was, however, rather reluctant as in the case of that of powdery chitosan, and the *ds* was only 0.07—0.09 after 48 h reaction at 100°C as shown by the curve in Figure 1.

Properties of the Hydroxyacylated Derivatives

The resulting products were obtained as almost colorless to pale yellow materials. The IR spectra showed characteristic amide I and II bands at 1665 and 1540 cm^{-1}, respectively, in contrast to a weak broad band at 1600—1630 cm^{-1} due to the free amino groups of the original chitosan. The spectra of the

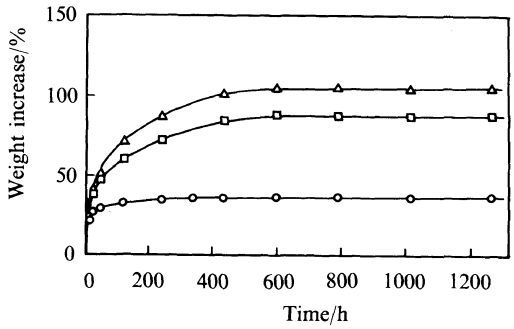


Figure 3. Water vapor absorption by chitosan and *N*-hydroxyacylated chitosans. (○), chitosan; (□), *N*-hydroxyacylated chitosan with a *ds* of 0.66 prepared in dispersion of powdery chitosan in DMSO; (△), *N*-hydroxyacylated chitosan with a *ds* of 0.65 prepared in a highly swollen state of chitosan in DMSO.

hydroxyacylated derivatives had no appreciable bands ascribable to carboxyl groups, though there are two possible ring-opening modes. The reaction of β-propiolactone with amines are dependent on many interrelating factors,[9,10] and no simple generalization has been established. The results obtained here with β-butyrolactone indicate that the carbonyl carbon is the favorable position to attack probably on account of the presence of the methyl group at the β-position.

Crystallinity change was studied by X-ray diffractiometry, and all the hydroxyacyl derivatives obtained here were amorphous, whereas the original chitosan showed characteristic crystalline peaks. This indicates efficient destruction of the tight arrangement of rigid chitosan molecules by hydroxyacylation.[11]

Although the hydroxyacylated chitosans were insoluble in ordinary organic solvents and aqueous acetic acid, those prepared in a swollen state showed higher swelling in polar solvents than those prepared in solid dispersion. The influence of hydroxyacylation on hygroscopicity was evaluated by following the water vapor absorption by chitosan and the derivatives prepared at 100°C for 48 h. The absorption by chitosan reached equilibrium at 36% after about 200 h as shown in Figure 3. The

hydroxyacyl derivatives prepared in DMSO turned out to be much more hydrophilic. As expected, the sample obtained in a swollen state was superior to that obtained in solid dispersion. This superiority is most reasonably attributable to the random distribution of the hydroxyacyl groups along the main chain in the samples prepared in a swollen state.[8,11] At equilibrium, weight increases were 105 and 88%, respectively.

CONCLUSION

N-Hydroxyacylation of chitosan with β-butyrolactone proceeded in a satisfactory manner in DMSO under appropriate conditions both in dispersion of powdery chitosan and in a highly swollen state. The resulting derivatives showed much improved hygroscopicity as expected, and those prepared in a swollen state were confirmed to exhibit remarkable hygroscopicity. The preparative methods reported here may be of wide applicability to the modifications with nucleophiles of poor reactivity.

Acknowledgments. This research was supported in part by a grant from Towa Shokuhin Kenkyu Shinkoukai, and also by Grant-in-Aid for Scientific Research (#63604584 and #01604595) on Priority Areas, New Functionality Materials—Design, Preparations, and Control—, from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. K. Kurita, in "Chitin in Nature and Technology," R. A. A. Muzzarelli, C. Jeuniaux, and G. W. Gooday, Ed., Plenum, New York, 1986, p 287.
2. K. Kurita, T. Sannan, and Y. Iwakura, *Makromol. Chem.*, **178**, 3197 (1977); T. Sannan, K. Kurita, and Y. Iwakura, *ibid.*, **176**, 1191 (1975); *ibid.*, **177**, 3589 (1976).
3. For example, P. P. Schorigin and E. Hait, *Chem. Ber.*, **68B**, 971 (1935); K. Kurita, T. Sannan, and Y. Iwakura, *Makromol. Chem.*, **178**, 2595 (1977); K. Kurita, S. Chikaoka, M. Kamiya, and Y. Koyama, *Bull. Chem. Soc. Jpn.*, **61**, 927 (1988).
4. M. Yalpani and L. D. Hall, *Macromolecules*, **17**, 272 (1984).
5. S. Otsuki and R. Iwamoto, *Kobunshi Ronbunshu*, **44**, 115 (1987).
6. T. L. Gresham and R. E. Benesch, *J. Am. Chem. Soc.*, **73**, 3168 (1951).
7. R. Trujillo, *Carbohydr. Res.*, **7**, 483 (1968).
8. K. Kurita, M. Kamiya, S. Nishimura, and Y. Koyama, *Chem. Lett.*, 1597 (1989).
9. T. L. Gresham, J. E. Jansen, F. W. Shaver, R. A. Bankert, and F. T. Fiedorek, *J. Am. Chem. Soc.*, **73**, 3168 (1951).
10. C. D. Hurd and S. Hayano, *J. Am. Chem. Soc.*, **74**, 5889 (1952).
11. K. Kurita, in "Progress in Biotechnology. 3. Industrial Polysaccharides," M. Yalpani, Ed., Elsevier, Amsterdam, 1987, p 337; K. Kurita, S. Chikaoka, and Y. Koyama, *Chem. Lett.*, 9 (1988); K. Kurita, Y. Koyama, and S. Chikaoka, *Polym. J.*, **20**, 1083 (1988); K. Kurita, Y. Koyama, S. Inoue, and S. Nishimura, *Macromolecules*, in press.