Studies on Chitin. 14. *N*-Acetylation Behavior of Chitosan with Acetyl Chloride and Acetic Anhydride in a Highly Swelled State

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N-Acetylation on chitosan has been studied in a highly swelled state with acetyl chloride in comparison with that with acetic anhydride under various conditions to establish the general possibility and scope of the acylation reactions on chitosan and other polysaccharides. A highly swelled chitosan precipitate prepared by pouring a chitosan solution in aqueous acetic acid/methanol into pyridine is quite suitable for efficient modifications, and the reaction with either acylating reagent proceeded smoothly and reproducibly under mild conditions. Although acetic anhydride is generally more appropriate in protic solvens, acetyl chloride is effective and advantageous especially in pyridine containing only small amounts of protic solvents.

Although chitin is structurally similar to cellulose and quite abundant in nature, it has been discarded and thus attracting much attention recently as an unutilized resource. It is considered of interest as a raw material of high potentials for developing new functional materials from the viewpoint of chemical modifications owing to the presence of the reactive amino groups in addition to hydroxyl groups.[1] Various types of reactions have been reported thus far for utilization of this amino polysaccharide, but severe conditions are generally necessary on account of the strong intermolecular hydrogen bonding which limits the solubility in ordinary organic solvents.[2,3] Among the modification reactions, acetylation is one of the most fundamental and important reactions to not only chitin but also many kinds of other polysaccharides to afford derivatives of a wide variety of attractive properties.

We have already reported the efficient acylation with acetic and some cyclic aromatic acid anhydrides in a highly swelled state by making use of the advantage of water-soluble nature of the 50% deacetylated chitin samples prepared under homogeneous hydrolysis conditions.[4,5]

Although acid anhydrides have been by far most commonly used for acylation, it is useful to establish the controlled acylation with more easily available and more reactive but less stable acyl chlorides under mild conditions. Several papers have dealt with the acylation of chitin, i.e., esterification at the hydroxyl groups, with a large excess of acyl chlorides,[6–10] but not much information has been obtained regarding the reaction on chitosan. Fujii and co-workers reported the acylation on chitosan where long-chain fatty acid chlorides were subjected to the reaction in a mixture of pyridine and chloroform under reflux to give polyacylated derivatives with degrees of substitution of up to 4.[11] These reactions have, however, been normally carried out under rather severe conditions, and thus difficulties are sometimes encountered in controlling the reactions. No attempt has been made to elucidate the conditions and course of the reaction on chitosan with varying amounts of acyl chlorides and also acid anhydrides for practical acylation. We have thus examined the progress of *N*-acetylation on chitosan with both acetyl chloride and acetic anhydride under mild conditions to provide a versatile modification for this naturally abundant amino polysaccharide.

Experimental

Chitosan with a degree of deacetylation of 88% was prepared by heating pulverized chitin in a 50% aqueous sodium hydroxide solution at 135 °C for 3.5 h under a nitrogen stream.[12] The degree of acetylation of the acetylated products obtained in this study was determined by IR spectroscopy.[13]

Acetylation of Chitosan with Acetyl Chloride in a Mixture of Aqueous Acetic Acid, Methanol, and Pyridine: Chitosan, 0.3 g, was dissolved in 8 ml of 10% aqueous acetic acid,

Table 1. Acetylation of Chitosan with Acetyl Chloride in a Mixture of Aqueous Acetic Acid, Methanol, and Pyridine

Chitosan/g	Acetyl chloride/ml	$-COCl/-NH_2$[a]	Yield/g	Degree of acetylation/%
0.304	5.0	44	0.263	16
0.304	16.8	147	0.240	25
0.301	35.0	307	0.258	34

a) Molar ratio.

Table 2. Acetylation of Chitosan with Acetyl Chloride after Repeated Replacement of the Solvent by Pyridine

Chitosan/g	Acetyl chloride/ml	$-COCl/-NH_2$[a]	Yield/g	Degree of acetylation/%
0.100	0.19	5.0	0.075	41
0.100	0.38	10	0.074	78
0.101	1.95	25	0.093	84

a) Molar ratio.

Table 3. Acetylation of Chitosan with Acetic Anhydride

Chitosan/g	Acetic anhydride/ml	$Ac_2O/-NH_2$[a]	Yield/g	Degree of acetylation/%
0.900	3.85	8.5	0.825	31
0.300	1.85	12	0.221	38
0.299	2.46	16	0.225	42
0.300	3.08	20	0.223	50
0.301	3.85	25	0.289	53
0.301	4.62	31	0.220	62

a) Molar ratio.

and the solution was diluted with 8 ml of methanol. The solution was poured into 100 ml of pyridine with stirring to afford a highly swelled precipitate of chitosan. The mixture was cooled at 0 °C in an ice bath, and a given amount of acetyl chloride was added dropwise with stirring. It was allowed to warm to room temperature, stirred for 16 h 40 min, and poured into 500 ml of a mixture of acetone and water (7:1) to coagulate the acetylated derivative. The product was isolated by filtration and washed with acetone and then with methanol. Without drying, it was treated with 100 ml of 1 M potassium hydroxide (1 M=1 mol dm^{-3}) in methanol at room temperature for 4 h. Filtration, washing with methanol and acetone, and drying in vacuo gave an acetylated product as a slightly yellow solid. The results are summarized in Table 1.

The reaction appeared to be fairly rapid and complete within 1 h, judging from the values of the degree of acetylation of the resulting products obtained after various periods of reaction. The reaction time was thus fixed at 3 h in the subsequent reactions.

Acetylation of Chitosan with Acetyl Chloride after Repeated Replacement of the Solvent by Pyridine: A solution of 0.1 g of chitosan in a mixture of 2.6 ml of 10% aqueous acetic acid and 2.6 ml of methanol was poured into 33 ml of pyridine. The mixture was centrifuged, and 25.5 ml of the supernatant was replaced by the same amount of fresh pyridine. After repeating this solvent replacement five times, acetyl chloride diluted with tetrahydrofuran to 5 ml was added dropwise at 0 °C with stirring. The mixture was allowed to warm to room temperature, and after 3 h the product was isolated in the same manner as described above. The results are listed in Table 2.

Acetylation of Chitosan with Acetic Anhydride: To 100 ml of pyridine was added a solution of 0.3 g of chitosan in a mixture of 8 ml of 10% aqueous acetic acid and 8 ml of methanol. Acetic anhydride was added, and the mixture was stirred at room temperature for 5 h. The product was isolated in the same manner. Table 3 shows the results.

Results and Discussion

Acetylations of chitosan with varying amounts of acetyl chloride and acetic anhydride were examined in order to make clear the general possibility and scope of the acetylation methods. The reactions were carried out in a highly swelled state brought about by pouring a solution of chitosan in a mixture of aqueous acetic acid and methanol into pyridine to enable efficient acetylation under mild reaction conditions. In the absence of methanol, the precipitate became rather sticky, and methanol helps disperse the swelled precipitate to realize an almost homogeneous mixture. Furthermore, methanol is effective to prevent phase separation of the reaction mixture on addition of hydrophobic organic reagents.

The resulting products with both acetyl chloride and acetic anhydride showed weak peaks in the IR spectra due to ester linkages formed by acetylation at the hydroxyl groups. The ester peaks were also observed even in the absence of pyridine with acetic anhydride. This indicates the reaction selectivity to the amino groups against the hydroxyl groups to be not sufficient. After the reaction, the products were thus treated with methanolic potassium hydroxide to ensure the removal of the ester groups and also to regenerate the amino groups lost by the salt formation with acetic acid. The treatment with 1 M methanolic potassium hydroxide for 4 h at room temperature was confirmed to be satisfactory for the purpose and not to harm the acetamide groups.

The resulting products showed medium to strong amide bands at 1650 and 1550 cm^{-1} and no distinct ester band at 1730 cm^{-1}.

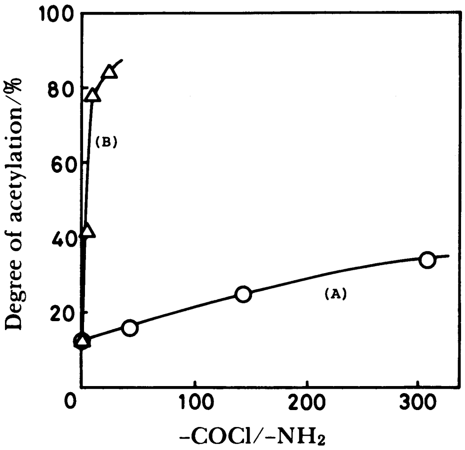

Fig. 1. Progresses of acetylation with acetyl chloride; (A) acetylation in a mixture of aqueous acetic acid, methanol, and pyridine, (B) acetylation after repeated solvent replacement by pyridine.

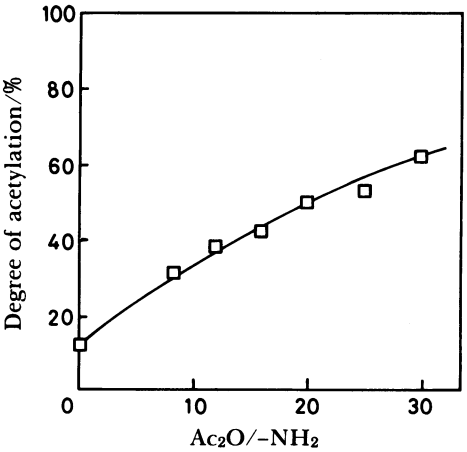

Fig. 2. Progress of acetylation with acetic anhydride in a mixture of aqueous acetic acid, methanol, and pyridine.

When acetyl chloride was added to a swelled precipitate of chitosan in aqueous acetic acid/methanol/pyridine, acetylation required a large excess of acetyl chloride as shown by the results in Fig. 1. With 307-fold excess acetyl chloride, an acetylated product with a degree of acetylation of 34% was obtained, indicating the acetyl chloride to have been consumed largely by some side reactions with the protic solvent components such as water and methanol during the expected reaction with the amino groups. Triethylamine was then added to the reaction mixture to regenerate the amino groups from the salt form for the facilitated reaction, but no improvement in the acetylation was observed. This is due to the high reactivity of acetyl chloride and suggested to decrease the contents of protic solvents for increased reaction efficiency.

The mixture containing a highly swelled chitosan precipitate was thus centrifuged, and the supernatant was replaced by fresh pyridine. After repeating this replacement procedure five times, the contents of protic solvents should have been reduced markedly, and noteworthily chitosan still remained highly swelled in the solvent yet. The reaction after this solvent replacement proceeded quite smoothly as expected, and in this way, a degree of acetylation of 78% was attained with only 10-fold excess acetyl chloride under these mild reaction conditions at 0 °C. The difference in the progress of the acetylation reaction under these different conditions is evident in Fig. 1.

The acetylation of chitosan was then examined with acetic anhydride in the same swelled state. Although the reaction was conducted in a mixture of aqueous acetic acid, methanol, and pyridine as in the first case of the reaction with acetyl chloride, acetylation proceeded fairly efficiently. As shown in Fig. 2, a 31-fold excess of acetic anhydride resulted in the formation of a derivative with a degree of acetylation of 62%. Qualitative solubility was examined in aqueous acetic acid, and the derivatives with degrees of acetylation below around 60% were soluble.

Consequently, these results indicate that 1) controlled acetylation on chitosan can be achieved effectively and reproducibly with either acetyl chloride or acetic anhydride under mild conditions in a highly swelled state, 2) a mixture containing highly swelled chitosan obtained by replacing the aqueous solvent by pyridine is especially suitable for efficient modifications of chitosan, and 3) acetyl chloride was found to be quite advantageous under these conditions, although acetic anhydride is generally more appropriate in the acetylation of chitosan in protic solvents.

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