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Enzymatic degradation of β-chitin: susceptibility and the influence of deacetylation

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Abstract

Susceptibility of β-chitin to lysozyme and the influence of deacetylation on the enzymatic degradation behavior have been elucidated. β-Chitin was degraded much more readily than α-chitin due to the weak intermolecular forces. Partially deacetylated β-chitins were prepared under mild conditions and subjected to lysozyme treatment. The degradation rate proved to be affected markedly by the extent of deacetylation and showed a maximum at about 50% deacetylation. The rate then decreased, and the derived chitosan with a degree of deacetylation of 0.97 was not degraded at all. Fine control of degradability thus became possible, indicating a high potential of β-chitin as a biodegradable specialty polymer with low toxicity.

Keywords: β-Chitin; Chitosan; Deacetylation; Lysozyme; Biodegradation

1. Introduction

Chitin is one of the most attractive biopolymers because of its unique physicochemical and biological properties (Muzzarelli, 1977; Roberts, 1992), and much attention has been paid recently to its potential applications in various fields. α-Chitin is the major form of chitin, and hence studies on chitin have been carried out mostly with this α-form. A serious drawback to using α-chitin is, however, its intractable nature ascribable to the strong intermolecular forces (Minke & Blackwell, 1978), and the studies are usually encountered by some difficulties in purification, structural identification and chemical modification. In sharp contrast, β-chitin is characterized by weak intermolecular forces arising from loose arrangement of the molecules (Gardner & Blackwell, 1975). It is also more tractable as anticipated from considerable affinity for solvents (Austin, Castle & Albsetti, 1989) and therefore might be important as an alternative chitin form. Actually, β-chitin exhibited high chemical reactivity compared to α-chitin as confirmed by several modification reactions (Kurita, Ishii, Tomita, Nishimura & Shimoda, 1994; Kurita, Tomita, Tada, Ishii, Nishimura & Shimoda, 1993a; Kurita, Tomita, Ishii, Nishimura & Shimoda, 1993b). These results suggest the high potential of this unutilized biomass resource, β-chitin, as a starting material for developing new applications.

Chitin has been degraded with lysozyme, and the specificity of enzymatic hydrolysis towards the sequence of its *N*-acetylglucosamine unit has been elucidated (Stokke, Vårum, Holme, Hjerde & Smidsrød, 1995; Vårum, Holme, Izume, Stokke & Smidsrød, 1996; Vårum & Smidsrød, 1998).

The recent strong interest in biodegradable materials for drug delivery systems and other biomedical uses as well as for environment protection has prompted us to evaluate β-chitin as a biodegradable polymeric material. β-Chitin is expected to show higher degradability than α-chitin; furthermore, controlled deacetylation of β-chitin would make possible finely tuned biodegradation, which is quite useful for some advanced applications, particularly in the biomedical field.

2. Experimental

2.1. General

The degree of deacetylation (dd) was determined by conductometric titration with a conductivity meter TOA CM-40S. The absorbance was measured with a JASCO Ubest-30 UV–VIS spectrometer. All the chemicals were of reagent grade and were used without further purification.

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Table 1
Preparation of partially deacetylated β-chitins (dd: degree of deacetylation)

dd of starting material	Reaction conditions			dd of product
	NaOH (%)	Temp (°C)	Time (h)	
0.11	20	100	2.5	0.47
0.11	20	100	3	0.57
0.47	20	100	3	0.66
0.11	30	100	3	0.84
0.11	40	80	3	0.80
0.80	40	80	3	0.94
0.94	40	80	3	0.97

2.2. Chitins and deacetylated chitins

α-Chitin isolated from shrimp shells was purified as reported (Kurita et al., 1994); the dd was 0.12. The chitin was treated with 30% aqueous sodium hydroxide at 110°C for 4 h to give a partially deacetylated chitin with a dd of 0.56.

β-Chitin was isolated from squid pens (Kurita et al., 1994), and the dd was 0.11. Deacetylation of the sample was carried out with 20–40% aqueous sodium hydroxide at 80 or 100°C to give partially deacetylated chitins with various dd values.

2.3. Enzymatic degradation

Chitins and deacetylated chitins were finely pulverized to 100 mesh pass and treated with lysozyme from egg white in pH 4.5 acetate buffer at 36°C in suspension. The amount of the resulting reducing ends formed by degradation was determined using ferricyanide as reported previously (Kurita, Yoshino, Nishimura & Ishii, 1993c).

3. Results and discussion

3.1. Preparation of partially deacetylated chitins

In order to elucidate the influence of the extent of deacetylation on enzymatic degradability, β-chitin was deacetylated under mild conditions to avoid discoloration (Kurita et al., 1993a). As summarized in Table 1, starting from β-chitin with a dd of 0.11, partially deacetylated chitins with a dd up to 0.97 were prepared.

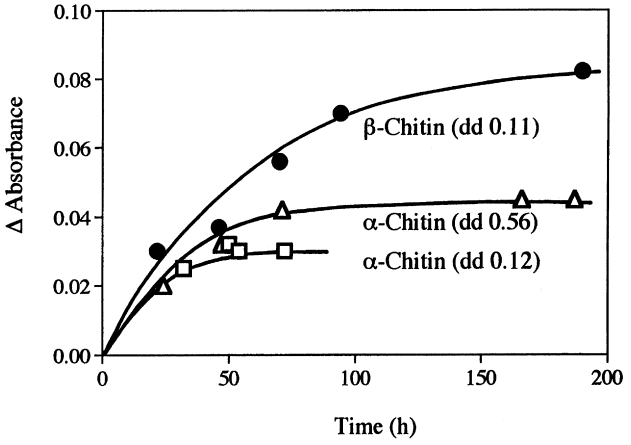

Fig. 1. Susceptibility of β- and α-chitins to lysozyme.

3.2. Enzymatic degradation

Susceptibility of β-chitin to lysozyme was first examined for comparison with that of α-chitin. The amount of reducing ends formed as a result of enzymatic degradation was determined using ferricyanide, and the differences in ferricyanide absorbance (*Δ*Absorbance) corresponding to the amount of the reducing ends were plotted as a function of time (Fig. 1). As anticipated from the loose arrangement of molecules (Gardner & Blackwell, 1975), β-chitin proved to be much more susceptible to lysozyme than α-chitin with a similar dd value. α-Chitin was degraded somewhat readily when the dd value was raised to 0.56, but the rate was still lower than that of β-chitin. The higher degradability of a moderately deacetylated α-chitin than a highly deacetylated one was suggested, in solution, from the viscosity measurement using acid-soluble deacetylated chitins (dd ≥ 0.66) (Sashiwa, Saimoto, Shigemasa, Ogawa & Tokura, 1990). The results in Fig. 1 show an improved susceptibility of a moderately deacetylated chitin compared to chitin with a low dd.

The present assay is based on the formation of reducing

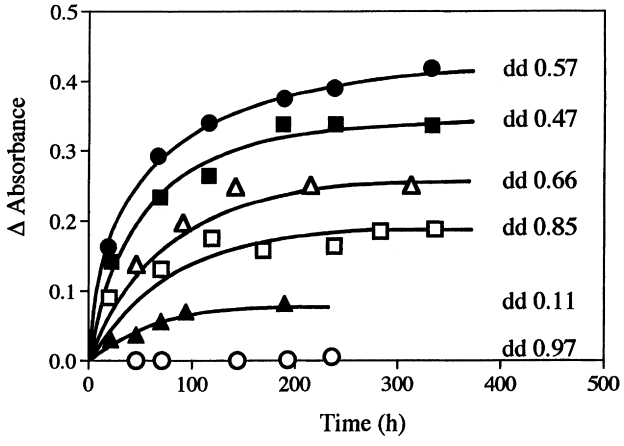

Fig. 2. Influence of the extent of deacetylation of β-chitin on the enzymatic degradation with lysozyme.

ends under heterogeneous reaction conditions and is hence applicable to any chitin samples independent of the deacetylation extent and solubility. The influence of deacetylation on the enzymatic degradability of β-chitin was thus examined over a wide range of deacetylation to establish fine control of degradation behavior. Partially deacetylated β-chitins with a dd of 0.11 to 0.97 were subjected to lysozyme degradation. The results are shown in Fig. 2. As evident in the figure, the susceptibility increased drastically with an increase in the dd value and reached a maximum at about 50% deacetylation. It then decreased with further deacetylation, and finally no apparent degradation was observed with a sample of dd 0.97.

The high susceptibility of a chitin sample with about 50% deacetylation is attributable to the improved hydrophilicity (Kurita, Kamiya & Nishimura, 1991; Sannan, Kurita & Iwakura, 1976; Sashiwa et al., 1990), obvious from the enhanced swelling brought about by partial deacetylation, and, therefore, to easy accessibility by the enzyme though under heterogeneous conditions. Extensive deacetylation above 80% resulted in marked swelling or even dissolution in the buffer solution during the enzyme treatment. However, despite this further improved hydrophilicity, the highly deacetylated samples exhibited only low or no susceptibility. This is attributable to the decrease in the *N*-acetylglucosamine sequences crucial as a substrate to be recognized by lysozyme (Vårum et al., 1996).

4. Conclusions

Deacetylation of β-chitin was accomplished under mild conditions to give an almost colorless sample with various extents of deacetylation. Enzymatic degradation studies with lysozyme indicated that β-chitin was much more susceptible to degradation than α-chitin, and moreover, the degradation behavior was highly dependent on the deacetylation extent. The degradation rate can thus be controlled. Furthermore, it is possible to prepare two kinds of samples with a similar degradation capacity and yet different solubility since the degradability showed a maximum at about 50% deacetylation; one with a low dd that is insoluble, and the other with a high dd that is soluble in acidic media. Partially deacetylated β-chitins are thus interesting as easily prepared biodegradable materials with finely tuned degradability along with low toxicity. They would be useful for developing advanced applications of chitin. The use of chitin as carriers for drug delivery systems, for instance, is considered promising.

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