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Carbohydrate Polymers 62 (2005) 316–320

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

N-Deacetylation and depolymerization reactions of chitin/chitosan: Influence of the source of chitin

Gemma Galed, Beatriz Miralles, Inés Paños, Alejandro Santiago, Ángeles Heras*

Instituto de Estudios Biofuncionales, Departamento de Quimica Física II, Facultad de Farmacia, Universidad Complutense de Madrid,
Pso. Juan XXIII, no 1. 28040 Madrid, Spain

Received 29 October 2004; revised 3 March 2005; accepted 15 March 2005

Abstract

The deacetylation and depolymerization reactions of chitin/chitosan from three crustacean species (*Paralomis granulosa*, *Lithodes antarcticus* and *Palinurus vulgaris*) were evaluated under the same conditions. The average molecular weight and the mole fraction of *N*-acetylated units were the parameters studied in the resulting chitosans. During the *N*-deacetylation process *P. granulosa*, *L. antarcticus* and *P. vulgaris* follow a *pseudo*-first order kinetics and their apparent rate constants are very similar. However, the degradation rate of chitosan in the first 45 min of this process is higher for *P. vulgaris*. The depolymerization process follows a *pseudo*-first order kinetics for the three species, but in the first 9 min *P. vulgaris* shows a slightly lower depolymerization rate. Hence, depending on the ash contents, crystallinity and the physicochemical characteristics of chitin from these sources, the obtained chitosans show different qualities.

Keywords: Chitosan; Crustacean species; *N*-deacetylation; Degradation; Degree of depolymerization

1. Introduction

Chitosan is a cationic biopolymer obtained from alkaline *N*-deacetylation of chitin where the A-units GlcNAc [β(1→4) 2-acetamido-2 deoxy-β-D-glucopyranose] and D-units Glc [β(1→4) 2-amino-2-deoxy-β-D-glucopyranose] have been shown to be randomly distributed along the chains.

Chitin has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae (Tolaimate, Desbriéres, Rhazi, Alagui, Vincendon, 2000). However, manufactured chitosan is usually obtained from crustaceans (crab, krill and crayfish), especially because a large amount of their exoskeleton is available as a by-product of food processing (Methacanon, Prasitsilp, Pitgsree & Pattaraarchachai, 2003)

The kinetics of heterogeneous alkaline deacetylation of chitin has been studied by several authors as Chang, Tsai; Lee and Fu; (1997) and it has been reported to be a *pseudo*-first order reaction. However, the effect of the biological source was not frequently mentioned.

Chitosan is, like other polysaccharides, susceptible to a variety of degradation mechanisms, including oxidative–reductive free radical depolymerization and acid, alkaline and enzymatic-catalysed hydrolysis. Degradation of polysaccharides occurs via cleavage of the glycosidic bonds. Controlling depolymerization of chitosan is useful in order to adjust properties like viscosity, solubility and biological activity (Rege & Block, 1999). Especially for biomedical applications high quality polymers of known molecular weight are mandatory as well as knowledge of the stability of chitosan over time in solution and in the dry state (shelf life). Roberts (1992); Vårum; Ottøy and Smidsrod, (2001) have reviewed the results on the degradation of chitin and chitosan. The degradation of chitosan has been mostly described for chitosan solutions but the influence of the species in this reaction has not been yet deeply studied.

Chitosans can be chemically depolymerized by different mechanisms (acid hydrolysis, oxidative-reductive and nitrous acid depolymerization). The nitrous acid depolymerization reaction mechanism has been found to be specific in the sense that HONO attacks the amino group

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* Corresponding author. Tel./fax: +34 91 394 32 84.
E-mail address: aheras@pluri.ucm.es (Á. Heras).

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doi:10.1016/j.carbpol.2005.03.019

of D-units, with the subsequent cleavage of the following glycosidic linkage (Allan and Peyron, 1995).

The physicochemical properties of chitosan, as well as its functionality, depend on two fundamental parameters: the degree of deacetylation and the degree of polymerization. These parameters can be influenced by the species and by the conditions used in both reactions.

The purpose of this study was to evaluate the influence of the crustacean source on the deacetylation and depolymerization reactions of chitin and the physicochemical properties of the resultant macromolecules. To this end, the hydrolysis of the *N*-acetyl linkage (*N*-deacetylation) of chitins and the *O*-glycosidic linkages (depolymerization) of partially *N*-acetylated chitosans were studied at fixed conditions for three different crustacean species (*Lithodes antarcticus, Paralomis granulosa* and *Palinurus vulgaris*). The kinetics of *N*-deacetylation and depolymerization reactions were investigated. The mole fraction of *N*-acetylated units (F_A) and average molecular weight ($\bar{M}_w$) of the by-products and the apparent rate constants (k') for these reactions were calculated.

2. Materials and methods

2.1. Materials

Chitins from the species *L. antarcticus, P. granulosa* and *P. vulgaris* were purchased from IDEBIO S.L. (Salamanca, Spain). Samples were milled and sieved. The particle size was between 0.5 and 1 mm. NaOH, KNO_2, acetone and HCl were analytical grade reagents from Panreac Química S.A. (Barcelona, Spain).

2.2. N-Deacetylation

Chitins from the three different species were treated identically with NaOH 50% (wt-%) (10 ml per g of chitin) at 110 °C for 4 h under a N_2 atmosphere (Joensen, 1999) in a batch reactor designed in our laboratory. Samples were taken out of the reactor every 15 min for 4 h (plus a sample at 360 min) and washed with distilled water until pH 7. All the experiments were done in triplicate.

2.3. Depolymerization

The chitosan products obtained from the *N*-deacetylation reaction above mentioned were dissolved (0.68%, wt%) in HCl 0.1 M, stirred for 12 h and filtered through 5 μm TMTP membrane. After that, 1.5 ml of 31.36 mM KNO_2 were added to the solutions and separated in eight different recipients for each selected time (3, 6, 9, 15, 30, 60, 90 and 120 min). The depolymerization was carried out by stirring at 35 °C and was stopped by precipitating the depolymerized chitosans with 80% (v%) acetone in distilled water, washing later with acetone 90% (v%) and pure acetone (Sannan, Kurita, & Iwakura, 1976). All the experiments were done in triplicate.

2.4. Characterization of samples

Chitins from the three sources were characterized by ^{13}C-CP-MAS NMR experiments in solid-state (Heux, Brugnerotto, Desbrières, Versali, & Rinaudo, 2000). The NMR experiments were performed on a Bruker WB DPX-400 spectrometer using the magic angle spinning (MAS) technique. The contact time was 1 ms, the sweep width 40 kHz, and the recycle delay 3 s for the ^{13}C. A typical number of 800 scans were acquired for each spectrum. The chemical shifts were externally referenced to those of adamantane (38.3–29.2 ppm). The spinning speed was set at 7 kHz for all samples. Moisture and ash contents analysis were carried out by gravimetric measures. Protein contents were determined according to the nitrogen content determined by elemental analysis, allowing for the acetylation degree of chitin and the chitin content of the sample (Lizardi et al., 2002).

Chitosans were characterized by measuring their F_A by first derivative UV-spectrophotometric method proposed by Muzzarelli; Rocchetti; Stanic and Weckx (1997). The $\bar{M}_w$ was calculated from the intrinsic viscosity values using the Mark-Houwink equation with the constants $a=0.93$ and $k=1.81\times10^{-3}$ cm^3/g (Roberts, 1992). Data of intrinsic viscosity were measured in an Ubbelohde viscometer (purchased from Schott-Gerate, Germany) at 25 °C using 0.1 M HOAc-0.2 M NaCl as solvent (Roberts, 1992). Two different viscosimeters were used for the characterization of the chitosan hydrolyzates obtained from

Table 1
F_A, Moisture, ash and protein contents and $\bar{M}_w$ of the chitins and chitosans from the three biological species

Samples	F_A	Moisture content (%)	Ash content (%)	Protein content (%)	$\bar{M}_w$ (kDa)
L. antarcticus (chitin)	0.82	1.32	6.25	0	–
P. granulosa (chitin)	0.87	5.94	5.25	5.52	–
P. vulgaris (chitin)	0.77	5.25	0.19	0	–
L. antarcticus (chitosan)	0.16	10.99	0.64	0	490.7
P. granulosa (chitosan)	0.16	9.02	1.64	0	518.0
P. vulgaris (chitosan)	0.14	8.96	0.05	0	423.7

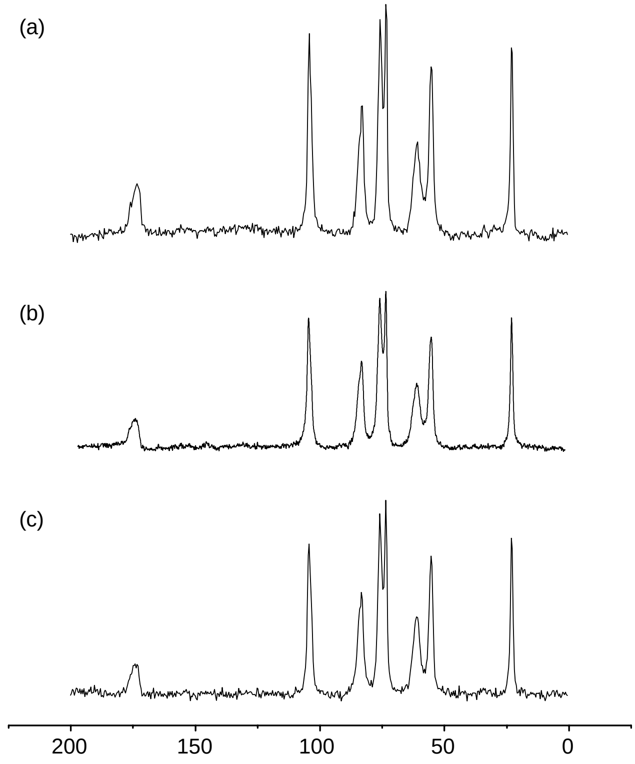

Fig. 1. Solid state ^{13}C-CP-MAS NMR spectra of the chitins obtained from (a) *Lithodes antarcticus*, (b) *Paralomis granulosa* and (c) *Palinurus vulgaris*.

the depolymerization reactions varying the diameter of their capillary (Nm. 525.20II and Nm. 525.13 Ic).

3. Results and discussion

3.1. Characterization of chitins and chitosans

Chitins isolated from the three crustacean species under study were characterized (Table 1). Fig. 1 shows the ^{13}C CP-MAS NMR spectra of the different chitins analysed. Signals in all cases were characteristic of chitins with high acetylation degrees. Single lines could be assigned in each spectrum to six of eight chemically distinct carbon atoms of the 2-deoxy-2-acetamide-D-glucose repeat unit. Spectra showed that the C3 and C5 carbon atoms give two partially resolved peaks for α-chitin (Tanner, Chanzy, Vincendon, Roux, & Gaill, 1990). Final chitosans obtained from these chitins by *N*-deacetylation were also characterized (Table 1).

3.2. N-Deacetylation

Experimental data of the *N*-deacetylation kinetics were studied between 0 and 360 min. Data were modelled via first- and second-order kinetic models and were best adjusted to a first order equation for the first stages of the reaction. The plots between the determined F_A and the deacetylation time are shown on Fig. 2. The apparent rate constants (k') for the deacetylation and correlation coefficients (R) from the regression analysis are shown in Table 2. All species present similar deacetylation rates and in the initial period the deacetylation reaction occurs at a very fast speed under the conditions studied in agreement with previous studies (Focher, Beltrame, Naggi, & Torri, 1990).

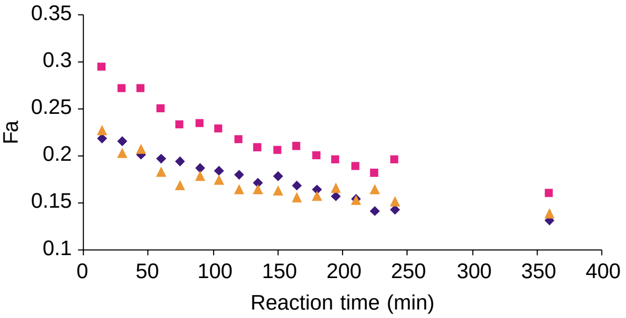

Fig. 2. Plot of the determined F_A versus deacetylation time for the three species (◆*L. antarcticus;* ■ *P. granulosa;* ▲*P. vulgaris).* Deacetylation conditions: NaOH 50% wt%; 110 °C.

Some authors report that the influence of the species is a factor that increases the standard deviation of the rate constant. However, differences in crustacean species and the chitin isolation procedure have shown to cause a variation in morphology or composition between chitin samples, leading to different behaviour during deacetylation (Chang, Viton, & Domard, 1997; Lamarque, Viton, Domard, 2004).

The degradation of the different chitosans along the deacetylation process was studied. Fig. 3 shows the time-course decrease of $\bar{M}_w$ versus deacetylation time. In the range 15–45 min a higher rate of degradation can be observed for *P. vulgaris* compared to *L. antarcticus* and *P. granulosa,* which present a similar trend. From this time a stabilisation of the relative decrease occurs in all cases. The difference among species can be confirmed by the plots of $\ln \bar{M}_{w0}/\bar{M}_{wt}$ versus deacetylation time up to 45 min. *P. vulgaris* presents an apparent rate constant of one order of magnitude higher than those for *L. antarcticus* and *P. granulosa* (Table 3).

In the presence of alkali, polysaccharide chains are found to undergo degradation because of the high concentration of reagents and prolonged reaction times required to obtain a complete deacetylation (Tolaimate et al., 2000). It is well

Table 2
Parameters of the *N*-deacetylation reaction for the three species according to pseudo-first order kinetics

Species	k' (min^{-1})	*R*
L. antarcticus	4.46×10^{-2}	0.8717
P. granulosa	3.89×10^{-2}	0.8950
P. vulgaris	4.45×10^{-2}	0.9004

k'; Apparent kinetic constant and R; correlation coefficient.

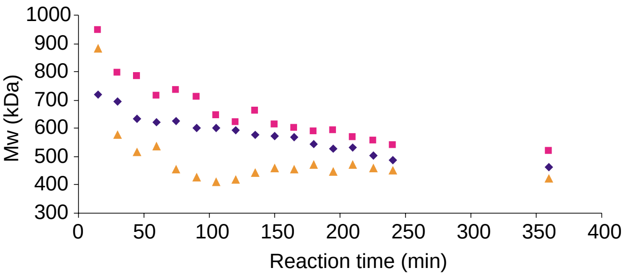

Fig. 3. Plot of the $\bar{M}_w$ decrease versus *N*-deacetylation time for the three species (♦ *L. antarcticus;* ■ *P. granulosa;* ▲ *P. vulgaris*). Deacetylation conditions: NaOH 50% wt%; 110 °C.

known that chitin degradation occurs inevitably during *N*-deacetylation although the molecular mechanism has not been elucidated. Our reaction is carried out in a nitrogen atmosphere to reduce the molecular weight modification caused by oxidative mechanisms, as some authors report (Mima, Miya, Iwamoto, & Yoshikawa, 1983).

The different degradation rates could be due to the different ash content and the cristallinity of samples. In our case, chitins with higher ash contents (Table 1, *L. antarcticus* and *P. granulosa*) presented slower degradation rates during deacetylation. The higher ash content can reduce the accessibility of the reagents to the raw chitin for both species. Some authors consider that the heterogeneous *N*-deacetylation might be controlled by either the reaction on solid surface, or the diffusion of reactant from bulk fluid to the solid surface influencing the rate of the reaction (Levenspiel, 1972).

3.3. Depolymerization

The kinetics of the depolymerization reaction for the chitosans obtained from the three species by nitrous acid was studied between 0 and 120 min. In the plots of $\bar{M}_w$ decrease versus depolymerisation time two domains were observed: a rapid decrease occurred up to 9 min and from this point the $\bar{M}_w$ leveled off (plots not shown).

Plots of ln $\bar{M}_{w0}/\bar{M}_{wt}$ versus depolymerization time in the range 0-9 min were adjusted to a first order equation behaviour (Table 4). For *P. vulgaris* the depolymerisation rate was lower than those of *L. antarcticus* and *P. granulosa*. The differences in the ash content of the initial chitosans cannot explain in this case the different reactivity with regard to the depolymerization reaction. Allan and Peyron (1995) under similar depolymerization conditions have described that chitosan exhibits significantly decreased depolymerization reactivity as the degree of deacetylation of the polymer increases but in the case of our chitosans the initial F_A was similar. These authors reported that the depolymerization rate is independent of the size of the chitosan substrate. This point should be further investigated focusing on other parameters as cristallinity indexes.

Table 3
Results of the *N*-deacetylation reaction for the three species studied, k' apparent kinetic constant for degradation up to 45 min, *R*: correlation coefficient

Species	k' (min^{-1})	*R*
L. antarcticus	4.1×10^{-3}	0.9646
P. granulosa	6.4×10^{-3}	0.9086
P. vulgaris	17.9×10^{-3}	0.9463

Table 4
Results of the depolymerization reaction of chitosans obtained from the three species according to the first order kinetics, k';kinetic constant for the first 9 min, *R*; correlation coefficient

Species	k' (min^{-1})	*R*
L. antarcticus	0.1506	0.9023
P. granulosa	0.1556	0.8916
P. vulgaris	0.1343	0.8901

F_A of the depolymerized samples showed relative standard deviations below 1.5%, so no changes in F_A were found throughout depolymerization (results not shown).

In conclusion, chitin from *P. vulgaris* has similar rate of *N*-deacetylation to the other two species while its degradation rate is one order of magnitude higher. Differences in the degradation rate during the *N*-deacetylation reaction can be attributed to the higher ash content of the chitins from *P. granulosa* and *L. antarcticus* compared to that of *P. vulgaris*. This is in accordance to the fact that the former belong to the same crustacean family (*Lithodidae*).

On the other hand, the rate of depolymerization of *P. vulgaris* is lower than that of *L. antarcticus* and *P. granulosa*. Differences in the depolymerization reaction remain unexplained although they could be related to the biological source of the initial chitosan samples.

Under the conditions studied, chitins and chitosans from *L. antarcticus* and *P. granulosa* presented analogous behaviour. These results show that the biological source of chitin is a key factor in the *N*-deacetylation and depolymerization rates and properties of the resulting chitosans.

Acknowledgements

Financial support from Project MAT2000-0037-P4-03 (Spanish Ministry of Science and Technology) and Idebio S.L. is acknowledged. B. Miralles was the recipient of a post-doctoral fellowship from the Spanish Ministry of Education.

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