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MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Acid Hydrolysis of Chitin and Chitosan

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Abstract—Acid hydrolysis of chitin and chitosan recovered from shells of sea Crustacea was studied by exclusion high-performance liquid chromatography.

Antiarthrosis preparations based on chitin oligomers and glucosamine salts attract growing attention [1, 2]. The yield and quality of glucosamine hydrochloride produced from chitin[1] depend in a complex manner on the chitin properties [3]. To understand this dependence, the mechanism of acid hydrolysis of chitin was studied in more detail.

Acid hydrolysis of chitin (CTN) and chitosan (CSN) was examined in [4–8]. The aim of this work was to determine the limiting degree of chitosan deacetylation suitable for preparing glucosamine. For this purpose, the influence of the degree of chitosan deacetylation on its acid hydrolysis was studied by exclusion high-performance liquid chromatography (HPLC).

EXPERIMENTAL

We used CTN prepared by deproteinization and demineralization of shell of North shrimp (*Pandalus borealis*) by the procedure in [9]. The chitosan content in the sample was 97 wt %; the ash content was lower than 0.1 %; the particle size was no larger than 2 mm.

The chitosan samples were prepared by deacetylation of CTN and CSN. Chitosan with the 68.0% degree of deacetylation (DD) (CSN-1) was obtained by treatment of CTN with 50% aqueous NaOH at 95 ± 5°C for 30 min. The samples with DD of 88.0 (CSN-2) and 92.0% (CTN-3) were prepared under similar conditions from CSN-1 and CSN-2, respectively.

Chitin and chitosan were hydrolyzed with 36.5% HCl at 50 and 70°C. The resulting hydrolyzates were diluted with distilled water, neutralized to pH 3, and filtered through a glass filter with pore diameter of no more than 10 μm (POR 10).

The degree of CSN deacetylation was determined by potentiometric titration of a 2% CSN solution with 0.1 M HCl by the procedure in [10]. The absolute error of the determination was 0.5%.

The hydrolysis products of CTN and CSN were separated by HPLC on an $LC10A_{vp}$ chromatograph (Shimadzu, Japan) with TSK-gel Alpha-2500 (30 × 0.78 cm) column and TSK-guardcolumn Alpha (6 × 0.4 cm) precolumn (TOSOH, Japan). The hydrolyzate samples were applied in the form of 0.1% solutions in the eluent (0.3 M NaCl acidified with HCl to pH 3). The hydrolysis products were detected by the absorption at 210 nm.

The UV detection of the hydrolysis products allowed us to monitor changes in the concentration of acetylated compounds and acetic acid. The considerably weaker absorption of deacetylated CSN oligomers and *D*-glucosamine (GA) was obscured by the strong band of the acetylated products. Monomeric *N*-acetylated glucosamine (AcGA) was identified by comparing its retention time (9.78 min) with that of pure GA (9.86 min). For this purpose, a 5% GA solution in the eluent was used.

Based on the published chromatographic data on CTN oligomers [5–7], we assigned the other chromatographic peaks to the acetylated oligomers (Fig. 1). If peak *2* is due to AcGA, then peaks *3* and *4* are assigned to *N*,*N*'-diacetylchitobiose and *N*,*N*',*N*''-triacetylchitotriose, respectively. The semilog plot of the molecular weight (MW) of these compounds on the elution time τ is linear. The nonlinear deviation for GA can be due to either characteristics of the linearity the column or structural difference of the deacetylated monomer from AcGA. Hence, GA cannot be used to construct the calibration curve.

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It is known [4, 8] that the rate of formation of AcGA and GA in the course of acid hydrolysis of CTN and CSN decreases with an increase in their DD.

The influence of DD on the rate of acid hydrolysis of CTN and CSN is seen from the chromatograms of their hydrolyzates. At 50°C, CTN is hydrolyzed incompletely to form a mixture of macromolecular and low-molecular-weight products (Fig. 2). As the hydrolysis time increases, the peaks of the macromolecular products shift toward lower molecular weights and decrease in the integral intensity; the intensity of the peaks of the oligomers and monomers increases. It should be noted, however, that the integral intensity of peaks cannot be a measure of chitin depolymerization, since depolymerization is accompanied by formation of deacetylation products whose molar extinction coefficient is lower than that of the acetylated products.

The content of the macromolecular compounds in the products of hydrolysis of CTN and CSN-1 (DD = 68%) at 70°C sharply decreases (Fig. 3). The products of hydrolysis performed for more than 10 min contain mainly oligomers, monomers, and acetic acid; the polymer concentration is very low.

In the products of hydrolysis of CSN-2 and -3 (DD 88 and 92%), the peak of macromolecular fractions is preserved even after hydrolysis for 300 min. It should be noted that a chitosan precipitate in the form of a fine dispersion and with the outward appearance of the initial sample was observed after 5-h hydrolysis of CSN-2 and CSN-3, respectively.

The degree of hydrolysis was estimated from the AcGA yield. The AcGA amount was recalculated to the number of acetyl groups in CTN with DD = 0%, taking the initial DD into account. The AcGA yield increases in the first hydrolysis steps owing to cleavage of glycoside bonds of the macromolecules and then decreases owing to deacetylation (Fig. 4). The AcGA yield in hydrolysis of CTN and CSN, performed for the same time, sharply decreases with an increase in DD of the polysaccharide (Fig. 5).

This result agrees with the fact that the glycoside bond in acetylated monomeric chains is more readily hydrolyzed than the bond in deacetylated chains [8]. Based on relative deceleration of depolymerization with respect to the deactylation rate at high acid concentrations, Varum *et al*. [8] suggested different hydrolysis mechanisms of *N*-acetyl and glycoside bonds. Probably the *N*-acetyl bond is hydrolyzed by the S_4N2 mechanism in which the rate-determining step is addition of water molecule to the carbocation. As the acid concentration increases, the concentration of water

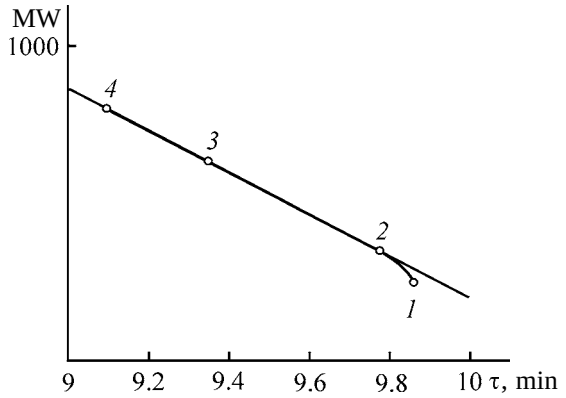

Fig. 1. Calibration curve for chromatographic determination of mono- and polysaccharides. (MW) Molecular weight and (τ) retention time. (*1*) GA, (*2*) AcGA, (*3*) chitobiose, and (*4*) chitotriose.

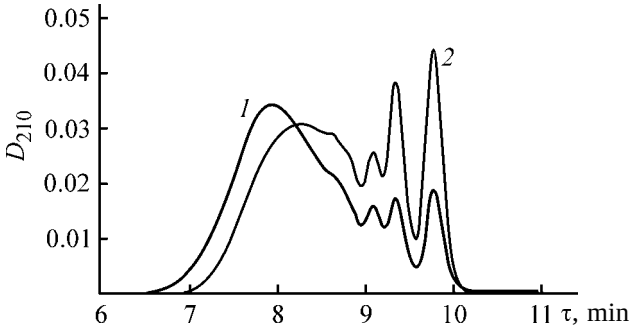

Fig. 2. Chromatograms of CTN hydrolyzates obtained at 50°C, normalized to the equivalent amount of acetyl groups corresponding to DD = 0%; the same for Fig. 3. (D_{210}) Optical density at 210 nm, and (τ) retention time. Hydrolysis time, min: (*1*) 20 and (*2*) 40.

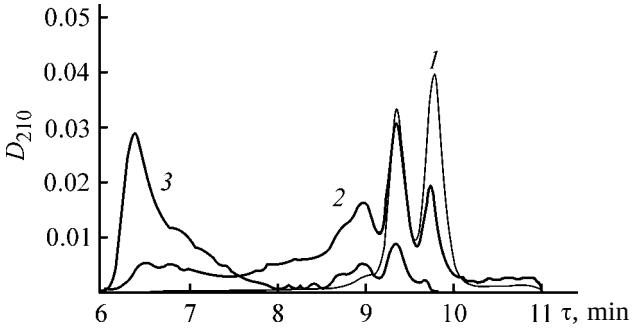

Fig. 3. Chromatograms of hydrolyzates of (*1*) CTN, (*2*) CSN-2, and (*3*) CSN-3 at 70°C (120 min). (D_{210}) Optical density at 210 nm and (τ) retention time.

molecules decreases and the reaction decelerates. The glycoside bond is hydrolyzed by the S_N1 mechanism in which the rate-determining step is the proton attack to form carbocation. In this case, the reaction rate is independent of the water concentration.

However, the influence of the acetyl substituent at the nitrogen atom of a CTN molecule on the depoly-

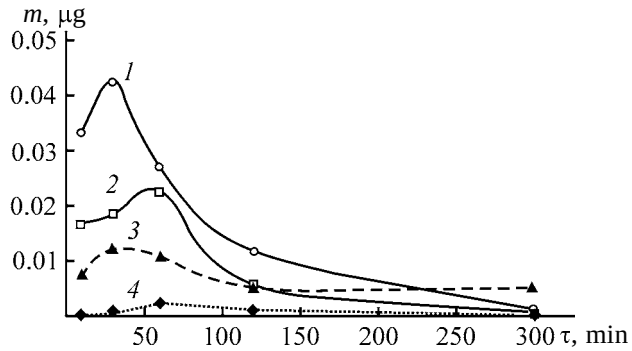

Fig. 4. Kinetics of AcGA formation in hydrolysis of (*1*) CTN, (*2*) CSN-1, (*3*) CSN-2, and (*4*) CSN-3. Temperature 70°C; the same for Figs 5 and 6. (*m*) AcGa weight and (τ) hydrolysis time.

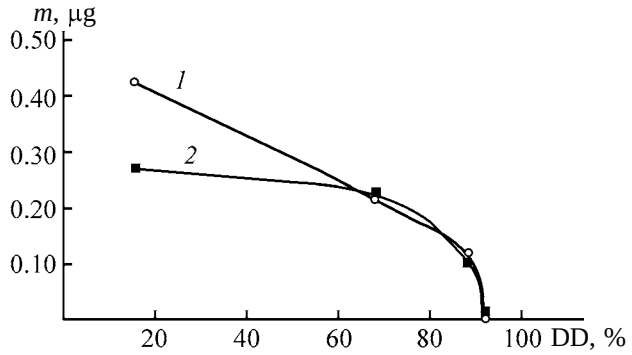

Fig. 5. Yield of AcGA as a functions of DD of CTN and CSN hydrolyzed for (*1*) 30 and (*2*) 60 min. (*m*) AcGa weight and (DD) degree of deacetylation.

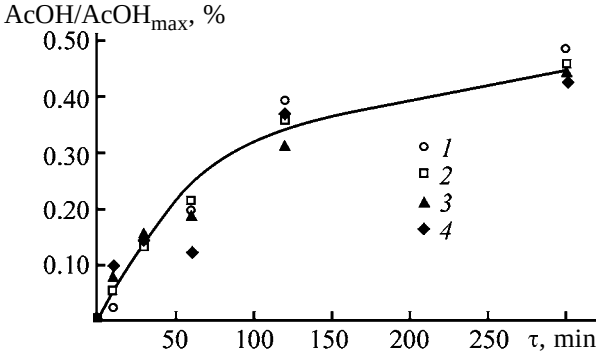

Fig. 6. Rate of AcOH formation in hydrolysis of (*1*) CTN, (*2*) CSN-1, (*3*) CSN-2, and (*4*) CSN-3. ([AcOH]/[AcOH]$_{max}$) Relative AcOH concentration and (τ) hydrolysis time.

merization rate cannot be explained within the framework of the proposed hydrolysis mechanism of the glycoside bond. Computer simulation of the electron density distribution in the molecule showed no appreciable increase in the excess negative charge on the atoms of the glycoside bond after acetylation of the amino group. This charge promotes the proton attack. To understand the hydrolysis mechanism of glycoside bonds in a chitin molecule, more comprehensive studies are required.

It is of practical importance that partially deacetylated CTN or CSN are completely hydrolyzed to AcGA and GA in a time longer than that required for hydrolysis of CTN with minimal DD.

The GA concentration in the CTN hydrolyzate is low, and this product cannot be detected by UV absorption since its molar extinction coefficient is small. Therefore, deacetylation of the acetylated products was monitored by the rate of acetic acid formation (Fig. 6).

The AcOH amount was estimated in relative units from chromatographic peak area (retention time 14.14 min). The amount of AcOH that could be released upon complete acid hydrolysis of CTN with DD = 0% was taken as the maximal. A sample containing the calculated amount of AcOH was introduced in the column, and the peak area was measured.

As seen from Fig. 6, the deacetylation rate is almost independent of the initial DD. This is also the case for the deacetylation of CTN and CSN in alkaline solution [11].

Thus, Figs. 4 and 6 show that depolymerization of CTN and CSN depends on the initial degree of their deacetylation. The higher DD, the lower is AcGA yield. The deacetylation course depends only on the experimental hydrolysis conditions and is independent of DD of CTN and CSN. Hence, the steps of deproteinization and demineralization of chitin production should be performed with care to avoid uncontrolled deacetylation affecting the GA yield.

The limiting degree of deacetylation providing optimal hydrolysis of CTN can be estimated from the dependence of the AcGA yield on DD, assuming that this dependence is linear in the DD range from 15 to 68%. We suggest that a decrease in the monomer yield in the course of hydrolysis should not exceed 10% of its yield in hydrolysis of commonly used CTN with DD ~15%. Our calculations show that the DD of CTN should not exceed 26%. The yield estimated by extrapolation to zero DD is higher by 14% than that of AcGA formed in hydrolysis of commonly used CTN. Hence, the production process of glucosamine can be further improved.

CONCLUSIONS

(1) The study of acid hydrolysis of chitin and chitosan by HPLC shows that accumulation of oligomers and monomers of chitin and chitosan decelerates with

increase in the degree of deacetylation and the deacetylation rate is independent of the initial degree of deacetylation of chitin and chitosan.

(2) The minimal degree of deacetylation of chitin suitable for glucosamine production is 26%.

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