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SHORT COMMUNICATION

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Chitosan structure in aqueous solution

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Abstract Chitosans having three different degrees of acetylation (*DA*) were studied in acid solution using the uranyl staining technique and electron microscopy. Strings of approximately spherical aggregates were seen. The aggregates were interpreted as micelle-like agglomerates formed by almost fully acetylated polysaccharide, interconnected by blocks of almost fully deacetylated polysaccharide stretched by electrostatic repulsion. These agglomerates include NH_3^+ groups which produce electrostatic swelling of the agglomerates, giving a radius proportional to the degree of deacetylation. The length of the strings are also proportional to the deacetylation degree. These strings are extended because of the electrostatic repulsion between charged ammonium groups.

Keywords Chitosan · Solution conformation · Acetylation degree · Uranyl staining

Introduction

Chitin is one of the most widespread of the naturally occurring polysaccharides. It is found in the exoskeleton of many arthropods and is composed of $\beta(1 \rightarrow 4)$-D-glucosamine units with a variable degree of *N*-acetylation (*DA*) [1]. The distribution of the *N*-acetyl groups along the polymeric backbone may control the solubility in a given solvent; chitin ($DA \sim 1$) is especially soluble in dimethylacetamide-lithium chloride mixtures and insoluble in aqueous solution. When the average *DA* is lower than approximately 0.5, the polymers are called chitosans and they become soluble in aqueous solutions in the presence of acids such as acetic acid [2]. For rational uses of chitosan, designed for specific applications, it is essential to understand its solution behavior.

The chemical structure has been ascertained with the help of methods for the determination of the *DA* and the distribution of acetyl groups along the linear copolymer. In contrast, a lot of contradicting results have been published on the chain conformation in solution [3]. A central point of the current debate is whether or not a varying degree of acetylation causes any changes in the expansion and stiffness of the chains or the tendency to aggregate [4]. Two effects have to be considered. On the one hand, a higher content of bulky acetyl groups may increase the stiffness of the chains for steric reasons. On the other hand, a lower *DA* automatically means a higher number of amino groups, which in acidic solution are protonated producing the stretching and some stiffness of the flexible chains because of the mutual electrostatic repulsion.

There is a full agreement that chitosans form single helicoidal stiff chains in acidic aqueous solution [3]. The Trondheim group has obtained a lot of evidence for a higher expansion and stiffness with increasing *DA* [5, 6, 7]. Nevertheless, the opposite effect, namely higher compactness for molecules of a higher *DA*, has been described by Errington et al [8].

The present study is an attempt to elucidate this controversy by direct visualization of chitosan structure in solution by uranyl staining and transmission electron microscopy (TEM).

It is sometimes argued that the morphology of aqueous aggregates is not preserved at the stage of the electron microscopic observation under high vacuum. However, for numerous biological samples it has been proven that staining agents can fix the aggregate morphology. Kunikate et al [9, 10]. have demonstrated that electron micrographs of negatively stained samples showed the morphologies of aggregates of a large number of surfactants in water in complete agreement with the findings with other techniques. In these experiments, the sample solution was mixed with an aqueous solution of staining agents and was allowed to spread on a carbon-coated Cu grid as a thin film and dried.

Chitosan has a remarkable ability to form specific complexes with a number of ions including transition and post-transition metal ions [11]. It is generally accepted that the free amino groups of chitosan are responsible for its complexing behavior with ions. As uranyl ion has a slight preference for ligands with nitrogen [12, 13], we assumed that it might be used as a staining agent for chitosan.

In the present work we report results on three commercial chitosans having different *DA*.

Experimental

Commercial samples of chitosan were obtained from Primex (TM818, *DA*% = 18; TM816, *DA*% = 7) and from Daras (*DA*% = 16). They were dissolved in acetic acid 0.5 M.

One milliliter of each sample solution (0.5 wt %) was mixed with 1 mL of 2% aqueous uranyl acetate and sonicated for approximately 20 s in an ultrasonic bath. Then the mixture was incubated in an ice-water bath for 30 minutes, applied to a carbon-coated Cu grid, and then dried under vacuum.

A JEOL 100 CX II transmission electron microscope was used for the measurement, operating at 100 kV with a magnification of 100,000×.

A measurement microscope by Chesterman (Sheffield, England), with a precision of 1/50 mm, was used for analyzing the micrographs.

Results

All the TEM micrographs showed that the structures of chitosan in solution are formed by a number of roughly spherical agglomerates intercalated with very thin straightened fibers (Fig. 1). The structure seems to be a long thread with some pearls inserted, which are not in contact among them.

The radii of the spherical structures and the length of the strings were measured on the photographs using the measurement microscope, and results are summarized in Table 1 and Figures 2 and 3.

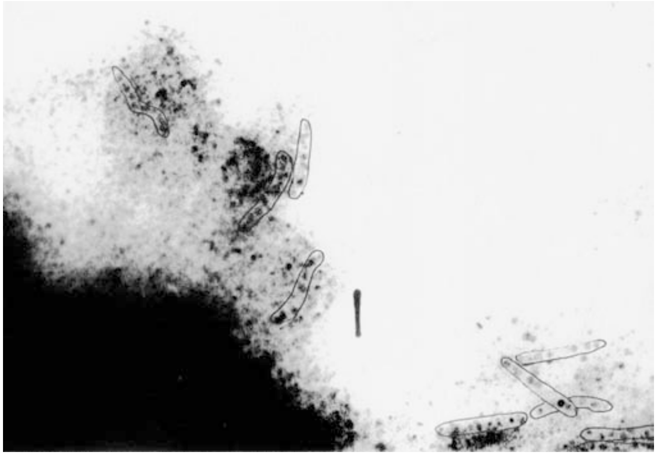

Fig. 1 TEM photomicrograph of uranyl stained Daras chitosan, *DA* = 16%, showing the strings of blocks. Some of the strings are marked on the photo. The bar length is 48.6 nm

Table 1 Influence of the *D*A on chain length and micelle size. The error values were computed with a confidence level of 0.90

Chitosan sample	DA (%)	Chain length (nm)	Micelle size (nm)
TM-816	7	209.5 ± 1.2	17.96 ± 0.07
Daras	16	141.5 ± 0.6	14.95 ± 0.08
TM-818	18	122.19 ± 0.85	13.74 ± 0.17

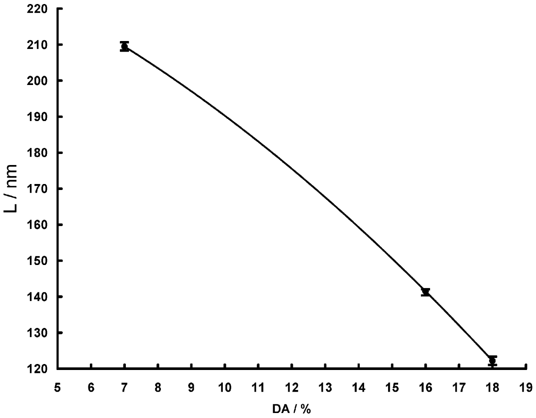

Fig. 2 Plot of the chain length *L* versus the degree of acetylation *DA*. The data fit to a parabolic curve with equation *L* (nm) = $-0.191*DA^2 - 3.16*DA + 239.8$ with $r = 1.0$

Discussion

The strings were interpreted as worm-like single chains of chitosan, as reported in the literature [3]. These chains can be considered as block-type copolymers, composed of blocks of almost 100% acetylated polysaccharide

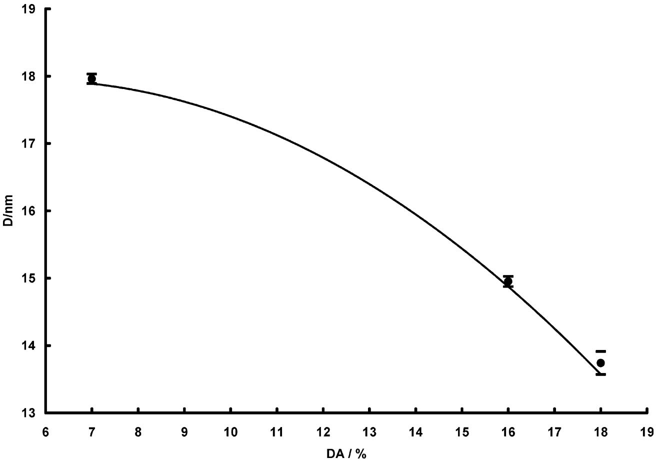

Fig. 3 Plot of the micelle size *D* versus the degree of acetylation *DA*. The data fit to a parabolic curve with equation L (nm) = $-0.0288*DA^2 + 0.326*DA + 17.0$ with $r = 1.0$

intercalated with blocks of highly deacetylated chains. This structure was also previously proposed in literature [14, 15]. The deacetylated chains are fully stretched by the electrostatic repulsion among the $-NH_3^+$ groups. The acetylated blocks are micelle-like agglomerates because of the hydrophobic forces.

The inverse relation between *DA* and string length is easily explained by the electrostatic stretching of the highly deacetylated chains, because a lower *DA* means a higher proportion of $-NH_3^+$ groups in the molecule.

The inverse relation between the micelle-like agglomerates' radius and the DA may be explained if it is assumed that the blocks are not fully acetylated. Assuming that some of the glucosamine units in the micelle-like region are deacetylated, and that their proportion increases with the total deacetylation, the resulting $-NH_3^+$ groups will produce swelling of the agglomerates because of their mutual electrostatic repulsion [16]. This is a reasonable explanation of the increase of the spheroidal agglomerates' radius with the total deacetylation of the molecule.

Conclusions

In solution, Chitosan is composed by a string of blocks of almost fully deacetylated polysaccharide stretched by electrostatic repulsion, intercalated with micelle-like agglomerates formed by almost fully acetylated polysaccharide. These agglomerates include $-NH_3^+$ groups which produce electrostatic swelling of the agglomerates, giving a radius proportional to the deacetylation degree. The length of the strings are also proportional to the deacetylation degree. These strings are extended because of the electrostatic repulsion between charged ammonium groups.

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