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STRUCTURAL INVESTIGATIONS OF CHITIN AND ITS DEACETYLATION PRODUCTS

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The structure of crab chitin and chitosan from it with various degrees of deacetylation (DDA) are studied by x-ray diffraction, IR spectroscopy, and microscopy. Deacetylation causes substantial destruction of the chitin crystal structure, makes it amorphous, increases the defectiveness of crystallites as the DDA increases, weakens intermolecular H-bonds, and eliminates the fibrillarity.

Key words: chitin, chitosans, structure.

Chitin and its derivatives (chitosans) are one of the most important and widely distributed natural polymers. They have been the subject of many investigations [1-7].

We systematically studied the structural features of chitin isolated from crustaceans of the Aral Sea, the shrimp *Palaemon elegans* and the crab *Phitrophanapeus harrissi*, and its variously deacetylated products, chitosans, as biologically active stimulants and fungicides during processing of cotton seeds and in several other areas [8-13].

X-ray diffraction of crab chitin and chitosans based on it with degrees of deacetylation (DDA) 8, 36, 57, and 81% showed that the x-ray diffraction pattern of chitosan with DDA 8% is almost the same as that of the starting chitin (Fig. 1). The starting chitin has the most common α-form with an orthorhombic cell. Several diffraction maxima of various intensity are observed at 2θ = 19.5, 26.5, 32.2, 34.6, and 39.0°. The strongest peak lies at 2θ = 19.5°. The interplanar spacings that are calculated from the Bragg equation at these angles are 0.46, 0.386, 0.33 nm, etc. α-Chitin is easily identified by them. Increasing the DDA to 36% destroys the structural regularity and decreases the degree of three-dimensional order in chitin. This is evident as a decrease in the degree of crystallinity (DC). The intensities of the principal equatorial reflections decrease markedly. Certain of them almost disappear (2θ = 32.2 and 26.5°). The strongest peak at 2θ = 19.5° also weakens and slightly broadens. This indicates a decrease in the dimensions of crystalline regions in chitin upon deacetylation. The sample with DDA 57% is still more amorphous. The diffraction patterns contain only one peak at 2θ = 19.5°. The remaining reflections are not observed. Chitosan with a high DDA (81%) gives only a weak and broad peak at 19.5°. Its half-width increases by 1.5 times, which indicates that the number and size of the crystalline regions have decreased further and that the number of defects increased. This is also indicated by results estimating the DC, which is 80, 78, 64, and 48% for chitosan samples with DDA 8, 36, 57, and 81%, respectively.

Thus, the results suggest that the chitin structure is continuously made more amorphous during the conversion to chitosan. The crystalline regions that remain in the chitin structure, although highly defective, do not form a new crystalline structure (chitosan lattice) although it has been reported in the literature that chitosan can crystallize and that it is amorphous [7]. Obviously, the ability of chitosan to crystallize should depend on the regular distribution of substituting groups along the chain, which in turn may be related to the method of converting chitin to chitosan.

Scanning-electron and optical microscopy of these samples revealed plate-like portions of various dimensions that are smooth or fibrillar. These are especially evident in the starting chitin. As the DDA increases, the quantity of the latter decreases and portions with a defective surface appear and increase in number. Certain portions, regardless of the DDA, are birefringent in polarized light. This is consistent with crystallinity or anisotropic structural elements (Fig. 2).

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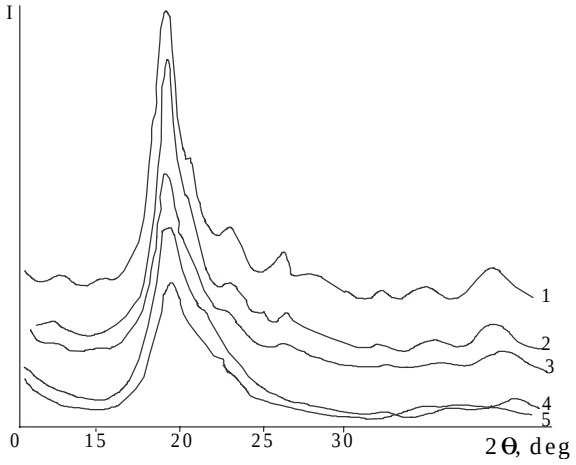

Fig. 1. X-ray diffractograms of chitin (1) and chitosans of various DDA: 8% (2), 36% (3), 57% (4), and 81% (5).

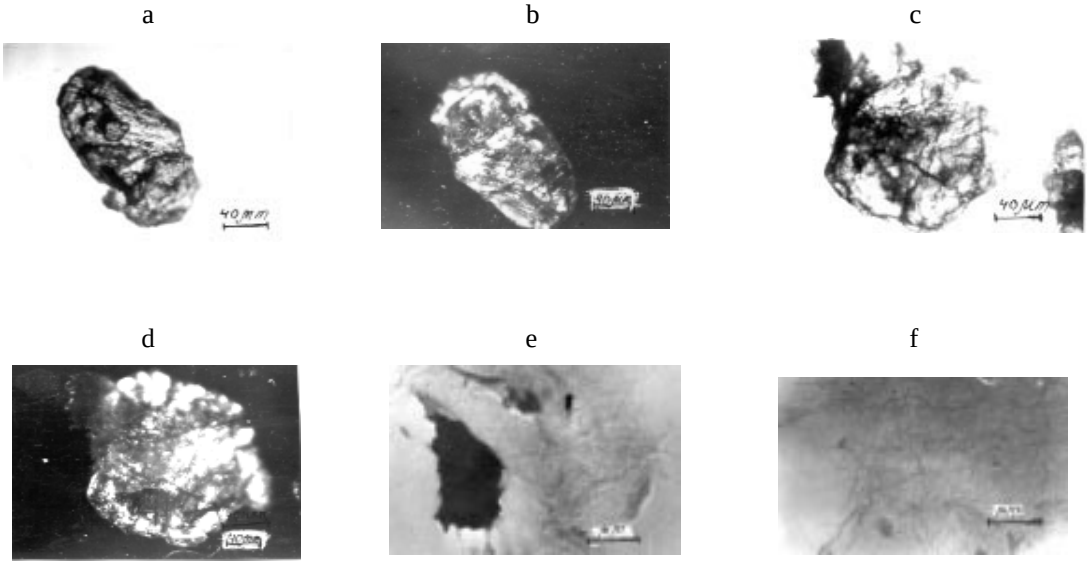

Fig. 2. Optical (*a* - *d*) and scanning electron - microscope (*e*, *f*) images of chitin and chitosans of various DDA: *a* - 8%, *b* - is the same as in polarizing light, *c* - 57%, *d* - is the same as in polyrizing light, *e* - hydrolyzed chitin, *f* - hydrolyzed chitosan - 81%.

Figure 3 shows transmission electron micrographs of dispersions of the hydrolyzed samples. It can be seen that layers of the fibrillar material and a large quantity of thin needle-like crystallites that are similar to cellulose crystallites remain in the starting chitin after removal of the amorphous fraction through hydrolysis. Starting at DDA 8%, the layers disappear in the chitosan samples owing to destruction of the regular chains and the H-bonding network. Only crystallites of the elongated needle-like shape are observed. The number of these decreases with increasing DDA. This is consistent with the decreasing DC that was seen in the x-ray diffraction studies.

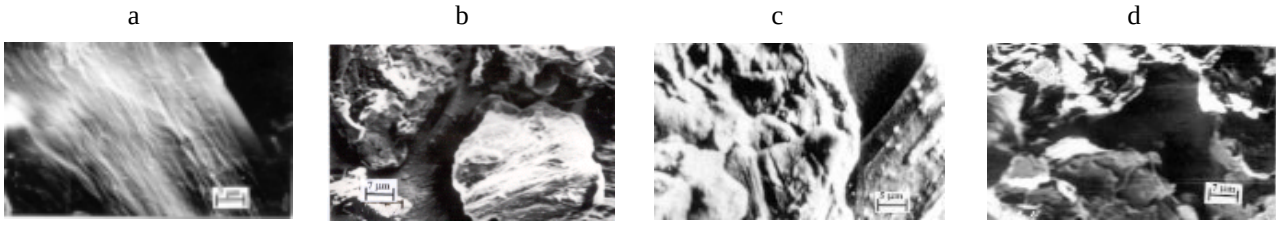

Fig. 3. Scanning electro-microscope images of chitin (*a*) and chitosans of various DDA (*b* - 36%, *c* - 57%, *d* - 81%).

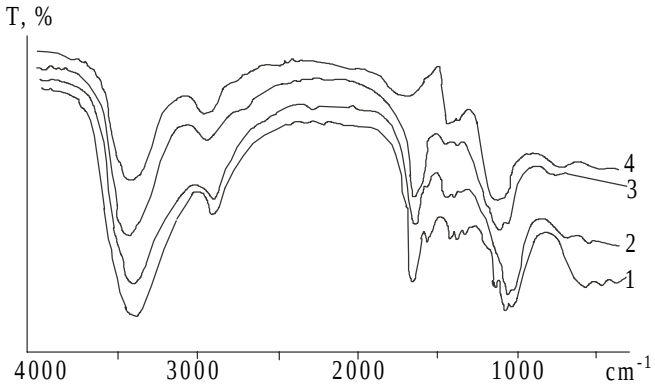

Fig. 4. IR spectra of chitosan of various DDA: 8% (1), 36% (2), 57% (3), and 81% (4).

This is also confirmed by IR spectroscopy of chitin with various DDA (Fig. 4). The IR spectrum of the sample with DDA 8% does not differ noticeably from that of untreated chitin. It contains absorption bands at 3200-3500 cm^{-1} (–NH and –OH stretching vibrations, including H-bonds), –CH and $–CH_2$ stretching vibrations at 2870-2845 cm^{-1}, an amide-I band at 1685 cm^{-1}, absorption bands due to –CH and –OH bending vibrations and C–CH and COC stretching vibrations at 1000-1500 cm^{-1}, and absorption bands in the so-called structurally sensitive region 500-900 cm^{-1}. Absorptions at 1665, 1415, 750, and 520 cm^{-1} identify α-chitin [2]. At DDA 36% and higher, the spectrum undergoes definite changes. The –OH and –NH absorptions, including H-bonds, at 3500-3250 cm^{-1} decrease in intensity. This is usually observed as the DC of polysaccharides decreases. This band also shifts to higher wavenumbers. This is a sign of weakening H-bonds and is related to amorphization of polysaccharides. Whereas this band has a maximum at 3380 cm^{-1} in the sample with DDA 8%, it is situated at 3430 cm^{-1} at DDA 36%. Furthermore, the intensity of the band at 1665 cm^{-1} decreases although its half-width remains constant ($\Delta\nu$ 1/2). Finally, no absorption bands are observed in general at 500-900 cm^{-1} in samples with DDA 57% and greater. This region for polysaccharides is known to be sensitive to the structure. The weakening and disappearance of these bands reflects structural changes (amorphization) of the samples [14].

Thus, definite changes in the molecular and structural features are detected during deacetylation of chitin and its conversion to chitosan. During the process the chitin becomes more amorphous as the DDA increases. This indicates a decreasing DC, fibrillar nature, crystallite dimensions, H-bond strength, etc. Chitosan does not crystallize at any DDA, which is consistent with an irregular distribution of $–NH_2$ groups in the macromolecule.

A parallel can be drawn between the structural changes of chitin during deacetylation and cellulose during acetylation [15]. In both instances the microfibrillar structure is destroyed and the crystallite dimensions decrease. These are accompanied by a decrease in the DC and amorphization of the polysaccharide. However, noticeable changes in cellulose are observed at a smaller degree of modification (acetylation). Obviously this is due to the higher crystallinity of chitin compared with cellulose.

EXPERIMENTAL

Chitosan of various DDA was prepared from crab (*Phitrophanapeus harrissi*) chitin by treatment with 50-60% NaOH at 140°C for 2-6 h.

X-ray diffraction was performed on a DRON 3M diffractometer using monochromatized CuK α-radiation in the range $2\theta = 10\text{-}35^\circ$. Samples were prepared as pellets by pressing powders. The DC was determined from the ratio of peak intensities:

$$DC(\%) = 100\% \times (I_{cr} - I_{am})/I_{cr},$$

where I_{cr} is the peak intensity at $2\theta = 19.5^\circ$ and I_{am} is the intensity of amorphous scattering.

IR spectra were recorded on a Specord-75 IR spectrometer in the range 500-4000 cm^{-1}. Samples were prepared as pellets by pressing powders with KBr.

Scanning electron microscopy (SEM) was carried out on a REM-200 instrument; transmission (TEM), on a PEM-100 electron microscope. A layer of Ag was placed on the samples in a VUP-4K vacuum chamber for SEM studies. Samples were hydrolyzed in boiling 2.5 N HCl for 2 h for TEM studies. A suspension of the hydrolyzed samples was washed with distilled water, placed on the screen, and coated with Pt.

Microscopic studies were performed on an MBI-6 optical microscope in transmitted and polarized light.

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