the polymer backbone may be an essential process. There are two possible processes for the solvation in polymeric media: one is the solvation by intrapolymer polar groups and the other is that by interpolymer polar groups. The former process may involve the cooperative interaction of the neighboring polar groups in the polymer backbone with an ion. We considered that the former process was more important for the carrier generation in polymeric media. The polar groups in PE-2,4 exist in high density, whereas those in PE-2,10 are far separated by octamethylene units. Thus, PE-2,4 may favor the former process. In contrast, PE-2,10 does not favor the former process because of the entropical instability. Thus, the ion dissociation in PE-2,10 may be suppressed, especially in the NaSCN and KSCN complexes, which may be responsible for the lower ionic conductivity.

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Binding Sites of Cu^{2+} in Chitin and Chitosan. An Electron Spin Resonance Study

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ABSTRACT: X-band ESR spectra of copper at high dilution in chitin and chitosan were measured at 77 and 297 K. Two bonding sites for Cu^{2+} in chitin were observed. Site 1, with $g_{\parallel}^{(1)} = 2.244$, $g_{\perp}^{(1)} = 2.069$, $A_{\parallel}^{(1)} = 188 \times 10^4$ cm^{-1}, and $A_{\perp}^{(1)} = 30 \times 10^{-4}$ cm^{-1} at 77 K, is dominant. Site 2 has approximately the same values of $g_{\perp}$ and $A_{\perp}$ as site 1 but $g_{\parallel}^{(2)} = 2.327$ and $A_{\parallel}^{(2)} = 162 \times 10^{-4}$ cm^{-1} at 77 K. One bonding site for Cu^{2+} in chitosan was observed, identical with site 1 in chitin. Analysis of the ESR parameters suggests that site 1 represents a square-planar arrangement of four nitrogen ligands around Cu^{2+}, while site 2 represents a tetrahedrally distorted structure of three N and one O *or* two N and two O ligands. Examination of the crystal structure of α-chitin suggests that a site consisting of two N and two O ligands is more probable. In both chitin and chitosan the suggested structures imply that modification of interchain distances occurs on chelation.

Introduction

Chitin is a naturally occurring polymer of the formula poly[(1→4)-*N*-acetyl-β-D-glucosamine], and chitosan is the corresponding N-deacetylated compound. The theoretical nitrogen percent is 6.89 in chitin and 8.70 in the completely deacetylated chitosan. In practice, because deacetylation is usually incomplete, chitin and chitosan represent a range of compounds with varying nitrogen content between the above limits. The ability of these compounds to bind selectively metal ions has been recognized for some time.[1] While the ability of chitin and chitosan to retain alkali and alkaline earth metal ions is extremely low, most transition-metal ions of the first row are strongly chelated, with zinc, nickel, and copper strongly and selectively favored.

Copper chelation has been studied extensively as a function of temperature, pH, time of contact with the metal solutions, and various degrees of chitin deacetylation[2,3] and polymer crystallinity. It was found that the chelating ability of chitosan is much greater than that of chitin, a result that has been associated with the higher amino group content in chitosan. Plots of percent copper ion collection vs. amino group content in chitosans with various degrees of deacetylation did not indicate a linear relationship, suggesting that other factors affect the ion binding.[3b] The role of the active hydroxy groups at the C_3 and C_6 positions in the chelation process is evident in a study that shows that the binding of copper and iron ions is reduced in chitin by methylation or acetylation.[4] Some evidence has been presented that more than one active binding site exists.[1,5] This conclusion was based on the fact that adsorption of a metal by chitin and chitosan from mixed-metal solutions was not proportional to the metal concentration in solution. No specific binding scheme or geometry was deduced or suggested in these studies.[5]

The purpose of this study is to obtain structural information on the metal ion ligation in chitin and chitosan using the technique of electron spin resonance (ESR). In many cases, ESR parameters of paramagnetic ions have been interpreted in terms of ligation to specific atoms in a variety of complexes, including complicated biological systems.[6-9] In this study we have detected for Cu^{2+} one binding site in chitosan and two binding sites in chitin. Specific coordination around the metal ion is suggested for each site.

Experimental Section

Chitin purified from crustacean shells and chitosan from crustacean chitin were obtained from Calbiochem-Behring Corp. of La Jolla, CA. Copper chelation was obtained by mixing a suspension of 0.1 g of chitin or chitosan in 20 cm^3 of D_2O and adding $CuSO_4 \cdot 5H_2O$ to an approximate molar ratio of 1/100 for Cu to monomeric chitin or chitosan. The suspension was stirred for 24 h, filtered, rinsed with D_2O, dried, and kept in a desiccator.[10]

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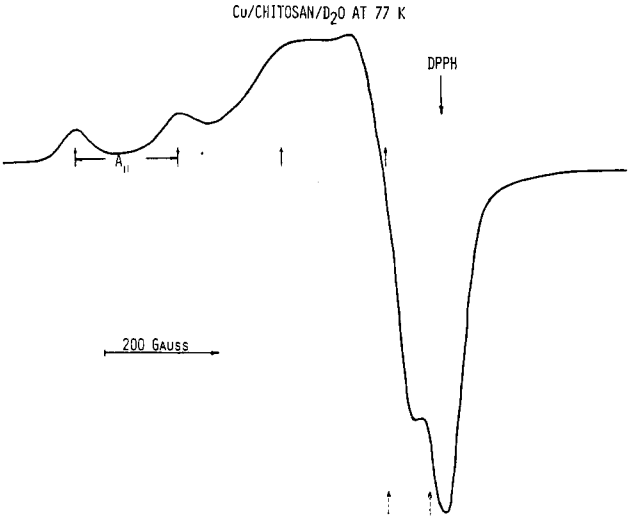

Figure 1. X-band ESR spectrum of Cu^{2+} in chitosan at 77 K. Solid upward arrows indicated the four components of the parallel orientation. Broken upward arrows indicate the positions of the extra transitions calculated in the Appendix.

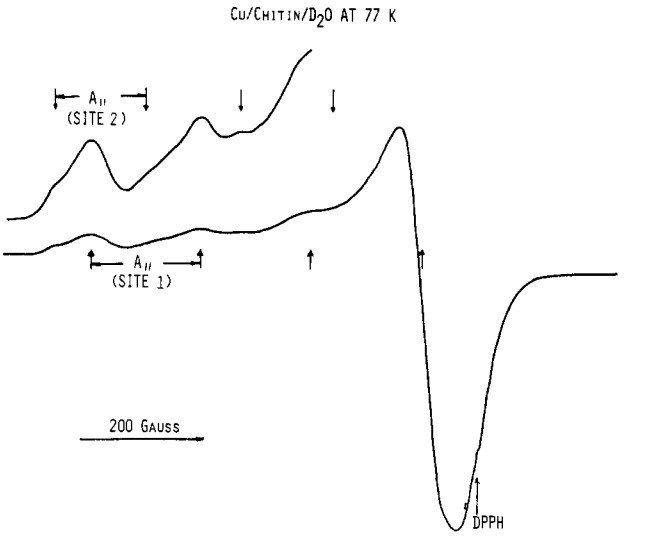

Figure 2. X-band ESR spectrum of Cu^{2+} in chitin at 77 K. The four components of the parallel orientation are indicated for sites 1 and 2.

Some preparations contained an additional amount of $ZnSO_4 \cdot 7H_2O$, to obtain a molar ratio 1/6 for Zn to monomeric chitosan, while maintaining the 1/100 molar ratio for Cu to chitosan. It was essential to maintain a low Cu^{2+} concentration in order to prevent the broadening of ESR lines by dipolar interactions.

For ESR measurements, samples were transferred to 4-mm-o.d. quartz tubes, evacuated to 10^{-4} torr, and sealed under vacuum. ESR spectra were measured with a Varian E-4 spectrometer operating at 9.3 GHz. Spectra at 77 K were taken in a liquid nitrogen Dewar flask inserted in the ESR cavity. DPPH (g = 2.0036) was used for the g value calibration. The scan in gauss was calibrated with a ^{55}Mn-doped MgO single crystal. The center interval of the hyperfine sextet was taken as 86.7 G. The microwave frequency was calibrated with a Hewlett-Packard 5342A microwave frequency counter.

Results

ESR spectra at 77 and 297 K are very similar for the samples studied. A typical spectrum for Cu^{2+} in chitosan at 77 K is shown in Figure 1, together with the "stick" diagram. $A_\parallel$, $g_\parallel$, and $g_\perp$ are read directly from the spectrum. $A_\perp$ is estimated to be $(30 \pm 5) \times 10^{-4}$ cm^{-1}. The strong absorption that appears at 3300 G is an "extra" transition of the type sometimes observed in powder spectra.[6,11] The position of this line, calculated in the Appendix, is in agreement with experimental results and provides an additional check for the measured ESR parameters for chitosan.

ESR spectra of Cu^{2+} in chitin at 77 K are shown in Figure 2 and can be analyzed in terms of two sites, differing in the values of $A_\parallel$ and $g_\parallel$. Site 1 is dominant and identical with the site observed in chitosan. Site 2 has approximately half the intensity of the dominant site, a larger value of $g_\parallel$, and a smaller value of $A_\parallel$. No differences in the values for $g_\perp$ and $A_\perp$ between the two sites were detected.

The intensity of the ESR signals in Cu^{2+} chitosan was higher than of those in chitin, in agreement with previous observations.[1-4,10]

Results for all the samples studied are summarized in Table I.

Table I
ESR Parameters of Cu^{2+} in Chitosan and Chitin

system	$g_\parallel$	$g_\perp$	$A_\parallel \times 10^4$, cm^{-1}	$A_\perp \times 10^4$, cm^{-1}	$g_\parallel/A_\parallel$, cm
Cu/chitosan/D_2O					
at 77 K	2.244	2.069	188	30	119
at 297 K	2.244	2.069	183	30	123
Cu, Zn/chitosan/D_2O					
at 77 K	2.250	2.059	183	30	123
at 297 K	2.053	2.063	178		127
Cu/chitin/D_2O					
site 1, 77 K	2.249	2.063	183	30	123
site 1, 297 K	2.241	2.065	177	30	127
site 2, 77 K	2.327		162	30	144
site 2, 297 K	2.304		166	30	139

Discussion

In this section we will analyze the ESR parameters and suggest binding sites for Cu^{2+} that are in agreement with experimental results obtained in this study. The crystal structures of chitin, chitosan, and chitosan–metal complexes will then be examined in order to deduce the most plausible location of chelated Cu^{2+} in these systems.

The most likely ligands for Cu^{2+} in the systems studied are oxygen and nitrogen. Oxygen ligation in chitin is supported by the qualitative observation that Cu^{2+} chelation is reduced by methylation or acetylation.[2,4] Chelation to the amino nitrogen in chitosan is a conclusion supported by the greater chelation ability of chitosan compared with that of chitin. The range of values for the g and A tensors measured in this study is within the range expected for Cu^{2+} bonded to four ligands in an *approximately* square-planar configuration.[6,11] The ligands can, in principle, be four nitrogen, four oxygen, or a combination of oxygen *and* nitrogen ligands. The dependence of the ESR parameters for Cu^{2+} on the ligation and charge has been extensively studied[12] and the Peisach–Blumberg (PB) plots derived have been very useful in choosing a structure that is compatible with the experimental results. The PB plots indicate the variation of $A_\parallel$ with $g_\parallel$ for various combinations of nitrogen and oxygen ligands in an approximately square planar arrangement as a function of the complex charge. For Cu–chitin and Cu–chitosan complexes, we expect ligation to amine nitrogen and hydroxyl oxygen attached to C^3 and C^6, as well as to the acetyl oxygen O^7, so that the overall charge is expected to be +2. For this charge, we can expect an increase in $g_\parallel$ and a decrease in $A_\parallel$ if a nitrogen ligand is replaced by an oxygen ligand. Site 1 detected in chitin is identical with the only site in chitosan. For a +2 overall charge, the ESR parameters for this site suggest ligation to *four* nitrogen

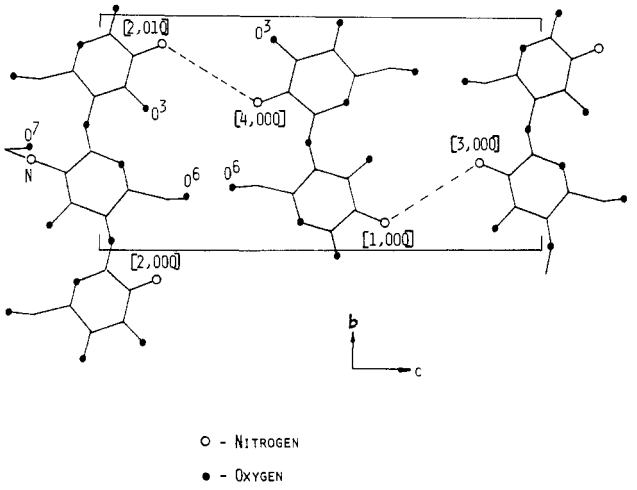

Figure 3. Projection of the unit cell of α-chitin in the *bc* plane, based on ref 2 and 16. The twofold helix axis is *b*. The nitrogen positions and the inter-nitrogen distances of 0.501 nm are indicated by broken lines.

ligands, according to the PB plots. These plots usually are very reliable and give an unambiguous choice for ligation to four oxygen or four nitrogen atoms. This choice is supported by the width of the parallel component, ~60 G, in accord with results in other CuN_4 complexes,[7] and reflects the unresolved hyperfine splittings from the N ligands.

Site 2 in chitin has a higher $g_\parallel$ value and a lower $|A_\parallel|$, indicating, in view of the PB plots, a smaller number of nitrogen ligands. The ESR parameters indicate either two oxygen and two nitrogen ligands or one oxygen and three nitrogen ligands, and it is hard to decide unambiguously on one of these two specific bonding schemes.

It has been suggested[13] that the ratio $g_\parallel/A_\parallel$ can be viewed as an empirical measure of the amount of tetrahedral distortion in Cu complexes. For square-planar geometry the range of 105–135 cm for this ratio has been suggested. As seen in Table I, the Cu complex in chitosan and site 1 in chitin fall well within these limits. Site 2, however, seems to suggest a strong tetrahedral distortion. Sites 1 and 2 are thus believed to be different in the nature of ligands *and* in their geometry.

It is interesting to compare the $g_\parallel/A_\parallel$ ratio in Cu/chitosan and (Cu,Zn)/chitosan systems. Values given in Table I for this ratio suggest slightly higher tetrahedral distortion with Zn present. This might be due to the fact that Zn forms a slighly distorted square-planar complex, and Cu, present in much lower concentration, is forced to adopt the same geometry. A similar effect has been observed before[9] in a study of a Cu-doped nickel complex with bacitracin A. In addition, changes in the location and coordination geometry of Cu^{2+} in X zeolites have been detected as a function of the nature and concentration of other cations present.[14]

We now examine crystal structures in order to deduce chelation sites for Cu^{2+} in chitin and chitosan.

Polymorphic structures have been detected for both chitin and chitosan by X-ray diffraction studies.[2] Three crystal structures are known for chitin, coded α-, β-, and γ-chitin. The most common and stable crystal structure is α-chitin.[15,16] For this form the most detailed crystallographic analysis has been published.[16] The space group is $P2_12_12_1$, and the unit cell contains two chains running in opposite directions, each chain consisting of two units of monomeric chitin related by a twofold helical axis. The orthorhombic unit cell has the dimensions a = 0.476 nm, b = 1.030 nm, and c = 1.885 nm; b is the fiber, or chain,

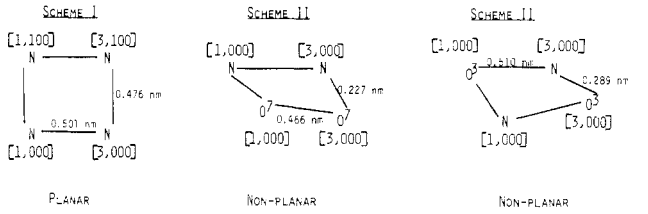

Figure 4. Three suggested ligand geometries in α-chitin. Scheme I is a planar agreement of four nitrogen ligands. Schemes II and III are nonplanar and consist of two nitrogen and two oxygen ligands. Scheme II is not applicable to chitosan because of the absence of O^7 ligands belonging to the $COCH_3$ groups.

axis. In Figure 3 the projection of the unit cell in the *bc* plane is shown and the positions of the nitrogen atoms are indicated. In the notation [1,000] the first digit specifies the symmetry position for the space group $P2_12_12_1$ and can be 1–4 because there are four monomeric units per unit cell; the other three digits specify a lattice translation.[17] For clarity, the oxygen in the $COCH_3$ group, O^7, is indicated only in one monomeric unit. Close approaches between the oxygen of type O^6 in adjacent chains are clearly seen in Figure 3. Each O^6 oxygen is at a distance of 0.476 nm respectively to one O^6 oxygen translated along the *a* axis, forming an oxygen quartet for possible Cu^{2+} chelation. No evidence for this type of ligation is observed in ESR experiments reported here, and we must conclude that O^6 oxygens do not provide a chelating site detectable by ESR measurements. Figure 4 indicates three possible arrangements of four N and O ligands in α-chitin. Scheme I consists of a planar arrangement of four nitrogen atoms, with distances between adjacent nitrogens of 0.501 and 0.476 nm. These are the closest inter-nitrogen distances in α-chitin. Schemes II and III consist of nonplanar arrangements of two nitrogen and two oxygen ligands. The cluster of N, O^3, and O^7 atoms shown in Figure 4 is the most likely candidate for Cu^{2+} chelation in α-chitin because it is in agreement not only with the type of ligand, N or O, as detected in ESR measurements but also with the deduced geometry of the ligands around Cu^{2+}. Scheme I is a planar site consisting of four N ligands, in agreement with the planarity of the complex in site 1. Schemes II and III are nonplanar arrangements of N and O ligands, in agreement with the tetrahedral distortion detected in site 2 in our ESR study.

Most Cu–N and Cu–O bond lengths are in the range 0.20–0.24 nm.[18] The maximum distance between adjacent ligands in a square-planar arrangement is thus 0.34 nm. This distance can be compared with inter-nitrogen distances of 0.476 and 0.501 nm and inter-oxygen distances of 0.466 nm, in Figure 4. The obvious conclusion is that considerable modification of the interchain distances in α-chitin occurs on binding. From this viewpoint, Scheme II involves less interligand distance modification and is thus favored over Scheme III for a nonpolar geometry, as detected for site 2 in chitin in our ESR study. The presence of other atoms in the acetamide group ($-NHCOCH_3$) is expected to interfere sterically with Cu^{2+} binding. This fact might explain the low chelating ability of chitin.

Three different crystal structures have been detected from X-ray diffraction studies on chitosan, and results indicate the presence of the twofold helix axis and the same unit cell symmetry as in α-chitin.[19,20] The most detailed X-ray diffraction results on chitosan have been recently published.[21] The orthorhombic unit cell has the dimensions a = 0.824 nm, b = 1.039 nm, c = 1.648 nm. The fiber axis b is identical with the b axis in α-chitin. The unit cell contains four chains, each chain consisting of two glucosamine units in a twofold helical conformation. Detailed

atomic coordinate positions have not been published. From the dimensions of the unit cell, the volume per monomeric unit is found to be 0.176 nm^3 compared with 0.231 nm^3 in α-chitin. The value for the fiber axis b is essentially the same in α-chitin and chitosan, and therefore the change in the volume of one glucosamine unit is due to a reduction in the interchain distances in the a and c axis directions. The inter-nitrogen distances shown for α-chitin in Figure 4 are expected to be reduced in chitosan, and a smaller modification of the interatomic distances is needed for Cu^{2+} chelation according to Scheme I.

Binding Scheme II, the most likely nonplanar arrangement in chitin, is not applicable to chitosan because of the removal of O^7 by deacetylation. Scheme I is the only binding scheme left in chitosan, in agreement with ESR results. The more compact packing of chains in chitosan and the absence of steric interference from the acetyl groups are very likely reasons for the increased ability of chitosan to bind Cu^{2+}, compared with chitin.

A preliminary X-ray diffraction study of metal–chitosan complexes has been published.[22] The results indicate that the twofold helical conformation of chitosan did not change by complex formation with a variety of transition-metal cations, including Cu^{2+}. Considerable change in the interchain distances were observed compared with chitosan. For chitosan–metal chloride complexes the orthorhombic unit cell consists of 16 glucosamine units, and the volume of one unit is 0.257 nm^3. In chitosan–metal sulfate complexes the unit volume is even larger, 0.336 nm^3. The metal concentration in these complexes was higher than in our ESR studies by a factor of 20–100. The large number of sulfate and chloride ions had to be accommodated in the structure studied. In addition, it has been mentioned that for the metal sulfate complexes the unit cell contains six molecules of water for each monomeric unit, and the dimension of the unit cell decreases with a decrease in the relative humidity. More details of the structure are needed in order to assess the local geometry of the chelated metal in these studies[22] and to compare with our ESR results.

Finally, results obtained from an optical study of Cu^{2+} chelation by chitosan oligomers[23] with a degree of polymerization between 9 and 17 indicate a Cu^{2+}/glucosamine ratio of 1:4. This result is in agreement with our ESR results which indicate chelation of Cu^{2+} by four nitrogen ligands in chitosan.

Conclusions

(1) ESR parameters suggest that in chitosan Cu^{2+} is ligated to four nitrogen ligands in a square-planar geometry.

(2) Some modification of the structure of chitosan is necessary in order to accommodate such a geometry.

(3) In chitin two binding sites for Cu^{2+} are detected. One site is identical with that in chitosan. The second site is a tetrahedrally distorted square planar arrangement of three nitrogen and one oxygen ligands *or* two nitrogen and two oxygen ligands. Examination of the crystal structure of α-chitin indicates that a site consisting of two O and two N ligands is more likely. Significant modification of interchain distances in α-chitin is necessary in order to ligate Cu^{2+} in both sites detected by ESR.

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Appendix

In powder spectra the intensity of the ESR absorption approaches infinity when the angle θ, which is the angle between the symmetry axis and the external magnetic field, is either 0° or 90°. For certain values of $A_\parallel$, $A_\perp$, $g_\parallel$, $g_\perp$, and m_I, discontinuities in the absorption are observed for values of θ other than 0° or 90°. "Extra" lines are thus obtained in the powder spectrum. The value of θ for which an extra line is expected and the corresponding resonant magnetic field H_{res} can be calculated with the following formula:[6,11]

$$\cos\theta = [\tfrac{1}{2}Bm_I^2(2A-1) + (A-1)/(2A-1)]^{1/2} \quad \text{(A1)}$$

with

$$B = \frac{(g_\parallel^2 A_\parallel^2 + g_\perp^2 A_\perp^2)(g_\parallel^2 + g_\perp^2)}{(h\nu)^2(g_\parallel^2 - g_\perp^2)} \quad \text{(A2)}$$

and

$$A = \frac{g_\parallel^2 A_\parallel^2}{g_\parallel^2 A_\parallel^2 + g_\perp^2 A_\perp^2} \quad \text{(A3)}$$

$$H_{res} = \frac{h\nu}{g\beta_e} - \frac{K}{g\beta_e} m_I \quad \text{(A4)}$$

$$K^2 = \frac{(A_\parallel^2 g_\parallel^2 \cos^2\theta + A_\perp^2 g_\perp^2 \sin^2\theta)}{g_\parallel^2 \cos^2\theta + g_\perp^2 \sin^2\theta} \quad \text{(A5)}$$

For $\theta = 63°$ and $m_I = -3/2$, an extra line is calculated at a resonant field of 3314 G for the spectrum shown in Figure 1, in excellent agreement with experimental results.

An additional extra line is expected for $\theta = 87°$ and $m_I = -1/2$ and is also indicated in Figure 1. This extra line is in the region of the ESR lines from the perpendicular orientation and is not resolved experimentally.

Registry No. $CuSO_4$, 7758-98-7; chitin, 1398-61-4; chitosan, 9012-76-4.

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