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Research Note

Hydrogen ion titration of chitosans with varying degrees of *N*-acetylation by monitoring induced ^{1}H-NMR chemical shifts

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Chitosan is a linear polysaccharide which is made from chitin and consists of β-(1->4)-linked 2-amino-2-deoxy-D-glucopyranose (GlcNAc). The conformation and solution properties of chitosan depend on the chemical structure, i.e. the degree of *N*-acetylation, F_A, and the distribution of GlcNAc and GlcN units along the polymeric chain. Moreover, the pH and ionic strength influences the physiochemical properties of chitosan (Anthonsen *et al.*, 1993). The pK_a value of chitosan has been reported to range from 6·2 to 7 (Park & Choi, 1983; Domard, 1987; Terbojevich *et al.*, 1989; Rinaudo & Domard, 1989) but no systematic study of the effect of widely differing values of F_A and ionic strength on the titration behaviour of chitosans has been undertaken. Different charge densities for chitosans having different values of F_A may result in different pK_a values, and we have, therefore, determined pK_a values of chitosans with F_A values of 0 and 0·5.

^{1}H-NMR spectra of chitosan (Domard *et al.*, 1987; Vårum *et al.*, 1991*a*; Hirai *et al.*, 1991), and GlcNAc (Perkins *et al.*, 1977; Boyd *et al.*, 1985) and GlcN oligomers (Tsukada & Inoue, 1981; Domard *et al.*, 1991) have been reported. In the present study, the chemical shift differences in the resonance of H-2 in GlcN units, in chitosans with $F_A = 0$ and 0·5, were monitored at varying pH values. It is convenient to monitor the resonance from H-2 of GlcN, as it is well separated from other resonances. Moreover, the proximity of H-2 of GlcN to the amino group results in high chemical shift sensitivity of its resonance towards changes in the ionization state of the amino group. It may be advantageous to use NMR instead of potentiometric titration to determine pK_a values, because potentiometric titrations demand that the amount of acid or base used to titrate water has to be corrected for, i.e., the amount of acid or base that actually titrates sites on the polymer has to be estimated. Also, for potentiometric titration, chitosans having different values of F_A have to be corrected to correspond to an equal number of charges at the start of the titration. Figure 1 shows the chemical shift differences between H-2 of GlcN and the internal reference TSP (sodium 3-(trimethylsilyl) propionate-d$_4$) as a function of pH for chitosan with F_A of 0 and 0·5. The pH dependence of TSP was corrected for by using a relation reported by De Marco (1977). Due to increased solubility and decreased line broadening in the NMR spectra, the chitosans were degraded to degrees of polymerization (DP) of 4 (F_A = 0·5), 5 (F_A of 0) and 14 (F_A of 0·5 and 0). The pK_a values determined from Fig. 1 are about 6·6, irrespective of F_A value and DP. This value agrees with previously reported pK_a values of chitosan (Park & Choi, 1983; Terbojevich *et al.*, 1989; Rinaudo & Domard, 1989). Also from chemical shift differences of H-2 at different pH values, the pK_a value of the GlcN monomer was found to be 7·7 (data not shown) as reported (Park & Choi, 1983). The lowered pK_a value of oligomeric chitosan relative to the GlcN monomer suggests a charge effect on the pK_a value of oligomeric chitosan. The charge effect may be due to either long range effects as discussed by Tanford (1961), or it may be due to short range effects occurring between neighbouring charges in the chitosan chain. When increasing the ionic strength from about 0·04 to 0·24 M, the pK_a value increased to 7·0 (for chitosan with F_A = 0·5 and DP = 14) thus approaching the monomeric pK_a value of 7·7. This suggests that the effect

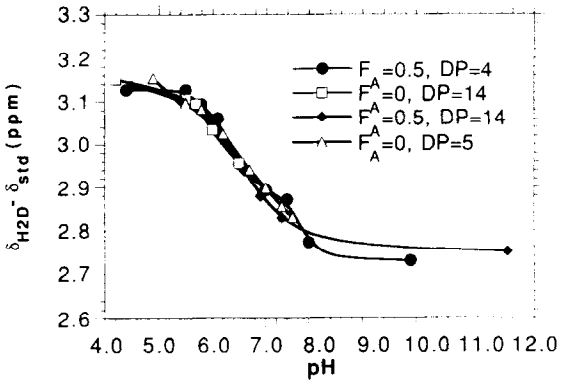

Fig. 1.

which results in a lowered pK_a value in oligomeric chitosan relative to the GlcN monomer is at least partly a long range charge effect.

For further evaluation of the long range, polyelectrolytic influence on the titration behaviour of chitosan, a plot according to Katchalsky and co-workers (1954) is shown in Fig. 2. The equation proposed by Katchalsky and his co-workers is as follows:

$$-\log[\alpha/(1-\alpha)] + pH = pK_{int} + \phi(\alpha)\cdot\alpha = (pK_a)_{apparent},$$

where α is the ionization degree, pK_{int} is the intrinsic pK_a value corresponding to an uncharged polyelectrolyte, $\phi(\alpha)$ is the electrostatic potential involved in moving an ion from a reference state at infinite distance to the surface of the polyelectrolyte, and $(pK_a)_{apparent}$ is the apparent pK_a value, i.e. the pK_a value when α is different from 0 (Tanford, 1961). The absolute value of $\phi(\alpha)\cdot\alpha$ increases with increasing charge density of the polyelectrolyte (increasing α). The term $\phi(\alpha)\cdot\alpha$ may be calculated for some limited cases in which only long-range electrostatic effects and monovalent ions are considered (Tanford, 1961). Moreover, ion pair condensation is neglected. Conformational changes which abruptly change the charge density with increasing α should change the value of the electrostatic potential, $\phi(\alpha)$. In Fig. 2, α is the degree of ionization determined from Fig. 1. The straight lines in Fig. 2 reveal their linear decrease in apparent pK_a values with increasing charge density, suggesting that no conformational changes occur during the titration. A constant value of is as expected for β-(1->4)-linked polysaccharides with relatively stiff backbones and low molecular weights. The value of pK_{int} of about 9 (extrapolated to zero charge density) is somewhat higher than the pK_a value of the GlcN monomer (7·7). Whether this discrepancy between pK_{int} and the monomeric pK_a value is due to experimental uncertainty or to shortcomings of the theoretical treatment cannot be decided.

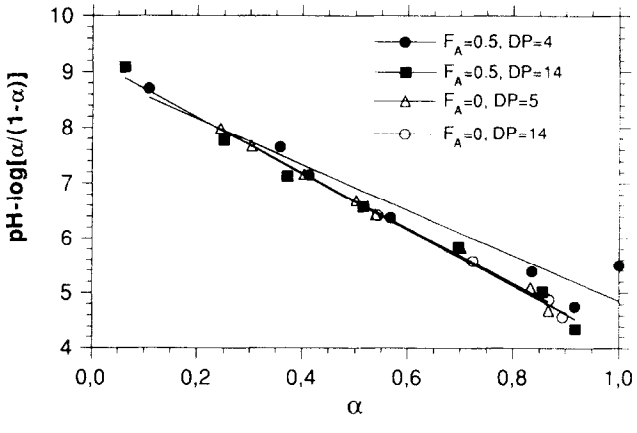

Fig. 2.

Our findings are useful when evaluating interactions of chitosan with other polyelectrolytes or charged species, which are the basis for many possible industrial and medical applications of chitosans. Capsules made from polyanion–polycation complexes may be of particular interest. In such cases long range electrostatic effects on chitosan may be completely swamped, or even reversed, if the polyanion is present in large excess. Therefore, in such cases the intrinsic pK_a value, pK_{int}, of about 9 is probably more relevant in discussing short range interactions between polyanion and polycation than the value of about 6·6 obtained for chitosans at low ionic strengths. Hence, good stability of such capsules should be expected at physiological pH values.

EXPERIMENTAL

Materials

The chitosan with F_A of 0·5, 'Seacure-Cl 313 No 208-490-03', was kindly provided by Pronova Biopolymers (Drammen, Norway). The chitosan with F_A of 0 was made in our laboratory as previously described (Anthonsen *et al.*, 1993). Values of F_A and random distribution of monomers in the samples were determined by ^{1}H- and ^{13}C-NMR (Vårum *et al.*, 1991*a*, 1991*b*). The chitosans were degraded according to a method of Allan and Peyron (1989), and the degrees of polymerization were estimated from the phenol–sulfuric acid method (Dubois *et al.*, 1956) using 2,5-anhydro-D-mannose ranging from 0 to 100 μg/ml as standard.

Measurements

Chitosans were dissolved in distilled water and titrated with NaOH (1, 0·5, or 0·1 M). The resulting chitosan concentrations in 5 mm tubes (O.D.), ranged from 10 to 14 mg/ml, including 7% (v/v) D_2O added for the frequency lock. NMR spectra were recorded at 9·4 T (399·65 MHz for ^{1}H) with a JEOL EX400 instrument.

Spectral widths of 4 kHz, 32 K datapoints and a pulse width corresponding to an excitation pulse of 40° were used. The spectra were recorded at 20°C, and the chemical shift values were referenced to internal sodium 3-(trimethylsilyl) propionate-d_4 (TSP) from Merck.

REFERENCES

Allan, G.G. & Peyron, M. (1989). In *Chitin and Chitosan*, eds G. Skjåk-Bræk, T. Anthonsen & P. Sandford. Elsevier Applied Science, London, pp. 443–466.

Anthonsen, M.W., Vårum, K.M. & Smidsrød, O. (1993). *Carbohydr. Polym.*, **25,** 13–23.

Boyd, J., Porteous, R., Soffe, N. & Delepierre, M. (1985). *Carbohydr. Res.*, **139,** 35–46.

De Marco, A. (1977). *J. Magn. Reson.*, **26,** 527–528.

Domard, A. (1987). *Int. J. Biol. Macromol.*, **9,** 98–104.

Domard, A., Gey, C., Rinaudo, M. & Terrassin, C. (1987). *Int. J. Biol. Macromol.*, **9,** 233–237.

Domard, A., Gey, C. & Taravel, F. (1991). *Int. J. Biol. Macromol.*, **13,** 105–109.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). *Analyt. Chem.*, **28,** 350–356.

Hirai, A., Odani, H. & Nakajima, A. (1991). *Polym. Bull.*, **26,** 87–94.

Katchalsky, A., Shavit, N. & Eisenberg, H. (1954). *J. Polym. Sci.*, **13,** 57–84.

Park, J.W. & Choi, K.-H. (1983). *Bull. Korean Chem. Soc.*, **4,** 68–72.

Perkins, S., Johnson, L.N., Phillips, D.C. & Dwek, R.A. (1977). *Carbohydr. Res.*, **59,** 19–34.

Rinaudo, M. & Domard, A. (1989). In *Chitin and Chitosan*, eds G. Skjåk-Bræk, T. Anthonsen & P. Sandford. Elsevier Applied Science, London, pp. 71–86.

Tanford, C. (1961). *Physical Chemistry of Macromolecules*. John Wiley, New York.

Terbojevich, M., Carraro, C., Cosani, A., Focher, B., Naggi, A.M. & Torri, G. (1989). *Makromol. Chem.*, **190,** 2847–2855.

Tsukada, S. & Inoue, Y. (1981). *Carbohydr. Res.*, **88,** 19–38.

Vårum, K.M., Anthonsen, M.W., Grasdalen, H. & Smidsrød, O. (1991*a*). *Carbohydr. Res.*, **211,** 17–23.

Vårum, K.M., Anthonsen, M.W., Grasdalen, H. & Smidsrød, O. (1991*b*). *Carbohydr. Res.*, **217,** 19–27.