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Pyrene fluorescence study of chitosan self-association in aqueous solution

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The change in peak III/I ratio of pyrene monomer fluorescence, a measure of the hydrophobicity of pyrene's environment, was used to examine the aggregation behaviour of chitosan in aqueous solution. The III/I ratio of 0·60 at chitosan concentrations below 0·1 mg/ml was very similar to that observed in water and other polar media. Increasing the chitosan concentration above 1·0 mg/ml, however, significantly increased the III/I ratio. The III/I ratio was 0·73 in chitosan-70 K and 1·12 in chitosan-750 K when the concentration was increased to 5·0 mg/ml. The values of 0·73 and 1·12 correspond to pyrene being localized in an environment similar to methylene chloride and isopropyl alcohol, respectively. The increased hydrophobicity suggests that chitosan chains were self-associated by intermolecular hydrophobic interactions. No difference in the III/I ratio was found between solutions made in 0·1 M acetic acid and 0·1 M hydrochloric acid. Changes in the ionic strength by addition of sodium chloride, however, did significantly affect the aggregation behavior.

INTRODUCTION

Hydrophobically modified water-soluble polysaccharides have found importance in various biomedical and pharmaceutical applications. Recent studies have shown that these systems could be used as viscosity modifiers, as matrices for immobilization of enzymes and drugs, and as support materials for hydrophobic chromatography (Akiyoshi *et al.*, 1993). Interesting physicochemical properties of these polymers are directly related to the intra- and intermolecular associations between the hydrophobic functionalities in aqueous solution within a certain concentration range (Sinquin *et al.*, 1993).

The peak III/I ratio of pyrene monomer fluorescence was used in the present study to examine the aggregation behavior of partially deacetylated chitosan. Chitosan, a linear polysaccharide, is composed of random repeating units of β-(1-4)-linked 2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) (Anthonsen *et al.*, 1993). Chitosan is obtained from N-deacetylation of chitin in alkaline media. Chitin, isolated from the exoskeleton of many crustaceans, is the second most abundant naturally occurring polymer (Li *et al.*, 1992). On the basis of the degree of deacetylation, chitosan in aqueous solution is expected to have the properties of an amphipathic molecule. In this study, chitosan solutions were prepared in 0·1 M acetic acid and 0·1 M hydrochloric acid. In addition, the effect of ionic strength on chitosan aggregation was examined.

MATERIALS AND METHODS

Partially deacetylated chitosan (≥70%) with average molecular weights of 70,000 (70 K) and 750,000 (750 K) were purchased from Fluka Chemika-BioChemika (Ronkonkoma, NY). According to the supplier, chitosan samples used in this study contained 0·06% protein and 0·77% ash as impurities. All other chemicals were reagent grade or better and were used as received. Deionized distilled water (NANOpure®, Barnsted/Thermolyne, Dubuque, IA) was used exclusively to prepare all aqueous solutions. Chitosan was dissolved in 0·1 M acetic acid or 0·1 M hydrochloric acid to prepare solutions with concentrations ranging from 0·001 to 10 mg/ml.

Pyrene, purchased from Sigma Chemical Company (St. Louis, MO), was purified by repeated recrystallization in methanol. Purified pyrene, dissolved in methanol, was added to filtered chitosan solution to give a final concentration of 2·0 μM. Previously, we have found that there is no interference from excimer emission at this low pyrene concentration (Amiji & Park, 1994). Pyrene emission spectra were obtained using a

Perkin-Elmer LS-50B fluorescence spectrophotometer (Norwalk, CT). The probe was excited at 343 nm and the emission spectrum was collected in the range of 360–500 nm at an integration time of 1·0 s. The excitation and emission slit openings were 15 and 2·5 nm, respectively.

RESULTS AND DISCUSSION

Being a hydrophobic molecule with low aqueous solubility (~0·3 μM), pyrene is expected to localize preferentially in the hydrophobic domains of amphipathic molecules (Dowling & Thomas, 1990). Pyrene monomer emission spectra are associated with vibronic fine structures whose intensities show a strong dependence on the polarity of the microenvironment (Dong & Winnik, 1982). In polar solvents, there is an enhancement in the intensity of peak I (at 372 nm), whereas no effect is seen on the intensity of peak III (at 384 nm). The III/I ratio, therefore, is used to study the change in environmental polarity of amphipathic molecules upon association in aqueous solutions (Dualeh & Steiner, 1990). Kalyanasundaram and Thomas (1977), for instance, used the III/I ratio to determine the critical micelle concentrations (CMC) of various surfactants.

Figure 1 shows the fluorescence spectra of pyrene in chitosan-750 K solution in 0·1 M acetic acid. The spectra correspond to chitosan concentrations of (A) 0·1; (B) 1·0; and (C) 5·0 mg/ml. The III/I ratio of 0·60 at chitosan concentrations of 0·1 mg/ml or lower was similar to the III/I ratio observed in water and other polar solvents (Kalyanasundaram & Thomas, 1977). At lower chitosan concentrations, there was no difference between the III/I ratio in chitosan-70 K and chitosan-750 K as shown in Fig. 2. When the chitosan concentration was increased to 1·0 mg/ml or above, however, the III/I ratio was 0·73 in chitosan-70 K and 1·12 in chitosan-750 K. The III/I ratio of 0·73 and 1·12 corresponds to pyrene being localized in an environment similar to methylene chloride and isopropyl alcohol, respectively (Kalyanasundaram & Thomas, 1977). The III/I ratio increased continuously with increasing chitosan concentration. The increase in hydrophobicity of pyrene's microenvironment at concentrations above 1·0 mg/ml suggests that the chitosan chains associated by intermolecular hydrophobic interactions between the GlcNAc groups. The higher III/I ratio in chitosan-750 K than in chitosan-70 K at the same concentration shows that the hydrophobic core of chitosan-750 K aggregates was more compact due to the increased number of GlcNAc groups in the polymer chain. Figure 3 shows the change in III/I ratio of chitosan-70 K and chitosan-750 K in 0·1 M hydrochloric acid. The III/I ratio in 0·1 M hydrochloric acid, at different chitosan concentrations, was exactly the same as that observed in 0·1 M acetic acid.

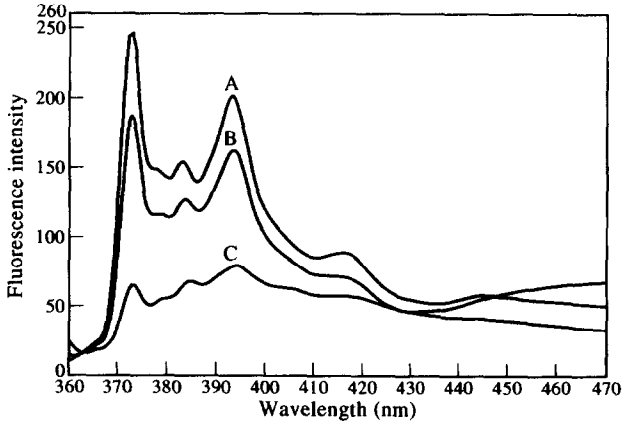

Fig. 1. Fluorescence spectra of pyrene in chitosan-750 K solution with concentrations of (A) 0·1 mg/ml; (B) 1·0 mg/ml; and (C) 5·0 mg/ml in 0·1 M acetic acid. The peak III/I ratio was calculated from the intensities of peak III (at 384 nm) and peak I (at 372 nm). Pyrene was dissolved in chitosan solution at a final concentration of 2·0 μM.

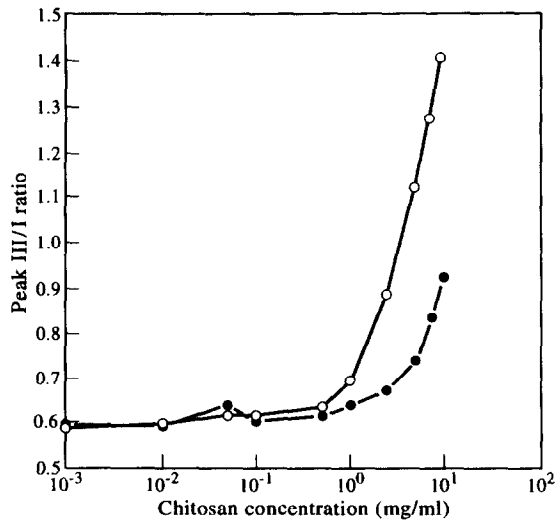

Fig. 2. The peak III/I ratio of pyrene fluorescence as a function of chitosan concentration. Chitosan-70 K (●) and chitosan-750 K (○) solutions were prepared in 0·1 M acetic acid.

Although there was no difference between the III/I ratio in 0·1 M hydrochloric acid, the aggregation behavior was significantly affected by changes in the ionic strength of the media as shown in Table 1. Sodium chloride, at different concentrations, was added to chitosan solutions to adjust the ionic strength. The III/I ratio did not increase when the sodium chloride concentration was 0·01 or 0·10 M. At 1·0 M sodium chloride concentration, however, the III/I ratio in chitosan-70 K was 0·797 in 0·1 M acetic acid and 0·85 in 0·1 M hydrochloric acid. An approximately 8·0% increase in the III/I ratio was observed in all cases at 1·0 M sodium chloride concentration. Since the interactions between chitosan chains occur primarily through the GlcNAc groups, increasing the

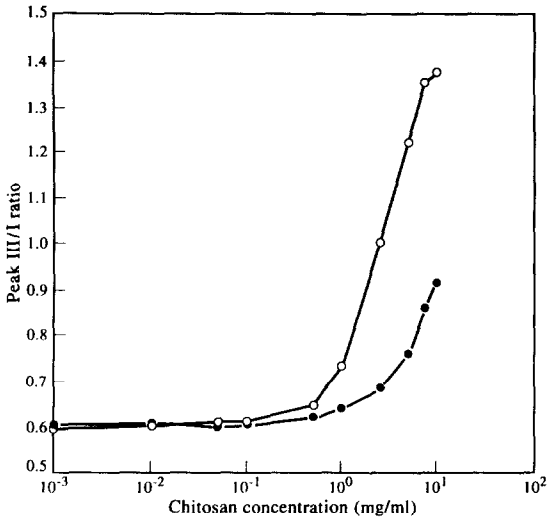

Fig. 3. The peak III/I ratio of pyrene fluorescence as a function of chitosan concentration. Chitosan-70 K (●) and chitosan-750 K (○) solutions were prepared in 0·1 M hydrochloric acid.

ionic strength of the medium does enhance the hydrophobic association.

The results of this study clearly show that chitosan chains self-associate in aqueous solution by intermolecular hydrophobic interactions. The association behavior, when correlated with the degree of N-deacetylation of chitosan, will have important implications in the future applications of this naturally abundant biopolymer.

Table 1. Effect of ionic strength on the peak III/I ratio of pyrene fluorescence in chitosan solutions[a]

Biopolymer	Sodim chloride concentration (M)			
	0·00	0·01	0·10	1·00
Chitosan-70 K in acetic acid[b]	0·73	0·73	0·74	0·79
Chitosan-750 K in acetic acid	1·12	1·13	1·15	1·22
Chitosan-70 K in hydrochloric acid	0·79	0·78	0·79	0·85
Chitosan-750 K in hydrochloric acid	1·22	1·21	1·23	1·29

[a]The final concentration of pyrene dissolved in chitosan solutions was 2·0 μM.
[b]Chitosan solution with a concentration of 5·0 mg/ml was prepared either in 0·1 M acetic acid or in 0·1 M hydrochloric acid.

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REFERENCES

Akiyoshi, K., Deguchi, S., Moriguchi, N., Yamaguchi, S. & Sunamoto, J. (1993). Self-aggregates of hydrophilized polysaccharides in water. Formation and characteristics of nanoparticles. *Macromolecules*, **26,** 3062–3068.

Amiji, M. & Park, K. (1994). Analysis on the surface adsorption of PEO/PPO/PEO triblock copolymers by radiolabelling and fluorescence techniques. *J. Appl. Polym. Sci.*, **53,** 539–544.

Anthonsen, M.W., Vårum, K.M. & Smidsrød, O. (1993). Solution properties of chitosans: conformation and chain stiffness of chitosan with different degrees of N-acetylation. *Carbohydr. Polym.*, **22,** 193–201.

Dong, D.C. & Winnik, M.A. (1982). The Py scale of solvent polarities. Solvent effects on the vibronic fine structure of pyrene fluorescence and empirical correlations with E_T and Y values. *Photochem. Photobiol.*, **35,** 17–21.

Dowling, K.C. & Thomas, J.K. (1990). A novel micellar synthesis and photophysical characterization of water-soluble acrylamide–styrene block copolymers. *Macromolecules*, **23,** 1059–1064.

Dualeh, A.J. & Steiner, C.A. (1990). Hydrophobic microphase formation in surfactant solutions containing an amphiphilic graft copolymer. *Macromolecules*, **23,** 251–255.

Kalyanasundaram, K. & Thomas, J.K. (1977). Environmental effects of vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems. *J. Am. Chem. Soc.*, **99,** 2039–2044.

Li, Q., Dunn, E.T., Grandmaison, E.W. & Goosen, M.F.A. (1992). Applications and properties of chitosan. *J. Bioactive Compat. Polym.*, **7,** 370–397.

Sinquin, A., Hubert, P. & Dellacherie, E. (1993). Amphiphilic derivatives of alginate: evidence of intra- and intermolecular hydrophobic associations in aqueous solution. *Langmuir*, **9,** 3334–3337.