Two Types of Hydrophobic Aggregates in Aqueous Solutions of Chitosan and Its Hydrophobic Derivative

Olga E. Philippova,* Evgenii V. Volkov, Natalia L. Sitnikova, and Alexei R. Khokhlov

Physics Department, Moscow State University, Moscow 117234, Russia

Jacques Desbrieres and Marguerite Rinaudo

Centre de Recherches sur les Macromolecules Vegetales, CNRS, affiliated with Joseph Fourier University, BP 53, 38041 Grenoble Cedex 9, France

Received December 8, 2000; Revised Manuscript Received April 24, 2001

The aggregation phenomena in aqueous solutions of hydrophobically modified (HM) chitosan, containing 4 mol % of *n*-dodecyl side chains, were studied by viscometry and fluorescence spectroscopy with pyrene as a probe. The results are compared with those for unmodified chitosan. Surprisingly, fluorescence data reveal the appearance of intermolecular hydrophobic aggregates both in chitosan and in HM chitosan. Nevertheless, these polymers exhibit quite different rheological properties: upon the formation of aggregates the viscosity of HM chitosan sharply increases, while that of unmodified chitosan raises only slightly. The aggregation models for both chitosan and its hydrophobic derivative were proposed. It was shown that in solutions of HM chitosan two types of hydrophobic domains exist: hydrophobic domains typical for different associating polymers with hydrophobic side chains and hydrophobic domains inherent to chitosan itself.

Introduction

The aggregation behavior of water-soluble associating polyelectrolytes, with its many industrial applications, has been the subject of numerous studies.[1−9] Most of these works were dealing with synthetic polyelectrolytes,[1−4] and there are only few reports on self-assemblies of naturally occurring polyelectrolytes and their derivatives.[5−9] At the same time, the use of nontoxic, biocompatible, and biodegradable naturally occurring polyelectrolytes with associative groups can essentially widen the area of practical applications of associative polyelectrolytes in such fields as food industry, pharmacy, cosmetology, and medicine. For naturally occurring polyelectrolytes, the aggregation behavior can be more complicated than that for the synthetic polyelectrolytes because normally there is a diversity of forces responsible for intermolecular interactions. At the same time, an understanding of the mechanism and forces involved in the aggregation has an obvious practical importance.

The aim of this work is to study the aggregation behavior of a cationic naturally occurring polyelectrolyte, chitosan, and of its hydrophobic derivative containing 4 mol % of *n*-dodecyl side chains (Figure 1).

To better understand the nature of the associations exhibited by these polymers in aqueous media the effects of hydrophobicity, temperature, and addition of urea, ethanol, and low molecular weight salt are considered both on macroscopic (rheological studies) and microscopic (fluorescence studies) scales.

Experimental Part

Materials. Chitosan from PROTAN (Norway) with molecular weight 190 000 and degree of deacetylation 88 mol % was used after purification. According to NMR data, in this polymer (Figure 1) the distribution of *N*-acetyl-D-glucosamine repeat units along the chains is random.

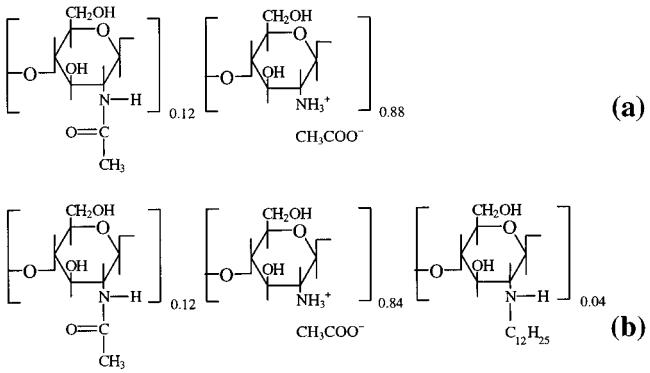

Figure 1. Chemical structures of chitosan (a) and HM chitosan (b) studied in this paper.

The HM chitosan was prepared by reductive amination of chitosan in homogeneous conditions using *n*-dodecylaldehyde. Details of chemical modification and characterization of this polymer are described elsewhere.[6] It was shown[6] that the chemical modification does not lead to the destruction of polymer. Therefore, HM chitosan has the same value of molecular weight as its precursor (190 000). The content of hydrophobic side chains in HM chitosan was 4 mol %.

Pyrene obtained from Aldrich was purified by repeated recrystallizations from absolute ethanol. Water was purified with a Milli-Q system (Millipore).

Solution Preparation. Polymer solutions were prepared by weighing the components and stirring during at least 24 h. All solutions of chitosan and HM chitosan were prepared

10.1021/bm005649a CCC: $20.00 © 2001 American Chemical Society
Published on Web 05/17/2001

Table 1. Values of Intrinsic Viscosities [η], Critical Overlap Concentrations C^*, and Huggins Constants in Aqueous Solutions of Chitosan and HM Chitosan at 25 °C

polymer	chitosan			HM chitosan		
solvent	0.3 M AcH	0.3 M AcH, 0.05 M AcNa	0.3 M AcH, 0.2 M AcNa	0.3 M AcH	0.3 M AcH, 0.05 M AcNa	0.3 M AcH, 0.2 M AcNa
intrinsic viscosity [η], L/g	2.10	0.78	0.55	2.42	0.66	0.35
overlap concentration C^*,[a] g/L	0.5	1.3	1.8	0.4	1.5	2.9
Huggins constant[b] k_H	0.31	0.33	0.41	2.1	2.7	2.8

[a] Calculated from intrinsic viscosity as $C^* = 1/[\eta]$. [b] Determined from the Huggins equation $\eta_{sp} = c[\eta] + k_H c^2[\eta]^2$.

in 0.3 M CH_3COOH, where the amino groups of polymers are fully protonated.[10]

The experiments were performed with dilute and semidilute solutions. The values of critical overlap concentrations C^* estimated from the values of intrinsic viscosity [η] ($C^* = 1/[\eta]$) are presented in Table 1.

Solutions for fluorescence measurements were prepared by first pipetting a small quantity of pyrene stock solution in ethanol (2×10^{-4} or 10^{-3} mol/L). Then 1.5 mL of polymer solution of a given concentration was added to the flask and stirred for 1 day (for the intensity ratio measurements) or for 3 days (for the determination of the concentration of hydrophobic aggregates). For the intensity ratio measurements the final concentration of pyrene in all the solutions was kept constant and equal to 8×10^{-7} mol/L. For the determination of the concentration of hydrophobic aggregates pyrene concentration was varied in the range $0.3-9.0 \times 10^{-6}$ mol/L.

Methods. (a) Viscosity Measurements. Viscosity measurements were carried out with an Ubbelohde viscometer with capillary diameter of 0.43 mm.

(b) Spectral Measurements. UV spectra were measured with a Hewlett-Packard 8452A photodiode array spectrometer.

Fluorescence measurements were performed on a Hitachi MPF-4 fluorescence spectrophotometer in a thermostated cuvette holder. The pyrene spectra were obtained by exciting the solutions at 338 nm and recording the emission over the range 350−550 nm at the scan rate of 15 nm/min. The slit width was set at 5 nm for the excitation and 1.5 nm for the emission. To increase the precision of the determination of the values of intensities of different vibronic peaks, the averaged (during at least 2 min) fluorescence intensities were recorded at the maximum of each peak.

The concentration of hydrophobic domains was estimated from the self-quenching of pyrene fluorescence as a result of the formation of excimers. To determine the concentration of hydrophobic domains, we fixed the total concentration of polymer (and hence the concentration of hydrophobic domains), and we varied the concentration of pyrene. With increasing pyrene concentration, the band of excimers at 480 nm appears. As most of pyrene molecules are localized inside the hydrophobic domains, the probability of excimer formation depends considerably on the concentration of the domains. The excimer formation will decrease with an increasing number of domains because of a lower probability of finding two pyrene molecules in the same domain.

According to the approach of Flynn and Goodwin,[11] the concentration of hydrophobic domains [Mic] can be determined from eq 1,

$$\ln\left[\frac{[Py_1]}{[Py_{total}]}\right] = -\frac{[Py_{total}]}{[Mic]} \quad (1)$$

where $[Py_1]$ is the concentration of monomer pyrene, $[Py_{total}]$ is the total concentration of pyrene (both in monomer and in excimer states). The total concentration of pyrene $[Py_{total}]$ was checked by UV absorption using 338 nm band. The concentration of monomer pyrene was determined from the half-sum of the emission intensities of allowed fluorescence bands of monomer pyrene at 383 (I_3) and 392.5 nm (I_5). The data fit well to eq 1, and a good linear relationship between $\ln[^1/_2(I_3 + I_5)/[Py_{total}]]$ and $[Py_{total}]$ values was obtained in all the cases (correlation factor, $r = 0.98$). The concentration of hydrophobic domains, [Mic], was estimated from the slope of the straight line. The error in the determination of the values of concentration of hydrophobic aggregates is estimated to be ca. 20%.

Results and Discussion

Onset of Aggregation. To study the hydrophobic aggregation on a molecular level we used a fluorescence spectroscopy with pyrene as a probe. The ratio of the intensity of first (371.5 nm) to third (383 nm) vibronic peaks I_1/I_3 in the fluorescence spectra of pyrene is quite sensitive to the polarity of the microenvironment of the probe.[12] This is due to the fact that increasing polarity of medium induces the increase of the intensity of the first peak I_1, corresponding to the forbidden transition, while the intensity of the third peak I_3, corresponding to the allowed transition, remains unchanged. As a result the value of the ratio I_1/I_3 ("polarity parameter") is higher in more polar media (e.g., in water (polar solvent) $I_1/I_3 = 2.0$, while in hexane (nonpolar solvent) $I_1/I_3 = 0.6$).[12] When in polar medium (water) the hydrophobic domains are formed, pyrene, being quite hydrophobic, is solubilized in their nonpolar interior, which leads to the decrease of the polarity parameter.

(A) HM Chitosan. Figure 2 shows that at low concentration of HM chitosan the polarity parameter of pyrene is close to that for pyrene in pure water, that is the hydrophobic domains are absent. Then the polarity parameter of pyrene decreases and reaches a steady value of 0.9 which is characteristic for pyrene incorporated into hydrophobic domains in aqueous solutions of some associative polymers[13,14] or into the micelles of some low molecular weight surfactants.[12]

The hydrophobic aggregation starts at polymer concentration of ca. 0.1 g/L. If we choose the inflection point of the

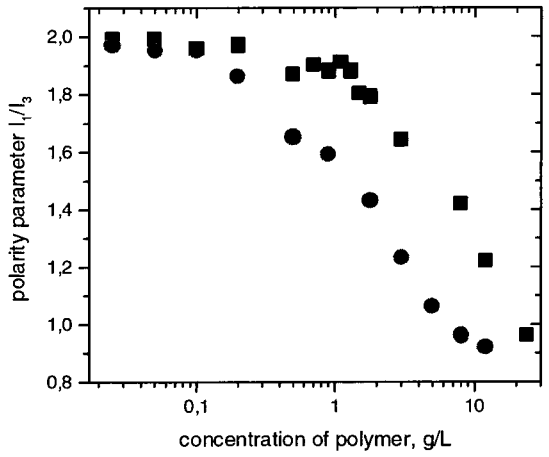

Figure 2. Polarity parameter I_1/I_3 of pyrene in aqueous solutions of chitosan (■) and HM chitosan (●) in 0.3 M CH_3COOH as a function of polymer concentration.

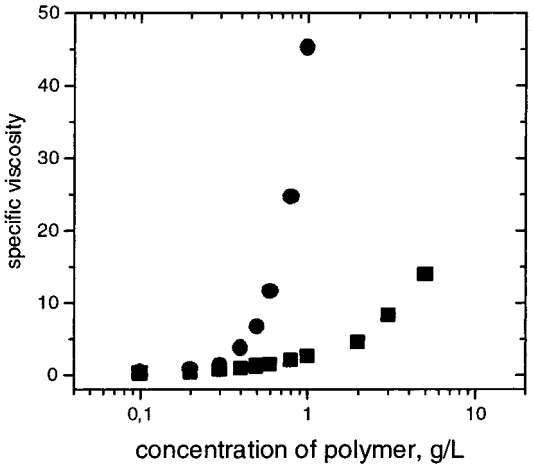

Figure 3. Specific viscosity of aqueous solutions of chitosan (■) and HM chitosan (●) in 0.3 M CH_3COOH as a function of polymer concentration.

curve (Figure 2) to estimate the critical aggregation concentration,[15] we find a value of ca. 1.5 g/L (or 3.5×10^{-4} mol/L, if calculate with respect to the concentration of hydrophobic side chains). This value is by 2 orders of magnitude lower than the critical micelle concentration (cmc) of ionic low molecular weight surfactants with the same hydrophobic groups. For example, the cmc value of cationic surfactant dodecyltrimethylammonium chloride equals to 1.5×10^{-2} mol/L.[16] Rather high cmc values for ionic surfactants are known to be related to electrostatic repulsion of charged surfactant heads, as well as to the losses of translational entropy of counterions, which compensate the charge of micelles. In the case of HM chitosan, there is no electrostatic repulsion of surfactants and the aggregation of hydrophobic side chains does not lead to the additional immobilization of counterions. Both these facts facilitate hydrophobic aggregation.

It should be noted that the onset of the formation of hydrophobic domains takes place at polymer concentrations close to the overlap concentration C^* (see Table 1) indicating mainly intermolecular character of the aggregation. This can be explained by the fact that the chitosan chains are strongly stretched because of their high degree of ionization, which hinders the intramolecular aggregation.

The formation of hydrophobic domains detected on a molecular level is accompanied by a pronounced increase of the viscosity of aqueous solutions of polymer (cf. Figures 2 and 3). Hence, we can suggest that these hydrophobic domains play a role of junction zones, which link multiple polymer molecules together in an intermolecular aggregate (Figure 4b). Therefore, HM chitosan demonstrates a behavior typical for many associating polyelectrolytes.

(B) Unmodified Chitosan. The data on the aggregation of HM chitosan were compared with those for the precursor polymer without hydrophobic side chains. Surprisingly, fluorescence data (Figure 2) clearly evidence that the hydrophobic domains are formed in this polymer as well despite the absence of hydrophobic side chains. But in chitosan the aggregates start to appear at much higher polymer concentrations (ca. 1 g/L) than in HM chitosan (ca. 0.1 g/L) (Figure 2). In both polymers, the hydrophobic

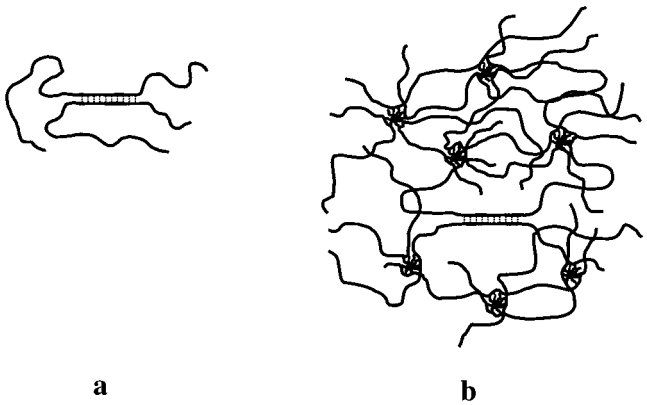

Figure 4. Schematic representation of aggregates formed in aqueous solutions of chitosan (a) and HM chitosan (b).

aggregates are mainly intermolecular (they are formed at polymer concentrations higher than C^*).

The formation of hydrophobic domains in unmodified chitosan seems to be rather unexpected. Nevertheless, our fluorescence results are consistent with the static[17] and dynamic light scattering data.[18,19] These data indicate the appearance of intermolecular aggregates in aqueous solutions of chitosan at the same concentration region at which fluorescence spectroscopy detects the appearance of hydrophobic domains. Therefore, our data show that the intermolecular aggregation in aqueous solutions of chitosan proceeds with the formation of hydrophobic domains capable to solubilize pyrene.

Usually upon formation of intermolecular aggregates the polymers demonstrate a significant increase of solution viscosity or gelation or precipitation. Here we show that the formation of intermolecular aggregates in unmodified chitosan does not lead to any of these effects. In particular, upon the formation of aggregates, the viscosity of unmodified chitosan rises only slightly (Figure 3). We can suggest two possible reasons for that. First, the aggregates may include only a rather small fraction of polymer chains, while most of the macromolecules remain nonaggregated. The second reason for a small influence of aggregation on the viscosity may be due to a more compact form of aggregates of chitosan in comparison with HM chitosan. More compact aggregates

Table 2. Concentration of Hydrophobic Domains in 0.3 M CH_3COOH Aqueous Solutions of Chitosan and HM Chitosan Calculated from the Fluorescence Data

concentration of polymer, g/L	concentration of hydrophobic microdomains $\times 10^{-6}$, mol/L	
	chitosan	HM chitosan
1.2		2
3	3	6
5	8	12
12	13	16
16	18	
24	24	

can be formed, for example, as a result of lateral arrangement of segments of different macromolecules (Figure 4a). Such aggregates were recently observed for some derivatives of another polysaccharide, cellulose.[20]

Concentration of Hydrophobic Aggregates. The concentration of hydrophobic domains, in which pyrene molecules are solubilized, was estimated by fluorescence spectroscopy as described in the experimental part. The results are presented in Table 2. It is seen that the concentration of hydrophobic domains is always somewhat higher for HM chitosan when compared with unmodified chitosan at the same polymer concentration, but the difference is not very significant. Therefore, the small influence of intermolecular aggregates on the viscosity of chitosan cannot be explained just by a small concentration of these aggregates. Most probably, it is due mainly to the compact form of the aggregates.

The values of the concentration of hydrophobic domains in HM polymers are usually used to estimate the mean aggregation number of domains under the assumption that all hydrophobic side chains are included in the domains.[14] Such an estimation, e.g., for the solutions of HM chitosan (5 g/L), gives the value of aggregation number to be equal to 175, which seems to be too high. Such a high value of the aggregation number can arise from the fact that not all the side chains are aggregated at these conditions. For instance, if we will assume that only ca. 5% of hydrophobic groups are aggregated, the mean aggregation number will have a realistic value of ca. 9.

The fact that not all hydrophobic side chains are included in the aggregates was recently observed for another associating polymer, HM poly(sodium acrylate) (PSA).[21] By NMR measurements, it was shown that in 5 g/L aqueous solutions of HM PSA (molecular weight 150 000) containing 3 mol % of hydrophobic *n*-dodecyl side chains only ca. 1% of hydrophobic groups are aggregated. The fraction of aggregated hydrophobic groups increases with increasing polymer concentration and becomes as high as 20% at polymer concentration equal to 100 g/L. The analysis of our fluorescence data allows one to suggest that in fully charged HM chitosan as in fully charged HM PSA only a small fraction of the hydrophobic groups are aggregated.

To gain more insight on the nature of forces involved in the formation of hydrophobic microdomains, the effects of 7 M urea, salt, ethanol, and temperature were examined.

Effect of Urea. Chitosan does not contain groups with very pronounced hydrophobic properties, like *n*-alkyl side chains. What can be the reason for the formation of hydrophobic domains in aqueous solutions of this polymer? One could suggest that hydrophobic domains are formed by nonionic *N*-acetyl-D-glucosamine repeat units, which are known to aggregate with each other due to hydrogen bonding.[22] But such aggregation is rather pronounced only when polymer chains have rather long sequences of *N*-acetyl-D-glucosamine units.[22−25] In the chitosan under study, NMR data demonstrate that the distribution of nonionic *N*-acetyl-D-glucosamine units along the backbone is not blocky. Therefore, we cannot expect significant aggregation of acetylated groups with each other. On the other hand, in chitosan, not only acetylated units but also deacetylated units possess both proton donor and proton acceptor groups capable of interacting with each other via hydrogen bonding. Thus, we can suggest that both types of chitosan units (*N*-acetyl-D-glucosamine and D-glucosamine units) can participate in the formation of hydrogen bonds.

Each monomer unit of chitosan contains both hydrophilic and hydrophobic groups, but only hydrophilic groups are involved in hydrogen bonding.[26] As a result of such bonding the interacting hydrophilic groups become "screened" from solvent, which effectively weakens the hydrophilicity of polymer.[27] The formation of cooperative hydrogen bonds between different macromolecules may be sensed by pyrene as a formation of hydrophobic domains as was shown for complexes between poly(methacrylic acid) and poly(ethylene oxide).[28]

To check this suggestion, we studied the effect of 7 M urea on the formation of hydrophobic domains. Urea is known to break effectively the hydrogen bonds.[29] The "side" effect of this property is the ability of urea to weaken the hydrophobic interactions in aqueous medium as well. Indeed, the driving force for hydrophobic association in aqueous systems is partially attributed to the need for the hydrophobic moieties to minimize the surface area of their contact with water and consequently minimize the amount of water that must be "structured" in order to solubilize them. The addition of urea to aqueous solutions disrupts the structuring ability of water, thereby weakening the hydrophobic interactions in the solution.[30] Therefore, urea can produce a double effect: it can destroy both the hydrogen bonds and the hydrophobic interactions in the system.

Figures 5 and 6 show the effect of 7 M urea on the polarity parameter of pyrene I_1/I_3 in aqueous solutions of chitosan and HM chitosan, respectively. It is seen that 7 M urea affects only scarcely the formation of hydrophobic domains in unmodified chitosan. This indicates that neither hydrogen bonding nor hydrophobic effects of the type that urea would destroy are responsible for the formation of hydrophobic aggregates in aqueous solutions of this polymer.

In contrast, the effect of 7 M urea on the aggregation of HM chitosan is very significant: in 7 M urea the hydrophobic domains appear only at polymer concentrations of ca. 2 g/L (instead of ca. 0.1 g/L in the absence of 7 M urea). Therefore, in HM chitosan, 7 M urea hinders the formation of hydrophobic domains but cannot prevent completely their appearance. It is interesting that the curves in 7 M urea are almost identical for HM chitosan and for its precursor

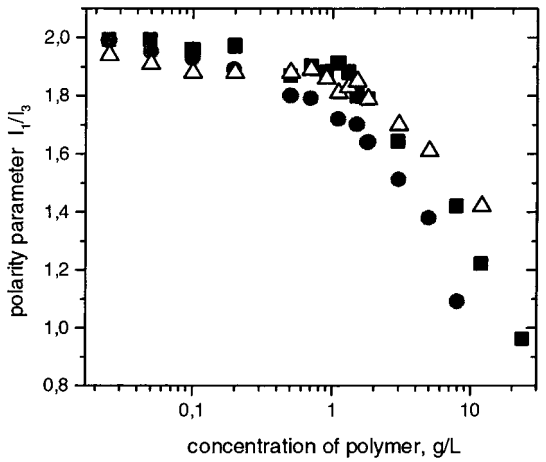

Figure 5. Effects of salt and urea on the dependence of polarity parameter I_1/I_3 of pyrene on concentration of chitosan. Solvents: 0.3 M CH_3COOH (■), 0.3 M CH_3COOH/0.2 M CH_3COONa (●), and 0.3 M CH_3COOH/7 M urea (△).

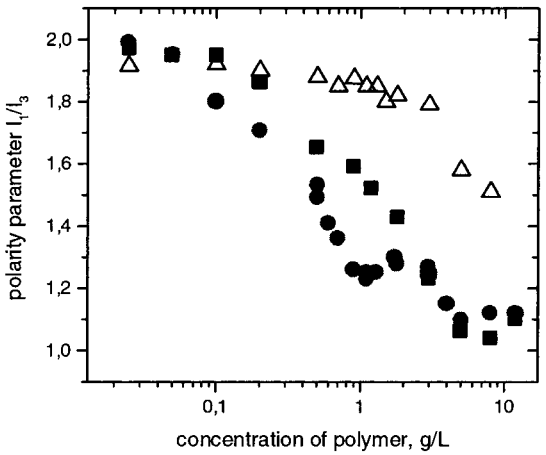

Figure 6. Effects of salt and urea on the dependence of polarity parameter I_1/I_3 of pyrene on concentration of HM chitosan. Solvents: 0.3 M CH_3COOH (■), 0.3 M CH_3COOH/0.2 M CH_3COONa (●), and 0.3 M CH_3COOH/7 M urea (△).

(Figures 5 and 6). This fact allows us to suggest that in HM chitosan two kinds of hydrophobic domains are formed (Figure 4b): (1) the domains inherent to HM chitosan (they include hydrophobic side chains) and (2) the domains inherent to chitosan itself. Here, 7 M urea disrupts only the first type of these domains, while the second type of domain (Figure 4a) remains almost unaffected, and HM chitosan behaves like its unmodified precursor.

Effect of Added Salt. As was mentioned above, both chitosan and HM chitosan are in a fully ionized state and contain a high fraction of charged repeat units (0.88 in chitosan and 0.84 in HM chitosan), which are responsible for the effective electrostatic repulsion, counteracting the hydrophobic aggregation. One can suggest that the addition of low molecular weight salt, which screens the electrostatic factors, will facilitate the formation of hydrophobic domains.

The effect of salt on the intrinsic viscosity of dilute solutions of chitosan and HM chitosan is shown in Table 1. It is seen that with increasing salt concentration the intrinsic viscosity of both chitosan and HM chitosan decreases, the decrease of viscosity being much more pronounced for the HM polymer. Finally, this results in the fact that at higher salt concentrations (0.2 M CH_3COONa) the viscosity of HM

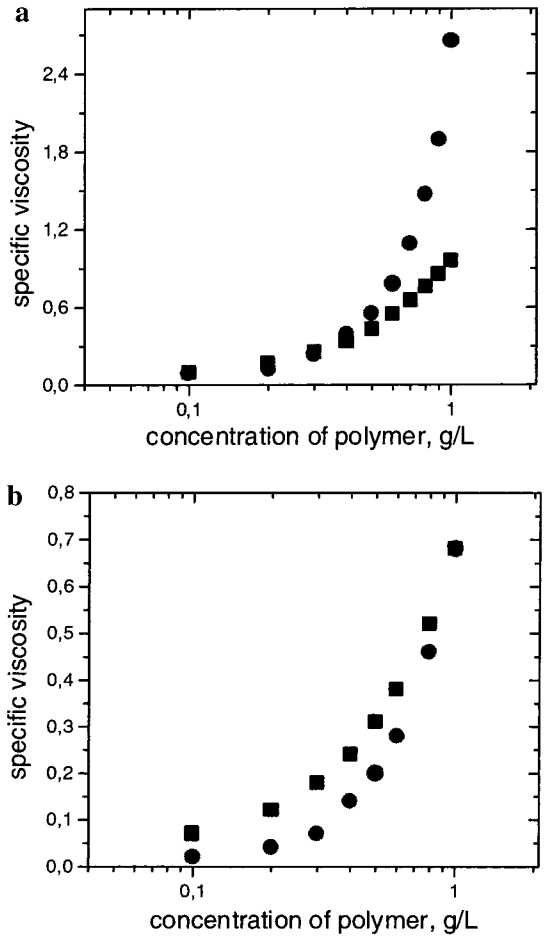

Figure 7. Specific viscosity as a function of polymer concentration for aqueous solutions of chitosan (■) and HM chitosan (●) in 0.3 M CH_3COOH/0.05 M CH_3COONa (a) and in 0.3 M CH_3COOH/0.2 M CH_3COONa (b).

chitosan becomes much lower than for its unmodified precursor (Figure 7b). This may be due to two reasons: (1) to the folding of highly charged polymer chains, when the electrostatic repulsion is screened by salt and (2) to the enhancement of hydrophobic interactions in the presence of salt. Both these effects facilitate the intra- and/or intermolecular aggregation of hydrophobic side chains. This suggestion is supported by very high values of Huggins constants calculated from the viscosity data for salt solutions of HM chitosan (Table 1). High values of the Huggins constant are usually interpreted as enhanced polymer−polymer interactions.[7]

At the same time, a recent study[17] did not reveal any indications to intramolecular aggregation in dilute solutions of HM chitosan induced by salt: the viscosity of HM chitosan was always somewhat higher than that of unmodified chitosan. Most probably, this is due to the low salt concentrations used in the study[17] (up to 0.01 M NaCl). Here we demonstrate that at higher salt concentrations (0.05−0.2 M) the salt is able to induce the intramolecular hydrophobic aggregation in HM chitosan.

Figures 5 and 6 show the effect of added salt, sodium acetate, on the fluorescence intensity ratio I_1/I_3 of pyrene in aqueous solutions of chitosan and HM chitosan, respectively. It is seen that the salt affects only slightly the formation of hydrophobic aggregates in unmodified chitosan. The salt

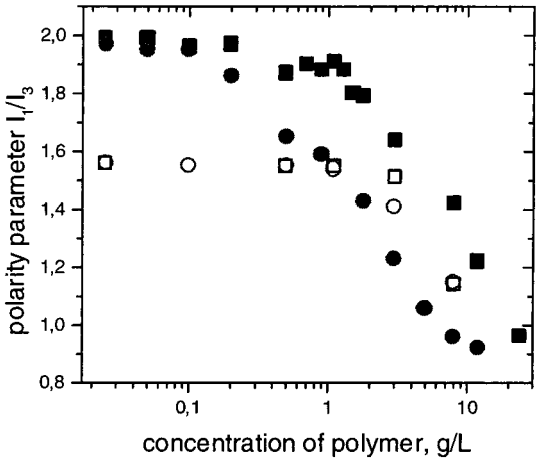

Figure 8. Polarity parameter I_1/I_3 of pyrene as a function of polymer concentration in solutions of chitosan (open and closed squares) and HM chitosan (open and closed circles) in 0.3 M CH_3COOH in water (closed symbols) and in water/ethanol mixture (1:1 v/v) (open symbols).

effect on hydrophobic aggregation of HM chitosan is more significant. In this case, the salt induces the formation of aggregates at lower polymer concentrations than in salt-free solutions. These data are in good agreement with the results of viscosity measurements and support our suggestion that the salt triggers the intramolecular hydrophobic aggregation.

It should be noted that in the case of HM chitosan the dependence of the polarity parameter of pyrene on the polymer concentration has a small plateau (Figure 6). Although the deviations of the points on plateau from the smooth curve are almost within the limits of experimental error, they look systematic. These data may reflect the process of two-step restructuration of hydrophobic domains: formation of loose domains consisting of a small number of hydrophobic side chains (where pyrene experiences rather hydrophilic environment) and then the growth of these domains with further increase of polymer concentration, when the amount of hydrophobic side chains becomes high enough to form domains of optimal size, which are able to screen effectively the hydrophobic groups from water.

Effect of Ethanol. Ethanol added to aqueous solutions should disrupt the aggregates formed exclusively due to hydrophobic interactions.

The effect of ethanol on the change of polarity parameter of pyrene I_1/I_3 with polymer concentration is shown in Figure 8. It is seen that in dilute solutions of both polymers in 0.3 M CH_3COOH in water/ethanol mixture pyrene experiences a less polar environment than in the corresponding polymer solutions in 0.3 M CH_3COOH in water (without ethanol). This is obviously due to the decrease of the polarity of medium in the presence of ethanol. It is seen that ethanol produces quite different effects on chitosan and on HM chitosan: it favors the formation of hydrophobic domains in chitosan solution, while at the same time it hinders the formation of hydrophobic domains in HM chitosan solution. As a result it turns out that the data for chitosan and for HM chitosan in this solvent lie on the same curve (Figure 8). This allows us to suggest that ethanol disrupts completely the domains formed by hydrophobic side chains of HM chitosan, and this polymer behaves as its unmodified

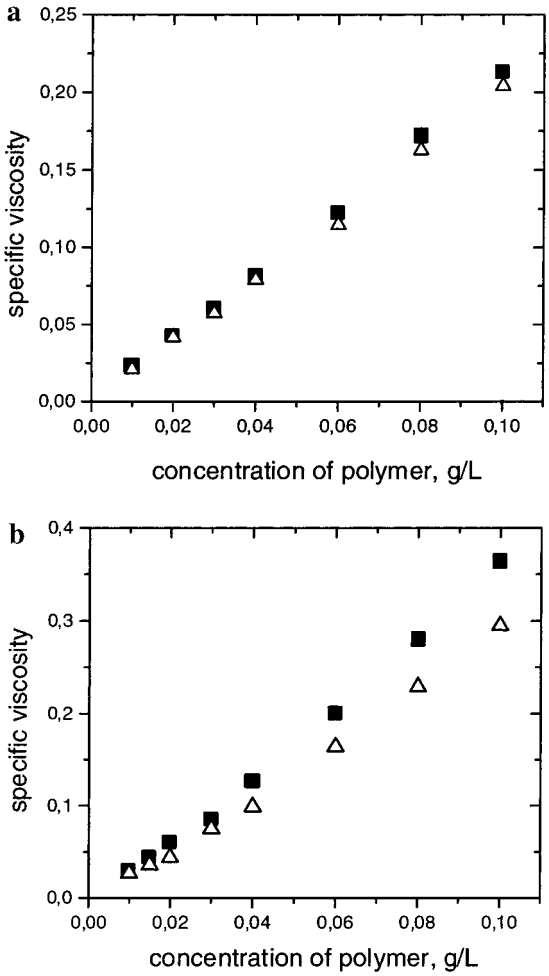

Figure 9. Effect of temperature on the dependence of viscosity of aqueous solutions of chitosan (a) and HM chitosan (b) in 0.3 M CH_3COOH: (■) 25 °C; (○) 40 °C.

precursor. As to unmodified chitosan, the fact that ethanol favors the appearance of hydrophobic domains in this polymer clearly evidences that these domains are not only due to hydrophobic interactions, but some other forces are responsible for their formation. It should be noted that for chitosan in 0.3 M CH_3COOH in water/ethanol mixture the formation of hydrophobic domains takes place just at the same concentrations of polymer as in 0.3 M CH_3COOH/0.2 M CH_3COONa aqueous solution (cf. Figures 5 and 8). This means that the effects of ethanol and of low molecular weight salt are quite similar. Most probably, they are due to the decrease of the electrostatic repulsion of similarly charged polymer chains. Indeed, ethanol being of lower polarity than water will induce the condensation of counterions on the charged groups of polymer chains, which will reduce the net charge density of macromolecules. Moreover, the ion pairs which are formed as a result of counterions condensation in ethanol can interact with each other due to the dipole−dipole attraction which additionally facilitates the aggregation. [31]

Effect of Temperature. The effect of temperature on the viscosity of dilute solutions of chitosan and HM chitosan in 0.3 M CH_3COOH is shown in Figure 9. It is seen that for both polymers heating leads to a small decrease of viscosity, that is, to the shrinking of macromolecules or their aggregates. This may be due to the strengthening of intra- and/or

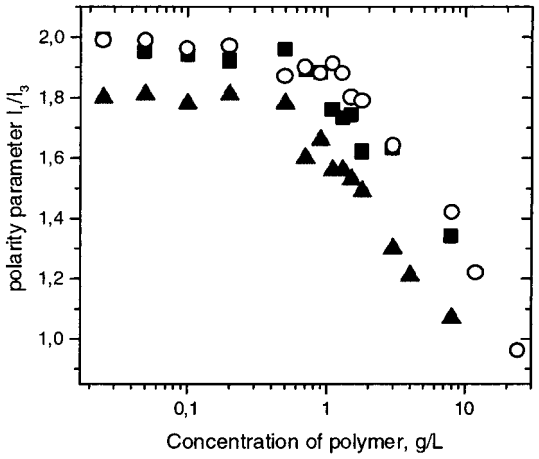

Figure 10. Polarity parameter I_1/I_3 of pyrene in aqueous solutions of chitosan in 0.3 M CH_3COOH as a function of polymer concentration at different temperatures: 15 (○), 25 (■), and 40 °C (▲).

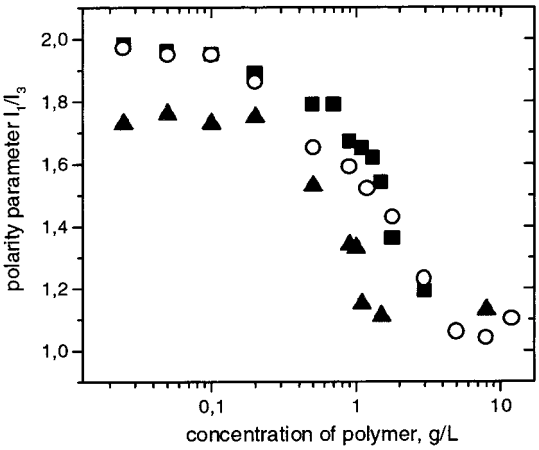

Figure 11. Polarity parameter I_1/I_3 of pyrene in aqueous solutions of HM chitosan in 0.3 M CH_3COOH as a function of polymer concentration at different temperatures: 15 (○), 25 (■), and 40 °C (▲).

intermolecular self-aggregation. The effect of temperature is more pronounced for HM chitosan than for its precursor (Figure 9).

Figures 10 and 11 show that in the dilute regime heating from 25 to 40 °C leads to a small decrease of the polarity parameter of pyrene from 2.0 to 1.8 (for chitosan) and 1.75 (for HM chitosan). Under the same conditions, the polarity parameter of pyrene in 0.3 M CH_3COOH (without polymer) decreases from 2.0 to 1.8, which may be due to the disruption of the network of hydrogen bonds between water molecules surrounding pyrene at heating. Therefore, the slight decrease of the polarity parameter of pyrene due to the hydrophobic aggregation of polymer is observed mainly for HM chitosan. Therefore, heating of the dilute solutions of chitosan and HM chitosan leads to some enhancement of hydrophobic aggregation, which is accompanied by shrinking of macromolecules, the effect being more pronounced for the HM polymer.

At the same time, in the semidilute regime, the effect of heating from 25 to 40 °C is clearly seen both for HM chitosan and for its precursor. Under these conditions, heating leads to the lowering of polymer concentrations, at which the polarity parameter of pyrene drops.

Conclusions

It was shown that HM chitosan demonstrates behavior typical for many associating polyelectrolytes. Upon formation of hydrophobic domains detected by the fluorescence probe method the viscosity of solutions of this polymer rises significantly, which is obviously due to the bridging of multiple polymer molecules by hydrophobic domains.

It is surprising that the precursor polymer (without hydrophobic side chains) is also able to form hydrophobic domains, but at much higher polymer concentrations. The hydrophobic domains in chitosan are very stable and only scarcely affected by heating and by the addition of salt, urea, and ethanol. This suggests that neither hydrogen bonds nor hydrophobic aggregation are responsible for the aggregation of chitosan. The nature of these aggregates still remains an open question, and it will be studied in our further investigations, in particular as such aggregates seem to be a general feature of many charged polysaccharides.[20]

An important observation consists of the fact that upon the addition of urea or ethanol to the solutions of HM chitosan the hydrophobic domains are partially disrupted and this polymer behaves like its unmodified precursor. This fact allows us to suggest that in HM chitosan two types of hydrophobic domains exist. The first type of domain is inherent to chitosan itself. The second type of domain is typical for different polymers with hydrophobic side chains.

Acknowledgment. This research was supported by the INTAS Grant No. 96-1193. O.E.P. gratefully acknowledges the financial support provided by Moscow Grant No. B-269 and RFBR Grant No. 99-03-33447. The authors are also grateful to Dr. G. A. Sukhadolski, Prof. V. A. Smirnov, and Dr. B. D. Ryzhikov for their help in the measurements of some fluorescence spectra and to Dr. V. G. Vasiliev for his advice in viscosity measurements.

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BM005649A