Notes

Ionization Characteristics of Polyelectrolyte Complex Gels: Analysis Based on Their Swelling Behaviors

Takaharu Sakiyama,* Tomoya Tsutsui, Etsuko Masuda, Koreyoshi Imamura, and Kazuhiro Nakanishi

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Okayama 700-8530, Japan

Received November 27, 2002
Revised Manuscript Received April 9, 2003

Introduction

When oppositely charged polyelectrolytes are mixed in a solution, strong electrostatic interaction between them leads to spontaneous complex formation. The product, whether soluble or insoluble, is called a polyelectrolyte complex (PEC). PECs have been artificially prepared from natural and/or synthetic polyelectrolytes for various applications.[1] For example, PECs were used as membranes for separation,[2−4] materials for the immobilization of enzymes[5] or cells,[6,7] and carriers for drug delivery.[8,9] Furthermore, PECs prepared from DNA and cationic polymers are expected to be useful as gene delivery tools.[10−12]

To develop PECs with the optimized properties for an application, characterization of PECs is necessary from various points of view. So far, a number of studies have been reported on physical and chemical properties of PECs,[1] including stoichiometry,[13] cluster size,[14] polymer conformation,[15] and swelling properties.[16−18] However, the information on ionization characteristics of PECs is scarce, especially for insoluble PECs, probably because of the problems associated with the measurement of pH inside PECs.

Here we report the analysis of the ionization characteristics of polyelectrolyte complexes composed of dextran sulfate and chitosan. Dextran sulfate is a strongly acidic polysaccharide with sulfate groups. Chitosan is a weakly basic polysaccharide with amino groups. Previously, we prepared PECs from the two polysaccharides as moldable gels and showed that they had pH-dependent swelling properties.[19,20] Because the pH dependence of gel volume is attributed to the change in ionization degree of the amino groups, analysis of swelling equilibrium data is expected to bring us information about the ionization characteristics of the amino groups. Here we studied pH-dependent swelling equilibria for two types of dextran sulfate/chitosan gels (DS/CH gels). We developed a theoretical model to estimate the net charge of DS/CH gels from the swelling equilibrium data. On the basis of those results, the effect of complex formation on the dissociation constant of protonated amino groups of chitosan was discussed.

* Corresponding author: Fax +81-86-251-8264; e-mail sakiyama@biotech.okayama-u.ac.jp.

Experimental Section

Chitosan with a deacetylation degree of 81.9% (8B; lot SJ03-M) was a product of Katokichi Co., Ltd. (Kagawa, Japan). The average molecular weight (MW), determined by viscosity measurement in 0.1 M acetic acid−0.2 M NaCl solution,[21] was 1.8×10^6. Dextran sulfate sodium salt (MW 5×10^5; lot 261208) was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Its sulfur content was 17%, indicating that the average number of sulfate group per glucose unit was 1.9. Other materials were of reagent grade.

Chitosan was dissolved in 2% acetic acid solution containing 10% NaCl. Dextran sulfate was dissolved in 10% NaCl solution. The presence of NaCl was necessary to avoid rapid aggregation on mixing the two solutions. Both solutions were heated at 80 °C to decrease their viscosity and then mixed with each other. At this stage, phase separation was not observed even under an optical microscope. The final concentration of chitosan was 2.25% while the final concentration of dextran sulfate was 1.50% or 2.19%. After being centrifuged at 3000 rpm for 10 min to remove air bubbles, the mixture was dropped into ice-cooled toluene/chloroform (5:3). After being kept at 4 °C overnight, the droplets were carefully transferred into a large volume of deionized water for desalting. The complex formation was completed through this desalting stage. The spherical gel beads thus obtained were rinsed in deionized water at least for 2 days.

For each type of the gel beads prepared, the solid content was measured by drying them under a reduced pressure. The amounts of amino and sulfate groups in each type of gel beads were calculated from the nitrogen and sulfur contents, respectively, measured by the elementary analysis (2400 series II, Perkin-Elmer, Shelton, CT) of the dry gel beads. Sodium content was measured with an atomic absorption spectrophotometer (150-50A, Hitachi, Tokyo, Japan) after the gel beads were dissolved in 0.1 N HNO_3.

Swelling experiments were performed as follows. In a 50 mL polystyrene flask, a gel bead with an initial diameter d_i was immersed in 30 mL of 10 mM NaCl solution, the pH of which was preliminarily adjusted to a specified value by adding dilute NaOH or HCl solution. The flask was purged with N_2 gas and sealed to avoid the dissolution of CO_2 before the incubation at 30 °C. After swelling equilibrium was attained, the equilibrium diameter of the gel bead, d_e, and pH of the ambient solution were measured. The equilibrium swelling ratio, defined as the ratio of the equilibrium volume to the initial one, was calculated as $(d_e/d_i)^3$.

Results and Discussion

For each type of DS/CH gel prepared, the contents of solid, amino group, and sulfate group together with the molar ratio of the sulfate group to the amino group (S/N) are listed in Table 1. The two types of DS/CH gels are referred to as DS/CH-0.78 and DS/CH-1.12 after their S/N values. DS/CH-0.78 had less sulfate groups than amino groups, while DS/CH-1.12 had more sulfate groups than amino groups. Figure 1 shows the results of the swelling experiments for the two types of DS/CH gels in 10 mM NaCl solution of various pHs. The initial

10.1021/ma021721g CCC: $25.00
Published on Web 06/03/2003

Table 1. Compositions of the DS/CH Gel Beads Prepared in This Study

type	solid (wt %)	amino group[a] (mol/cm^3)	sulfate group (mol/cm^3)	S/N[b]
DS/CH-0.78	1.00	27	21	0.78
DS/CH-1.12	8.09	190	212	1.12

[a] Acetylated amino groups were excluded. [b] S/N is referred to as the molar ratio of the sulfate group to the amino group.

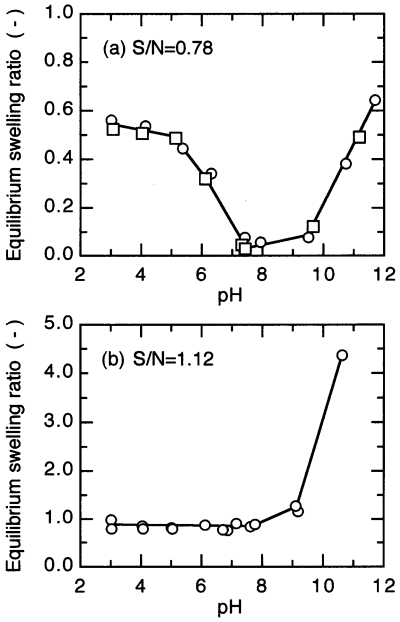

Figure 1. Effect of pH on the equilibrium swelling ratio of DS/CH gels of S/N = 0.78 (a) and of S/N = 1.12 (b) in the presence of 10 mM NaCl. The initial diameters of the gel beads were 3.63 ± 0.22 mm (○) or 6.52 ± 0.34 mm (□) for DS/CH-0.78 and 3.33 ± 0.18 mm for DS/CH-1.12.

diameters of DS/CH-0.78 beads were 3.63 ± 0.22 or 6.52 ± 0.34 mm, and those of DS/CH-1.12 beads were 3.33 ± 0.18 mm. When the gel beads were transferred from deionized water into 10 mM NaCl solution without preliminary pH adjustment, they shrunk owing to the increase in ambient osmotic pressure. Thus, the equilibrium swelling ratio around pH 5.5 was lower than 1 for the both types of gels. As shown in Figure 1a, the equilibrium volume of DS/CH-0.78 decreased with increasing pH from 5 to 7 and reached the minimum at a pH between 7 and 8. With increasing pH from 8, the equilibrium volume became closer again to the initial one. The bead diameter had no effect on the equilibrium swelling ratio. On the other hand, the equilibrium volume of DS/CH-1.12 increased with increasing pH above 8 as shown in Figure 1b.

These pH-dependent behaviors are qualitatively explained by the modes of dissociation illustrated in Figure 2. In the initial state, the amino groups of DS/CH gels are protonated since they were prepared under acidic conditions. In DS/CH-0.78, the protonated amino groups are classified into the following two types: one is bound to a sulfate group with electrostatic interaction (type N1), and the other is free from the electrostatic interaction (type N2). The sodium content measurement showed that DS/CH-0.78 initially contained a negligible amount of Na^+ (data not shown), suggesting that almost all of the sulfate groups were electrostatically bound to the protonated amino groups of type N1 in the initial state. Thus, DS/CH-0.78 initially has a net positive charge and is in a swollen state. When it is immersed

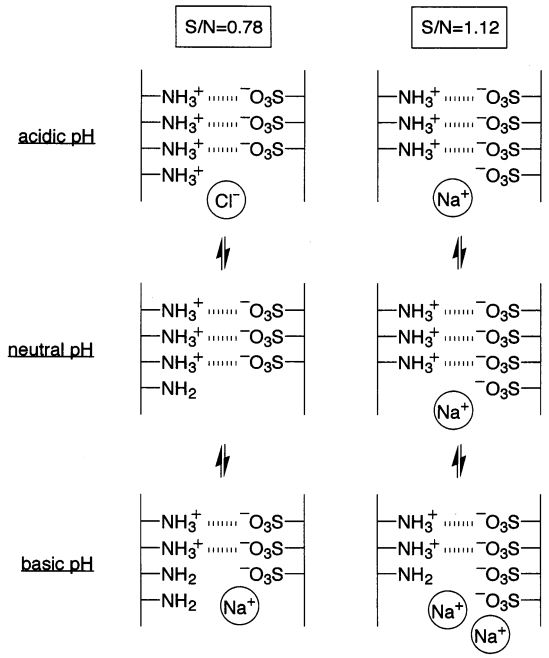

Figure 2. Schematic illustration of the change in ionization state of DS/CH gels.

in a solution of pH around 7, the protonated amino groups of type N1 are deionized. This is reasonable considering that pK_a ($-\log K_a$) was reported to be 6.4,[22] where K_a was the dissociation constant of protonated amino groups of chitosan. The net charge thus decreases to make the gel shrink. The protonated amino groups of type N2 probably need a higher pH to become deionized because of the presence of negatively charged groups in their close vicinity.[23] Owing to the deionization of the protonated amino groups of type N2 at basic pHs, DS/CH-0.78 becomes negatively charged and the gel volume thus increases. In the case of DS/CH-1.12, part of the sulfate groups are electrostatically bound to the protonated amino groups, and the rest remains free from the electrostatic interaction. Thus, DS/CH-1.12 initially has a net negative charge. The protonated amino groups, belonging to type N2, are deionized only at alkaline pHs. The deionization of the amino groups increases net negative charge of DS/CH-1.12, leading to increase in the equilibrium volume.

On the basis of the above discussion, we developed a theoretical model to estimate the net charge of DS/CH gels. To begin with, we consider the cases where the molar content of amino groups is greater than that of sulfate groups, namely S/N < 1. The dissociation constants of the two types of protonated amino groups are probably different as discussed above. Therefore, we discriminated between them using suffix N1 and N2. In the following derivation, it is assumed for simplicity that the dissociation constants are independent of the degree of ionization and that the activity coefficients of ions are unity. The charge balance equation for the gel is given as follows.

$$\sum_i Z_i C_i^G - \alpha_S C_S^G/X + \alpha_{N1} C_{N1}^G/X + \alpha_{N2} C_{N2}^G/X = 0 \qquad (1)$$

where C_i^G is the concentration of mobile ion i in the gel, Z_i is the charge on ion i, C_S^G is the initial concentration of sulfate group in the gel, C_{N1}^G and C_{N2}^G are the initial concentrations of the two types of amino groups, and X

is the ratio of the equilibrium gel volume to the initial one. The degree of ionization of sulfate groups, α_S, can be assumed as unity unless the pH is extremely low. The degrees of ionization of amino groups, α_{N1} and α_{N2}, are related to the dissociation constants of the protonated amino groups, K_{N1} and K_{N2}, as follows:

$$K_{N1} = C_H^G(1 - \alpha_{N1})/\alpha_{N1} \quad (2)$$

$$K_{N2} = C_H^G(1 - \alpha_{N2})/\alpha_{N2} \quad (3)$$

where C_H^G is the concentration of proton (hydronium ion) in the gel. Assuming the Donnan potential created by the fixed charge on the gel, mobile ions are distributed between the gel and the external solution as described in the following equation[24]

$$C_i^G/C_i^S = K^{Z_i} \quad (4)$$

where C_i^S is the concentration of mobile ion i in the external solution. The Donnan ratio, K, is identical for all ion species though it depends on such conditions as pH and ionic strength. On the basis of eq 4, eqs 2 and 3 can be rewritten as follows:

$$\alpha_{N1} = KC_H^S/(K_{N1} + KC_H^S) \quad (2')$$

$$\alpha_{N2} = KC_H^S/(K_{N2} + KC_H^S) \quad (3')$$

Using eqs 2′, 3′, and 4, eq 1 is expressed in terms of the external concentrations of mobile ion species as follows:

$$\sum_i Z_i K^{Z_i} C_i^S - \frac{C_S^G}{X} + \frac{KC_H^S C_{N1}^G}{(KC_H^S + K_{N1})X} + \frac{KC_H^S C_{N2}^G}{(KC_H^S + K_{N2})X} = 0 \quad (5)$$

We obtained the value of K by solving eq 5 with the experimental values for C_i^S, C_S^G, C_{N1}^G, C_{N2}^G, and X. The dissociation constants, K_{N1} and K_{N2}, should be known for the calculation. As for a chemically cross-linked chitosan resin, the results of potentiometric titration was reported to be well predicted by using the dissociation constant of protonated amino groups of chitosan.[25] Thus, it is reasonable to assume that K_{N1} is equal to the dissociation constant of protonated amino groups of chitosan, and hence $pK_{N1} = -\log K_{N1} = 6.4$. For K_{N2}, we assumed an appropriate value as described later. Substituting the obtained value for K in eqs 2′ and 3′ yielded values of α_{N1} and α_{N2}, from which the net charge was calculated. For the case where the molar content of amino groups is equal to or less than that of sulfate groups, namely S/N ≥ 1, eq 5 is still valid if C_{N1}^G is taken as 0.

Figure 3a shows the pH dependence of the net charge calculated for DS/CH-0.78. The net charge, expressed as the value relative to the molar content of amino groups, decreased as pH increased. The gel volume is expected to be minimum when the net charge is zero. If pK_{N2} was assumed to be equal to $pK_{N1} = 6.4$, the net charge became zero around pH 5.8. This is not in accordance with the fact shown in Figure 1a that the minimum gel volume was attained at a pH between 7 and 8. We calculated the net charge assuming higher

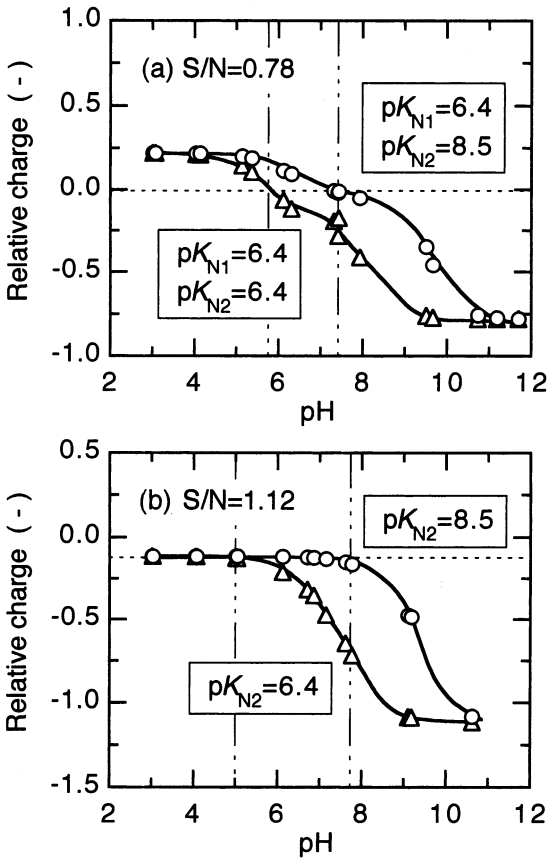

Figure 3. Results of the net charge estimation for DS/CH gels of S/N = 0.78 (a) and of S/N = 1.12 (b). The symbols (○, ●) represent the values of net charge relative to the molar content of amino groups calculated from the experimental data shown in Figure 1 on the assumptions indicated in the boxes. The solid and broken curves are to guide the eye.

values for pK_{N2} and found that the net charge became zero at a pH between 7 and 8 if we assumed $pK_{N2} = 8.5$. Figure 3b shows the pH dependence of the net charge calculated for DS/CH-1.12. In this case, the net charge was negative at any pH because of the excess sulfate groups. When the deionization of the protonated amino groups occurred, the net negative charge increased, causing the increase in the gel volume. If pK_{N2} was assumed to be equal to $pK_{N1} = 6.4$, the decrease in the net charge would be found even at pHs below 6. If pK_{N2} was assumed to be 8.5, the decrease in the net charge was found only at pHs above 8. The assumption that pK_{N2} is 8.5 thus explained the results of swelling experiments for DS/CH-1.12 as well as for DS/CH-0.78.

Above pH 9, however, the increase in equilibrium volume was more drastic than the increase in negative charge for both types of gel beads. The complex disintegration is a probable reason for this because the disintegration lowers the degree of cross-linking between the two polyelectrolytes.

The value of pK_a for the protonated amino groups was shown to increase, owing to the electrostatic interaction with the sulfate groups. The increase of pK_a implies the stabilization of the protonated state of amino groups. On the basis of the assumption that pK_{N2} is 8.5, the standard Gibbs free energy of the stabilization, $\Delta\Delta G°$, would amount to 12 kJ/mol at 300 K according to the relation $\Delta\Delta G° = -2.303RT(pK_{N2} - pK_{N1})$, where R and T are gas constant and absolute temperature, respectively. Although the value of $\Delta\Delta G°$ is not necessarily

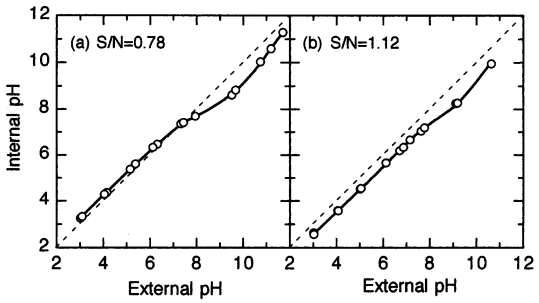

Figure 4. Internal pH values estimated for DS/CH gels of S/N = 0.78 (a) and of S/N = 1.12 (b). The symbols (○) represent the values of internal pH calculated from the experimental data shown in Figure 1 assuming $pK_{N1} = 6.4$ and $pK_{N2} = 8.5$. The solid curves are to guide the eye.

definite because the calculation was based on the assumed value of pK_{N2}, it can be said that the stabilization of the protonated amino groups is supported by a relatively large free energy. The presence of a negatively charged group in close vicinity of the protonated amino group probably makes a major contribution to the relatively large energy of stabilization. Another contribution may come from a cooperativity effect; the presence of a number of pairs of oppositely charged groups along the polymer chains would inhibit each protonated amino group from leaving its counterpart.

On the basis of the above analysis, it is also possible to estimate the pH value inside the DS/CH gels using the relation $-\log C_H^G = -\log(KC_H^S)$, derived from eq 4, together with the value of K calculated for each swelling experiment. Figure 4 shows relationships between the internal and external pH values estimated for DS/CH-0.76 and DS/CH-1.12 assuming $pK_{N1} = 6.4$ and $pK_{N2} = 8.5$. The results suggest that the internal pH value is different from the external pH value. Furthermore, the change in internal pH is suggested to be smaller than the change in external pH, especially for DS/CH-0.76, in the pH range in which the dissociation of the amino groups is assumed to occur. These may cause a difficulty in determining the precise values of pK_{N1} and pK_{N2} by potentiometric titration, in which only the external pH is evaluated.

In conclusion, we analyzed the dissociation behavior of protonated amino groups in DS/CH gels. Although further confirmation may be necessary, this is the first report that quantitatively discussed the decrease in dissociation constant of protonated basic groups upon complex formation with strongly acidic groups. The strategy of analysis adopted in this study may be useful for investigating the ionization characteristics of various PECs as long as their volume change can be measured.

Acknowledgment. Part of this work was financially supported by Okayama Foundation for Science and Technology.

References and Notes

(1) Philipp, B.; Dautzenberg, H.; Linow, K.-J.; Kötz, J.; Dawydoff, W. *Prog. Polym. Sci.* **1989**, *14*, 91−172.
(2) Hung, P. D.; Masawaki, T.; Tone, S. *J. Chem. Eng. Jpn.* **1998**, *31*, 484−487.
(3) Kim, S.-G.; Lim, G.-T.; Jegal, J.; Lee, K.-H. *J. Membr. Sci.* **2000**, *174*, 1−15.
(4) Iwatsubo, T.; Kusumocahyo, S. P.; Shinbo, T. *J. Appl. Polym. Sci.* **2002**, *86*, 265−271.
(5) Dumitriu, S.; Chornet, E. *Biotechnol. Prog.* **1997**, *13*, 539−545.
(6) Chu, C.-H.; Kumagai, H.; Nakamura, K. *J. Appl. Polym. Sci.* **1996**, *60*, 1041−1047.
(7) Yoshioka, T.; Hirano, R.; Shioya, T.; Kako, M. *Biotechnol. Bioeng.* **1990**, *35*, 66−72.
(8) Kono, K.; Kawakami, K.; Morimoto, K.; Takagishi, T. *J. Appl. Polym. Sci.* **1999**, *72*, 1763−1773.
(9) Mi, F.-L.; Sung, H.-W.; Shyu, S.-S. *Carbohydr. Polym.* **2002**, *48*, 61−72.
(10) MacLaughlin, F. C.; Mumper, R. J.; Wang, J.; Tagliaferri, J. M.; Gill, I.; Hinchcliffe, M.; Rolland, A. P. *J. Controlled Release* **1998**, *56*, 259−272.
(11) Hill, I. R. C.; Garnett, M. C.; Bignotti, F.; Davis, S. S. *Anal. Biochem.* **2001**, *291*, 62−68.
(12) Liu, W. G.; Yao, K. D.; Liu, Q. G. *J. Appl. Polym. Sci.* **2001**, *82*, 3391−3395.
(13) Karibyants, N.; Dautzenberg, H.; Coelfen, H. *Macromolecules* **1997**, *30*, 7803−7809.
(14) Pogodina, N. V.; Tsetkov, N. V. *Macromolecules* **1997**, *30*, 4897−4904.
(15) Paradossi, G.; Chiessi, E.; Malovìkovà, A. *Macromolecules* **2001**, *34*, 8179−8186.
(16) Sakiyama, T.; Chu, C.-H.; Fujii, T.; Yano, T. *J. Appl. Polym. Sci.* **1993**, *50*, 2021−2025.
(17) Sakiyama, T.; Chu, C.-H.; Yano, T. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 717−719.
(18) Zhumadilova, G. T.; Gazizov, A. D.; Bimendina, L. A.; Kudaibergenov, S. E. *Polymer* **2001**, *42*, 2985−2989.
(19) Sakiyama, T.; Takata, H.; Kikuchi, M.; Nakanishi, K. *J. Appl. Polym. Sci.* **1999**, *73*, 2227−2233.
(20) Sakiyama, T.; Takata, H.; Toga, T.; Nakanishi, K. *J. Appl. Polym. Sci.* **2001**, *81*, 667−664.
(21) Roberts, G. A. F.; Domszy, J. G. *Int. J. Biol. Macromol.* **1982**, *4*, 374−377.
(22) Ikeda, S.; Kumagai, H.; Sakiyama, T.; Chu, C.-H.; Nakamura, K. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 1422−1427.
(23) As for the dissociation behaviors in mixed solutions of chitosan and an acidic polymer, xanthan, Ikeda et al.[22] showed that the apparent pK_a value of the protonated amino groups of chitosan increased from 6.4 up to 7.0 as the xanthan/chitosan ratio increased.
(24) Validity of eq 4 was shown by Ikeda et al.[22] for polyelectrolyte complex gels composed of xanthan and chitosan.
(25) Matsumoto, M.; Matsui, T.; Kondo, K. *J. Chem. Eng. Jpn.* **1999**, *32*, 190−196.

MA021721G