Polyelectrolyte Complexes of Chitosan with Sodium Carboxymethyldextran

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Mixing of chitosan with sodium carboxymethyldextran having opposite charge gave rise to the formation of novel water-insoluble precipitates. The mole ratios of N/COO^- in the polyelectrolyte complexes thus prepared were estimated to be 0.248—1.658. It was found that pH, mole ratio of N/Na and the order of mixing play an important role in determining the composition ratio of N/COO^- in the polyelectrolyte complexes. IR spectroscopic studies, blood clotting test, elementary analyses, the color reaction with Toluidine Blue and studies on solubility of polyelectrolyte complexes revealed that there is no noticeable interactions between –COOH groups and –OH groups. However, the $-NH_3^+$ groups in chitosan participate in the binding with carboxymethyldextran, probably through its $-COO^-$ groups, in the polyelectrolyte complexes. It seems that the number of binding sites between $-NH_3^+$ and $-COO^-$ groups in the polyelectrolyte complexes at pH 3.0 is smaller than those prepared at pH 6.5. Both the polyelectrolyte complexes prepared at pH 3.0 and 6.5 suppressed the coagulation of blood. This anti-thrombogenic property should be related to elution of anti-thrombogenic carboxymethyldextran.

A solution of charged polyelectrolyte reacts with one of oppositely charged polyelectrolyte to form a water-insoluble hydrous complex whose composition, in general, is sensitive to the composition of reaction mixture, order of mixing and the polyion concentration. Reports in this field have been given by some investigators.[1,2]

The mechanism of polyion interaction, the probable structure of the polyelectrolyte complex (PEC), and their properties have been investigated in detail by Michaels *et al.*[3,4] However, only a few papers[5] deal with the polyion interaction between polysaccharides, or polysaccharide and synthetic macromolecules, in spite of its importance concerning biological system, membranes and industrial and biomedical application.[6]

We have reported on chemical reactions of chitosan with dextran[7,8] and of [2-(diethylamino)ethyl] dextran with dextran.[9] This paper deals with a novel chemical reaction of chitosan with sodium carboxymethyldextran (CMD) and the results of our studies on the polyelectrolyte complex thus prepared.

Experimental

Chitosan (Tokyo Kasei Co., Ltd., nitrogen content 7.65%) and CMD (Meito Sangyo Co., Ltd., sodium content 7.38%; degree of substitution 0.70/A.G.U.; viscosity of its 1% aqueous solution 970 centipoise at 20 °C) were used. Chitosan was purified by reprecipitation from its acidic solution with 20% sodium hydroxide solution; CMD was used without purification.

When the pH of solution is below 2 no precipitate is formed by mixing chitosan with CMD and when the pH of chitosan solution is higher than 6.5, chitosan remains insoluble. Thus, the reactions were carried out at pH 3.0 and pH 6.5, respectively. Purified chitosan was dissolved in 0.1% hydrochloric acid solution. The pH of solution was then adjusted to 3.0 and 6.5 with sodium hydroxide solution. CMD was dissolved in water (pH 6.5), and the solution was adjusted to pH 3.0 with hydrochloric acid solution. These chitosan solutions were added dropwise to CMD solutions, adjusted to the same pH as those of chitosan solutions, respectively, with stirring under the conditions given in Table 1. Water-insoluble precipitate (PEC) was thus obtained. After being left to stand for a half hour, the precipitate was washed with 0.01 M hydrochloric acid solution, water and methanol, and then separated by centrifugation, dried *in vacuo* for a week, until a constant weight was attained.

The blood clotting test was carried out according to the procedure of Imai and Nose.[10] 160 mg PEC was pressed (5 t/4.5 cm^2) with poly (vinyl chloride) powder, a reinforcing agent, in a vacuum for 10 min. The blood (Red Cross Hospital Blood Center) was kept in a thermostat at 4—6 °C for one or two months. The ACD blood was prepared by adding the blood to an anticoagulant citrate dextrose solution consisting of sodium citrate, citric acid and dextrose.

Elementary analyses of the PEC were performed at the Institute of Physical and Chemical Research, the infrared spectra being taken by the KBr method. Purple coloration appears by the reaction of CMD with a solution containing Toluidine Blue.[11] This is utilized for the demonstration of CMD elution from PEC.

Results and Discussion

Experimental conditions, yield and elementary analyses for the PEC are given in Tables 1 and 2, respectively. The yield of PEC becomes smaller with an increase in the mole ratio N/Na of the reaction mixture. The nitrogen content in PEC prepared at pH 6.5 (B series) is greater than that prepared at pH 3.0 (A series), while the nitrogen content in PEC of A-2 and B-2, prepared in the mixing order, CMD to chitosan, is greater than that of PEC prepared in the reverse order, chitosan to CMD. The nitrogen content in the PEC becomes greater with increase in the mole ratio N/Na of the reaction mixture.

The data on the infrared spectra and elementary analyses suggest that the $-NH_3^+$ groups in chitosan participate in the binding with CMD, probably through its $-COO^-$ group in PEC. Thus, the mole numbers of $-COO^-$ groups in PEC were calculated from its nitrogen content. The mole ratios of N/COO^- in PEC were determined (Table 2), by assuming that the sodium or chlorine content in PEC is approximately zero. The mole ratios of N/COO^- in PEC, A-1 to B-4, were estimated to be 0.248 to 1.658. The pH value, the mole ratio of N/Na in the reaction mixture and the order of mixing, play an important role in determining the

Table 1. Conditions and yields in the preparation of polyelectrolyte complexes[a]

Sample code	Volume of chitosan solution (ml)	Volume of CMD solution (ml)	Mixing order	Mole ratio of N/Na in the reaction mixture	Yield (mg/100ml chitosan solution)
A-1[b]	100	170	Chitosan to CMD	1.0	364
A-2[b]	100	170	CMD to chitosan	1.0	386
A-3[b]	25	212.5	Chitosan to CMD	0.2	1323
A-4[b]	200	68	Chitosan to CMD	5.0	78
B-1[c]	100	170	Chitosan to CMD	1.0	441
B-2[c]	100	170	CMD to chitosan	1.0	302
B-3[c]	25	212.5	Chitosan to CMD	0.2	392
B-4[c]	200	68	Chitosan to CMD	5.0	126

a) Concentration of chitosan and sodium carboxymethyldextran (CMD): 2.0 g/l. b) Both solutions adjusted to pH 3.0. c) Both solutions adjusted to pH 6.5.

Table 2. Elementary analyses and composition of polyelectrolyte complexes

Sample code	Nitrogen[a] content (%)	Sodium[b] content (%)	Chlorine[b] content (%)	Mole ratio of N/COO^- in the polyelectrolyte complex
A-1[c]	1.45	0.055	0.085	0.397
A-2	2.12	/	/	0.648
A-3	0.98	/	/	0.248
A-4	1.72	/	/	0.491
B-1[d]	2.69	0.065	0.210	0.914
B-2	3.81	/	/	1.658
B-3	2.33	/	/	0.740
B-4	3.49	/	/	1.406

Sample codes correspond to those in Table 1.
a) Nitrogen analysis carried out according to Kjeldahl method. b) Analyses performed at the Institute of Physical and Chemical Research. c) Carbon content: 40.09%, hydrogen content: 6.05%. d) Carbon content: 38.19%, hydrogen content: 6.23%.

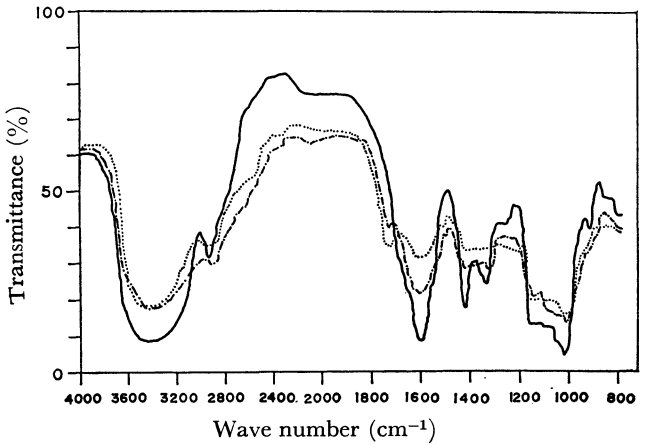

Fig. 1. IR spectra of polyelectrolyte complexes and polymer blend of chitosan with sodium carboxymethyldextran.
——: Polymer blend of chitosan with sodium carboxymethyl-dextran (mole ratio of N/Na=1.0), ·····: polyelectrolyte complex of A-1, -·-·-: polyelectrolyte complex of B-1.
Sample codes, A-1 and B-1, correspond to those in Table 1.

ratio of the reactive group of CMD to that of chitosan in the PEC. The mole ratios of N/COO^- in the PEC of series A were smaller than those of series B. Only the PEC of series A, A-1, A-2, and A-4, having not more than 0.5 of the mole ratio N/COO^-, gave slight purple coloration with Toluidine Blue. It seems that the number of binding sites between $-NH_3^+$ and $-COO^-$ groups in the PEC of series A is smaller than those of series B, and CMD in the PEC of series A should be liable to detach itself from the PEC chain.

The IR spectra of PEC are roughly similar to those of a mixture of CMD and chitosan, differing from each other in detail. PEC has an absorption band around 1740 cm^{-1}, [12] assigned to -COOH group. This is absent in the mixture of CMD and chitosan. The smaller the ratio of N/Na in the reaction mixture, or the smaller the nitrogen content in PEC, the stronger the absorption band around 1740 cm^{-1}. The absorption band in the PEC of A-2 and B-2 prepared in the mixing order, CMD to chitosan, is stronger than the band prepared in the reverse mixing order, chitosan to CMD. The stronger the absorption band around 1740 cm^{-1}, the weaker the absorption band around 1600 cm^{-1}. Though neither the shift in absorption band around

Table 3. Solubilities of polyelectrolyte complexes

Sample code	Ternary[a] solvent-I		Ternary[b] solvent-II		Formic acid		Dimethyl sulfoxide	
	R	H	R	H	R	H	R	H
A-1	△	△	△	△	△	○	×	×
A-2	△	△	△	△	△	○	×	△
A-3	△	△	△	△	△	○	×	△
A-4	△	△	△	△	△	○	×	△
B-1	△	△	△	△	△	○	×	×
B-2	△	△	△	△	△	○	△	△
B-3	△	△	△	△	△	○	△	△
B-4	△	△	△	△	△	○	△	△

Sample codes correspond to those in Table 1. All the polyelectrolyte complexes were insoluble, even on heating, in *N,N*-dimethylformamide. R: Room temperature, H: Heating. ○: Almost soluble, △: Partially soluble, ×: Insoluble. a) Aceton/potassium bromide/water (20: 20: 60 wt%). b) Hydrochloric acid/dioxane/water (45: 50: 5 wt%).

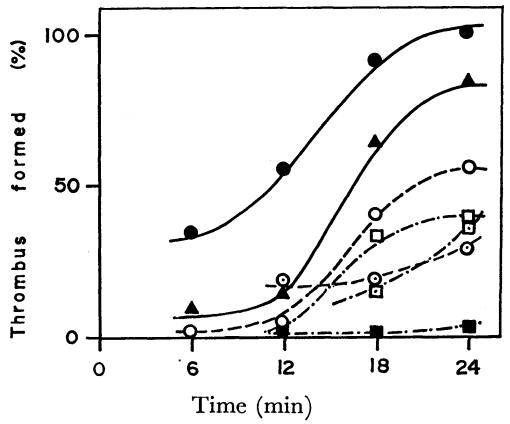

Fig. 2. Percentage of thrombus formed on polyelectrolyte complexes compared with that on glass.
●: Glass, ▲: polyvinyl chloride, ⊙: B-2, ○: B-4, ⊡: A-1, □: A-2, ■: A-2 (without reinforcing agent). Sample codes A-1, A-2, B-2, and B-4 correspond to those in Table 1.

3500 cm^{-1} assigned to –OH groups nor the change in its absorption intensity about PEC were observed appreciably in the mixture of CMD and chitosan blended in the same ratio as that of the PEC composition, there would be no appreciable interaction between the –OH group and other reactive groups, especially carboxyl group. On the other hand, there is no absorption around 1500 cm^{-1}, which is assigned to $-NH_3^+$ group,[13] in the mixture of CMD and chitosan. The sodium and chlorine contents in the PEC are distinctly smaller than those in CMD or chitosan. The results suggest that the $-NH_3^+$ groups in chitosan participate in the binding with CMD through its $-COO^-$ groups.

PEC are insoluble or hardly soluble in dimethyl sulfoxide or *N,N*-dimethylformamide, partially soluble in a ternary solvent mixture, and soluble in formic acid on heating (Table 3). The difference in solubility between the PEC of series A and those of series B is hardly discernible. However, the PEC of series B is soluble in dimethyl sulfoxide.

The blood clotting test was performed for the PEC (Table 1) by gravimetric measurement[10] of the amount of thrombus at appropriate time intervals, after addition of calcium chloride solution (0.1 M, 0.02 ml) to ACD blood (0.2 ml) which had been in contact with samples. PEC has an anti-thrombogenic character; it suppresses the coagulation of blood. The amount of thrombus on the PEC is 20—50% of that on glass (Fig. 2). The difference in anti-thrombogenic behavior between the PEC of series A and that of series B is hardly discernible. The elution of anti-thrombogenic CMD from PEC into the blood might be related to our results.

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