In-vitro Clot Forming Properties of Polyelectrolyte Complexes of [2-(Diethylamino)ethyl]dextran Hydrochloride with Poly(sodium-L-glutamate) and of Sodium Carboxymethyldextran with Chitosan

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Synopsis. Polyelectrolyte complexes prepared at higher hydrogen ion concentrations may form clots in higher quantities than those prepared at lower hydrogen ion concentrations, irrespective of the content of anticoagulant reagents. This difference is attributable to the difference in molecular structure of polyelectrolyte complexes, especially to the presence of carboxyl groups.

We have reported[1,2] on novel chemical reactions, structures and properties of polyelectrolyte complexes (PEC) and their *in-vitro* clot formation and we have also reported[3] that different characters of blood coagulation are attributable to the difference in molecular structure among PECs, with an emphasis on the fact that formation of sulfo or carboxyl groups in PECs plays an important role in increasing the quantity of clot. This note deals mainly with attractive results of clot formation *in-vitro* by PECs of [2-(diethylamino)-ethyl]dextran hydrochloride with poly(sodium-L-glutamate),[4] and by carboxyl-containning PECs of sodium carboxymethyldextran of low molecular weight (about 5000) with chitosan.

Experimental

Chitosan (Tokyo Kasei Co., nitrogen content 7.89%, intrinsic viscosity 1.50 dl/g in 1 M (1 M=1 mol dm^{-3}) NaCl of pH 3.0 at 30 °C), sodium carboxymethyldextran (CMD) (Meito Sangyo Co., degree of substitution 1.38 mol/A.G.U., molecular weight about 5000), poly(sodium-L-glutamate) (PSLG), and [2-(diethylamino)ethyl]dextran hydrochloride (EA) were used. Properties of PSLG and EA are given in a previous paper.[4] When the pH of solution is below 4.0 or 3.0, no precipitate is formed by mixing PSLG with EA (system A) or chitosan with CMD solution (system B), respectively, and when the pH of chitosan solution is higher than 6.0, chitosan remains undissolved. Thus, reactions were carried out at pH 5.0, 6.0, and 8.0 (system A) and pH 3.5, and 5.5 (system B). General conditions, the apparatus and chemical analyses, and the blood clotting test used are the same as those described in previous papers.[1-4]

ACD bloods of types O and A (Red Cross Hospital Blood Center) were kept in a thermostat at 4—6 °C for 2—6 d.

Conditions, yields and results of elemental analyses for PECs prepared in such a way that a PSLG or CMD solution was added to EA or chitosan and *vise versa*, are given in the previous paper[4] and in Table 1.

In system B, all the PECs prepared at pH 5.5 have higher nitrogen contents, which represent the molar ratio CMD/chitosan, than the PEC prepared at pH 3.5 because of the higher dissociation of chitosan at pH 3.5 and similarly, in system A, all the PECs prepared at pH 5.0 have lower PSLG/EA molar ratios than the PECs prepared at pH 6.0 and 8.0 and those prepared by reagent addition in the reverse order have higher ratios as shown in the previous paper.[4]

IR spectra of PECs are similar to those of their corresponding mixtures of PSLG and EA or of CMD and chitosan.

Table 1. Experimental conditions[a], polymer yield, and elemental analyses of polyelectrolyte complexes[b] of chitosan[c] with CMD[d]

Sample code[e]	V_{CMD}[f] / cm^3	R[g]	Y[h] / g	N[i] / %
A-1	442	4.0	0.96	3.3
A-2[j]	298	2.7	0.96	3.3
A-3[k]	274	2.5	0.93	3.6
A-4	221	2.0	0.74	3.8
B-1	442	4.0	0.71	4.5
B-2[j]	88	0.8	0.69	4.8
B-3[k]	76	0.7	0.67	4.8
B-4	55	0.5	0.44	4.9
C-1	442	4.0	0.98	3.5
C-2	298	2.7	0.97	3.8
C-3	274	2.5	0.93	3.6
C-4	221	2.0	0.76	3.4
D-1	442	4.0	0.75	4.6
D-2	88	0.8	0.67	4.4
D-3	76	0.7	0.60	4.2
D-4	55	0.5	0.42	4.9

a) Concentrations of chitosan and sodium carboxymethyldextran (CMD), 2 and 4 g/L. b) Nitrogen analyses carried out according to the Kjeldahl method. c) Chitosan: Nitrogen content, 7.89%; intrinsic viscosity, 1.50 dl/g in 1 M NaCl of pH 3.0 at 30 °C. d) CMD: Degree of substitution, 1.38 mol/A.G.U.; molecular weight, about 5000. e) A, B series: CMD solution was added dropwise to chitosan solution; C, D series: Chitosan solution was added dropwise to CMD solution; A, C series: Both chitosan and CMD solutions were adjusted to pH 3.5; B, D series: Both chitosan and CMD solutions were adjusted to pH 5.5 (see text). f) V_{CMD} is one amount of CMD solution in ml/200 ml chitosan. g) R is the molar ratio of Na(CMD)/N(chitosan) for solution. h) Y is the yield of polymer. i) N is the nitrogen content. j) End of coagulation range. k) Beginning of coagulation range.

However, the PEC prepared at pH 5.0 (system A) or pH 3.5 (system B) has an absorption band around 1740 cm^{-1} which appears neither in the PEC prepared at pH 8.0 (system A) or pH 5.5 (system B) nor in the mixture of PSLG and EA or of chitosan and CMD; the PEC prepared at pH 6.0 (system A) has weaker absorption bands than the one prepared at pH 5.0. These absorption bands have been assigned to carboxyl groups.[5] Furthermore, the absorption band around 3500 cm^{-1} (assigned to the hydroxyl group) of the PEC prepared at pH 5.0 (system A) or pH 3.5 (system B) is caused to shift to lower wave numbers in both systems. In view of the formation of hydrogen bond between the hydroxyl and other functional groups, it is reasonable to expect such a shift in frequency.

The PECs differ appreciably from one another in such

properties as the degree of swelling, color reaction with Toluidine Blue, and solubility depending on experimental conditions of hydrogen ion concentration and molar mixing ratio of constituents, although they have common constituents. It appears that the degree of dissociation and the conformation of PSLG, EA, chitosan, and CMD change with hydrogen ion concentration.

The PECs of the same nitrogen content, representing the CMD content in PEC, is similar to one another, in such properties as IR spectra, solubility, swelling, and color reaction with Toluidine Blue, under the same conditions. Consequently, blood clotting tests were performed on PEC tables 1-B, 2-B, 3-B, 1-b, 2-b, and 3-b as reported in the previous paper,[4] and on PEC tablets A-1 A-2, B-1, B-2, C-1, D-3, and D-4 as given in Table 1, which were selected arbitrarily from within the nitrogen content range 3.3—4.9 according to the gravimetric procedure of Imai and Nose.[6] No change in IR absorption intensity for one carboxyl group was detected for the PECs which had long been kept dipped at pH 7.3. No firm clot was formed even after 10 min dipping with PEC tablets. Percentage formation of thrombus for the PECs (system A: 1-B, 2-B, and 3-B; system B: A-2, C-1, and D-3) is given in Figs. 1 and 2. For the PECs (system A: 1-b, 2-b, and 3-b; system B: A-1, B-1, B-2, and D-4) were obtained similar reactions between the percentage of thrombus formed and the molecular structure as well as results of observation similar to the above-mentioned. All the PEC tablets suppress the coagulation of blood. Moreover, quantities of clots for the PEC tablets prepared at pH 5.0, 6.0 (system A) and 3.5 (system B) are more than those for the PEC tablets prepared at pH 6.0 and 8.0, 8.0, and 5.5, respectively, although the content of PSLG or CMD (suppression of coagulation) in the PEC tablets prepared at pH 5.0 and 6.0 and 3.5 are higher than that in those prepared at pH 8.0 and 5.5, respectively. We have reported an experimental work on clot formation of PEC consisting of EA, CMD, and sodium dextran sulfate, pointing out a definite correlation between the character of blood coagulation and the molecular structure and the significance of formation of carboxyl or sulfo groups in PECs. Thus, the present experimental results have confirmed that the difference in the characters of blood coagulation is attributable to the difference in molecular structures of the PECs prepared; especially, the formation of carboxyl group in PECs has a great contribution to the increase in the quantity of clots as well as the above-mentioned experimental results.[3]

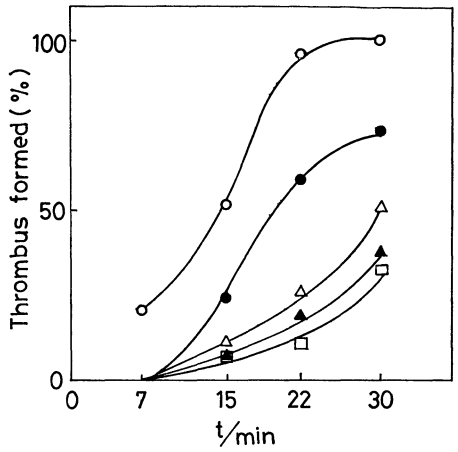

Fig. 1. Percentage of thrombus formed on polyelectrolyte complexes (PSLG→EA) compared with that on glass.
Storage time of A type blood: 6 d, ○: glass, ●: poly(vinyl chloride), △: 1-B, ▲: 2-B, □: 3-B. Sample codes 1-B, 2-B, and 3-B correspond to those in Table 1 in the previous paper.

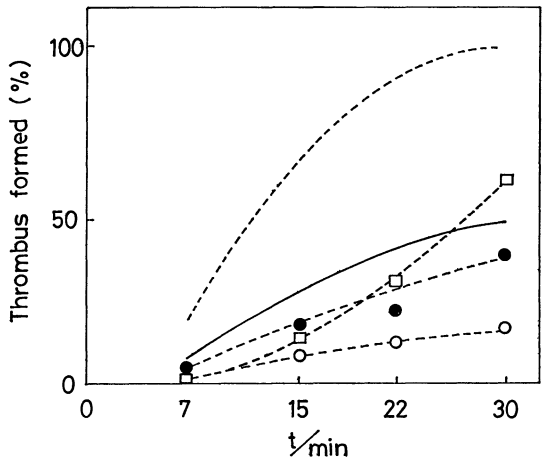

Fig. 2. Percentage of thrombus formed on polyelectrolyte complexes (EA–CMD) compared with that on glass.
Storage time of A type blood: 3 d, ----: glass, ——: poly(vinyl chloride), □: A-2, ●: C-1, ○: D-3. Sample codes A-2, C-1, and D-3 correspond to those in Table 1.

The mechanism of increasing the quantity of clots makes it likely that facters effective for blood coagulation are activated delicately by interaction of carboxyl groups in PEC with blood. On the other hand, the mechanism for the suppression of coagulation suggests the possibility that negative charges such as the carboxylate group may exist active on the surface of PEC.[7]

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