Properties of Polyelectrolyte Complexes Consisting of [2-(Diethylamino)-ethyl]dextran Hydrochloride, Carboxymethyldextran, and Sodium Dextran Sulfate for Clot Formation *in Vitro*

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Synopsis. A polyelectrolyte complex (PEC) prepared at higher hydrogen ion concentration enhanced the coagulation of blood, whereas PEC prepared at lower hydrogen ion concentration prevented coagulation of blood. This difference sholud be attributed to the difference in the molecular structures of the PEC prepared.

The mixing of oppositely charged polyelectrolytes in solution leads to the formation of a complex.[1] Many reports[2,3] have dealts with the polyelectrolyte interaction between polysaccharides, or between polysaccharide and synthetic macromolecules and their properties. The biomedical characteristics are important in relation to biological systems, membranes, and industrial applications.[4]

We have reported the novel chemical reaction, structure, and properties of polyelectrolyte complexes containing three materials,[5-7] and the clot formation of these polyelectrolyte complex *in vitro*.[5,6] This note deals with the attractive results for clot formation *in vitro* of the novel polyelectrolyte complexes (PEC) consisting of three materials, which have been reported in a previous paper.[7]

Experimental

The materials of [2-(diethylamino)ethyl]dextran hydrochloride (EA), sodium dextran sulfate (DS), and sodium carboxymethyldextran (CMD) and the general experimental procedures, the apparatus and the chemical analyses were the same as those described in the previous paper.[7]

ACD blood samples of O and A type were provided from the Red Cross Hospital Blood Center, Japan and kept in a thermostat at 4—6 °C. The storage time of 0 type ACD blood was 10 d; that of A type ACD blood was 5 d. The ACD blood was prepared by adding the blood to an anticoagulant citrate dextrose solution consisting of sodium citrate, citric acid and dextrose. The blood test was carried out according to the procedure of Imai and Nose[8]: a polymer blend of 80 mg PEC and 320 mg poly (vinyl chloride) in 1-C and 1-D (Table 1 in ref. 8) was pressed (8.5 t/4.9 cm^2) under vacuum for 5 min to make a sample tablet; in series 2—5, the PEC of 5 mg was coated on a sample tablet by pressing (8 t/4.9 cm^2) under vacuum for 5 min after 400 mg poly (vinyl chloride) was pressed (2 t/4.9 cm^2) for 15 s under vacuum.

Experimental conditions and yields in the preparation of PEC, and elementary analyses and composition of PEC, are given in Tables 1 and 2 of the previous paper.[7] As seen in these tables, the hydrogen ion concentration and the mole ratio of mixture solution (DS+CMD) to EA solution in the reaction mixture both played important roles in changing the ratio of the mixture solution to that of EA in the PEC produced. However, all the PEC were found to consist of the three materials, EA, CMD, and DS, by IR spectra, even though precipitates did not form in the reaction mixtures of EA and CMD at higher hydrogen ion concentration of the reaction system (pH<2).

The IR spectra and content in CMD of PEC were described in the previous paper.[7] That is, the localized interactions between -OH and $-OSO_3H$ groups in the 1, 2, and 3 series would result from the formation of $-SO_3H$ groups. In other words, the long chains on the three materials having the same dextran ring and the intermolecular hydrogen bonds between OH groups for PEC may be broken at high hydrogen ion concentraiton and consequently bonds will form between $-OSO_3H$ and -OH groups. Furthermore, the carboxy groups in the PEC of 1, 2, and 3 series existed as undissociated -COOH. Actually, the PEC in 4 and 5 series differed appreciably from the PEC in 1 and 2 series in such properties as sulfur content, solubility, and color reaction with Toluidine Blue, as described previously. From those results, the structures of PEC were estimated as follows: the PEC prepared at higher hydrogen ion concentration consisted of both COOH groups and a great number of $-OSO_3H$ groups (4%>1%> pH 2 HCl solution of reaction system); the molecular weight of the PEC prepared became smaller because of the breaking of long chains of the materials; those prepared at pH 6.5 or 11.0 were not composed of the -COOH and $-OSO_3H$ groups in PEC; and in addition, the content of CMD in PEC became greater with an increase in hydrogen ion concentration.

The results in three-component solvents show that there is a small region in the solvent composition field where the complex remain in solution to yield a homogenous, transparent, viscous syrup. Phase diagrams were not obtained in the PEC

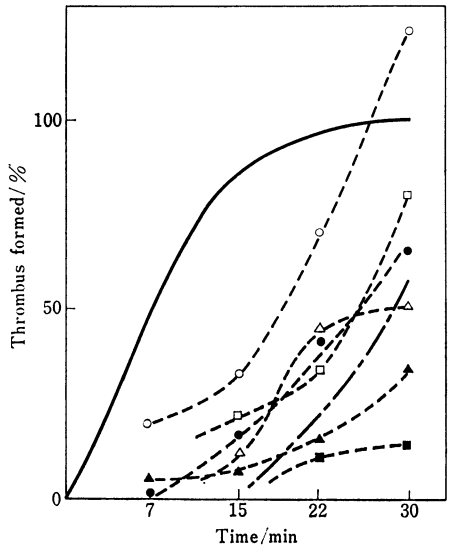

Fig. 1. Percentage of the thrombus formed on polyelectrolyte complexes compared with that on glass after a lapse of 30 min as a criterion.
Storage time of 0 type blood: 10 d, ——: glass, — — —: poly (vinyl chloride). ···□···: 1-C, ···○···: 1-D, ···●···: 2-D, ···▲···: 3-D, ···■···: 4-D, ···△···: 5-C. Sample codes 1-C, 1-D, 2-D, 3-D, 4-D, and 5-C correspond to those in Table 1 in the previous paper.

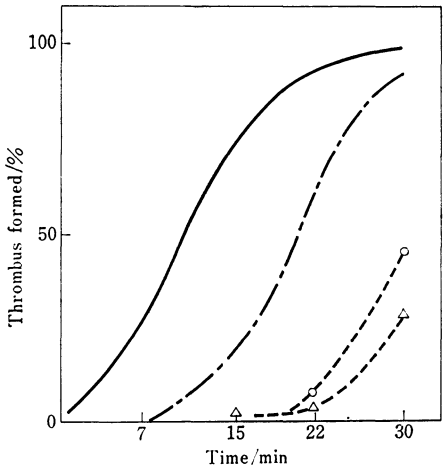

Fig. 2. Percentage of the thrombus formed on polyelectrolyte complexes compared with that on glass after a lapse of 30 min as a criterion.
Storage time of A type blood: 5 d, ——: glass, ——: poly (vinyl chloride). ···○···: 3-C, ···△···: 5-D. Sample codes 3-C and 5-D correspond to those in Table 1 in the previous paper.

except for 1-C and 1-D, but the PEC in 2-D dissolved partially in the three-component solvent metnioned above. The experimental results in which the PEC in 1 series or 2 series dissolved or partially dissolved in the three-component solvent, and also the colored reaction with Toluidine Blue[7] in PEC, strongly support the difference in the molecular structure according to the experimental conditions of hydrogen ion concentration mentioned above.

Blood tests were performed on PEC (1-C, 1-D, 2-D, 3-C, 3-D, 4-D, 5-C, 5-D) listed in the previous paper[7] by measuring gravimetrically[8] the amount of thrombus formed at appropriate time intervals, after adding $CaCl_2$ solution (0.1 M, 0.02 ml;=1 mol dm^{-3}) to ACD blood (0.2 ml, O or A type, storage time: 10 or 5 d) which had been in contact with test samples. The blood clotting test by coating on PEC tablet could not be performed on the PEC prepared in 4% hydrochloric acid solution (1-C, 1-D) because of the soaking of blood into the tablet. Therefore, tests were performed on a tablet of polymer blend of PEC and poly(vinyl chloride). A firm clot did not form even after 10 min with PEC of 3, 4, or 5 series except with 1-C, 1-D, and 2-D complexes. It may be noted that the PEC in 3,4, and 5 series suppressed the coagulation of blood considerably. On the other hand, the PEC in 1 series somewhat enhanced the coagulation of blood, as seen in Figs. 1 and 2, considering the amount of poly(vinyl chloride) in a tablet was 4 times as much as the PEC and the amounts of thrombus were larger than that formed on poly (vinyl chloride) tablets. Moreover, it was found that the quantities of the clot on the PEC tablet formed in high hydrogen ion concentration are somewhat greater than those on that formed in lower hydrogen ion concentration, although the contents of DS of PEC in 1, 2, and 3 series were greater than those in 4 and 5 series. Similarly, these experimental results strongly support the difference in molecular structure according to the experimental conditions of hydrogen ion concentration mentioned above. Thus, it has been well confirmed by the experimental results that the difference in the character of coagulation of blood should be attributed to the difference in the molecular structure of PEC prepared as mentioned above.

The likely mechanism of enhancement of coagulation appears to be the following: the factors of coagulation in blood were activated by the interactions of -COOH and $-OSO_3H$ groups with blood in PEC. On the other hand, as seen in the colorimetric reaction of Toluidine Blue, the mechanism of suppression of coagulation may be related to the possibility that negative charges such as $-COO^-$ and $-SO_3^-$ groups exist actively on the surface of PEC.[9]

Finally, it is concluded that the preparation of the described PEC offers an important reservoir of materials whose properties may be tailored according to each requirement.

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