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Complex Formation of Heparin or Sulfated Cellulose with Glycol Chitosan

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Formation of polyelectrolyte complexes was investigated as a function of pH by using glycol chitosan as the polycation component, and two polysaccharides with high charge density, heparin and sulfated cellulose as the polyanion component. From turbidimetric and metachromasy measurements, it was found that glycol chitosan-heparin system almost obeys stoichiometry but glycol chitosan-sulfated cellulose system does not. Such a result was interpreted by taking into account the molecular chain conformations of component polymers and the distribution of ionizable groups along the chains.

INTRODUCTION

Since Kossel[1] observed that proteins interact with nucleic acids and form precipitates when mixed in aqueous solution, extensive studies have been made on the interactions of oppositely charged polyelectrolytes. Such intermolecular reaction is of great interest as models of the prebiological organization[2,3] and macromolecular complexes such as nucleoproteins and protein-polysaccharide complexes[4,5] which play important roles in the living systems. In addition, since polyelectrolyte complexes synthesized from linear synthetic polyelectrolytes possess characteristic physical, chemical, and electrical properties,[6] they must be useful materials for selective membranes and for biomedical uses.[7]

However, these interactions are very complicated because they are influenced by a number of factors. One of the important subjects in complex formation is whether the electrochemical stoichiometry is adopted for reaction between polycation and polyanion under given conditions.

Katchalsky[8] pointed out that the reaction of polylysine hydrochloride with polymethacrylic acid is stoichiometric, that is, the precipitation occurs when the total number, βC_{pc}, of the positive groups in a basic polyelectrolyte (where C_{pc} is the molar concentration of the basic polyelectrolyte and β is its degree of dissociation) equals the total number, αC_{pa}, of the negative groups of an acidic polyelectrolyte. Such electrochemical stoichiometry has been found to occur in many combinations of polyanion and polycation,[9~11] and afforded a basis on the analytical method as is termed the colloid titration.[12]

On the other hand, systems which do not obey the stoichiometry were also found by some authors.[13,14] Their results suggest that intermolecular reactions may be conducted not only by electrochemical but also by conformational natures of the

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component polyelectrolytes.

In this sense, complex formations in which polysaccharides participate are worth noting, because ionic polysaccharide molecules include bulky pyranose rings in their backbone chains, and highly stereoregular in backbone chain configuration. However, few papers have been reported on the interactions between oppositely charged polysaccharides.[12,15,16]

The purpose of this research is to elucidate the conformation-directing interactions occur between two polysaccharides mutually bearing opposite charges. Glycol chitosan (GC) was chosen as the polycation component and two polysaccharides with high charge density, heparin (Hep) and sulfated cellulose (SCS), as the polyanion components.

Glycol chitosan is a copolymer of N-acetyl-D-glucosamine-6-glycol and D-glucosamine-6-glycol, and is prepared from N-acetyl-D-glucosamine (chitin) by de-N-acetylation after glycolation. Heparin is an alternating copolymer of L-iduronic acid which is frequently O-sulfated at C2 and D-glucosamine-N-sulfate with an additional O-sulfate group on C6. SCS is a polymer of β (1, 4)-D-glucose sulfate.

EXPERIMENTAL

Materials

Glycol chitosan (Lot: LM9931) was purchased from Wako Junyaku Co., Kyoto, and was used after dialysis followed by deionization through an ion exchange resin, Amberlite MB-1. Heparin-Na (Lot: M4B1355) was purchased from Nakarai Chemicals Co., Kyoto, and was used after dialysis. Sulfated cellulose-Na (Lot: KELCO-LV-1), a product of Kelco Co., was dialysed after precipitations with ethanol before use.

Characterization of Polymer Samples

The molecular weights M of these polymer samples were estimated from the limiting viscosity numbers $[\eta]$ in specified solvent systems.[17~19]

Contents of ionizable groups were measured by conductometric titrations with a Yanagimoto Conductivity Outfit Model MY-8 after passing through MB-1 ion exchange column for GC and DOWEX 50 for Hep and SCS. The experimental results were shown in Table I.

The NH_2 content in GC was 0.62 per pyranose ring. This figure corresponds to

Table I. Characterization of Polysaccharides

Polymer	$[\eta]$ (dl/g)	M	Remarks on ionizable groups	Purity (%)
GC	0.612 (in 0.1 N NaOH)	105,000[17]	0.62 NH_2/pyranose	
Hep	3.10 (in H_2O)	10,200[18]	HSO_3: COOH=2.1: 1.0	95
SCS	0.065 (in 2 N NaOH)	67,000[19]	2.5 HSO_3/pyranose	

the degree of deacetylation of the parent polymer, chitin. The ratio of the numbers of two ionizable groups, *i.e.*, the number of sulfonic group to that of carboxyl group, in heparin chains was shown in the forth column of Table I. The purity cited in the fifth column designates the % of COOH observed to the theoretical amount of COOH. The concentrations of ionizable groups mentioned hereafter are based on these purities. Finally, the content of ionizable group HSO_3 in SCS was 2.5 per pyranose ring.

The degree of dissociation of NH_2 group in GC and of COOH group in Hep were determined by potentiometric titrations. The initial concentration of polyelectrolyte was about 0.003 mol ionizable groups per liter. Titrations were performed in the presence of about 0.005 M NaCl under N_2 atmosphere on both the polyelectrolyte solution and a reference solution having the same composition except the absence of polyelectrolyte. The degree of dissociation was obtained from the difference between two curves at given pH values. The apparatus used for potentiometric tirtations was a Hitachi-Horiba pH-Meter Model F–7_{ss}. In Fig. 1, the degree of dissociation was plotted against the pH for NH_2 group of GC (curve 1), and COOH group of Hep (curve 2). With respect to SO_3H group, a strong electrolyte, the degree of dissociation is unity (curve 3) independent of pH as a matter of course. The apparent dissociation constants pK_a estimated from Fig. 1 were 6.0 for NH_2 group in GC and 4.9 for COOH group in Hep.

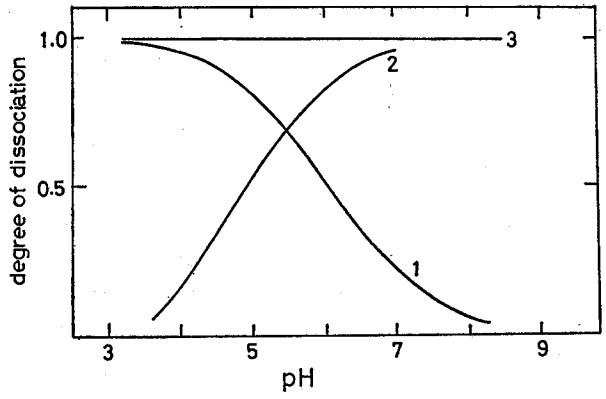

Fig. 1. Degrees of dissociation plotted against pH for NH_2 groups of GC (1), for COOH groups of Hep (2) and for SO_3H groups (3).

Formation of Polyelectrolyte Complexes

The polysaccharides mentioned above were dissolved at about 0.0004 mol/l based on ionizable groups in various buffer solutions of different pH and of ionic strength of ca. 0.005. The polyanion solution at a fixed pH was then added in different ratios to the polycation solution having the same pH with stirring. The pH differences between before and after the addition of polyanion were within 0.02. Complex formation was followed by the measurements of turbidity and metachromasy of the mixtures. Turbidity measurements were carried out at a wave length of 430 nm with a Shimadzu-Kotaki Photoelectric Nephelotitrator Type NT 3H. Metachromasy

measurements were carried out as follows. After the polyanion solution was added to the polycation solution, the precipitates if any were removed by centrifuge, and Toluidine Blue was added to the supernatant solution. Thus the color change of Toluidine Blue was measured by means of the visible spectrum with a Hitachi Spectrophotometer Model EPS–3T. The turbidity τ of the mixtures and the absorption metachromasy were plotted against the mixing ratio of polycation to polyanion.

RESULTS AND DISCUSSION

As indicated in Table I, GC contains 0.62 NH_2 per pyranose residue, Hep contains 2.0 HSO_3 and 0.95 COOH per 2 pyranose residues, and SCS contains 2.5 HSO_3 per pyranose residue. Accordingly, the total numbers of ionizable groups of polycation and polyanion components in a given system can be represented on the basis of the numbers of pyranose residues for polycation and polyanion component, respectively. Such designation is useful for quantitative treatment of the effect of backbone chain conformations on the reactions. The quantities defined so were denoted by PC_{py} and PA_{py}, respectively, for polycation and polyanion components.

When a polyanion was added to a polycation solution, the mixing ratio R was defined as

$$R=\frac{PC_{py}}{PC_{py}+PA_{py}} \tag{1}$$

In Fig. 2, the turbidity τ of the mixture of GC with Hep was plotted against the mixing ratio at different pH.

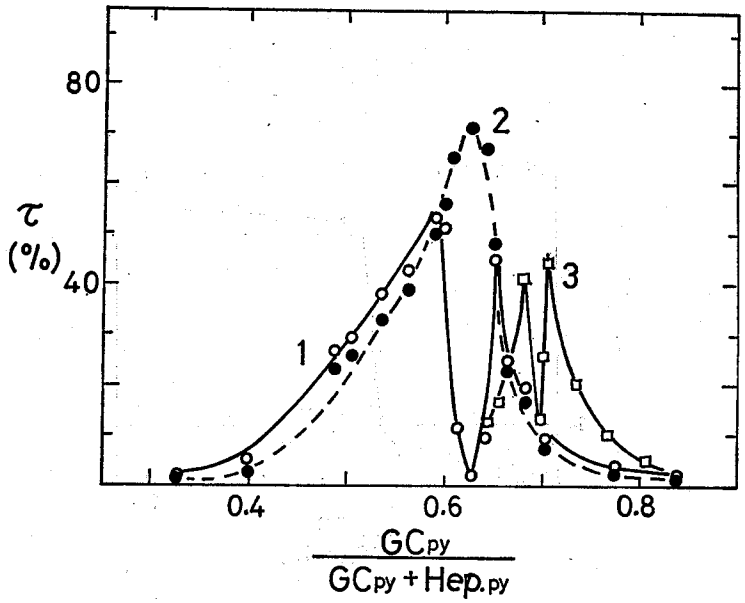

Fig. 2. Turbidity *vs.* mixing ratio for GC-Hep system at pH=2.75 (1), (2), and 5.05 (3). (2): at 15 min after mixing, (1): at 24 hr after mixing.

The effect of time of standing after the mixing on the turbidity was also shown in Fig. 2. The maximum in turbidity curve appeared at an early stage of standing transferred to a minimum with increasing time of standing. Such decrease in turbidity is due to the sedimentation of the complex particles suspended in the solvent. It should be noted that the mixing ratio R at the maximum exactly coincides with that at the minimum. Maximum or minimum point could be determined within an accuracy of ± 0.005 on R. As shown in Fig. 3, similar behavior was observed on GC–SCS system.

In many works, the mixing ratio of maximum or minimum turbidity was regarded as the composition of the polyelectrolyte complex formed at the given pH. To confirm the recognition, complex formation was pursued also with metachromasy, parallel to the turbidity measurement. Acidic polysaccharides are known to exhibit metachromasy. Figure 4 shows the relation between the absorption ratio at 635 nm to

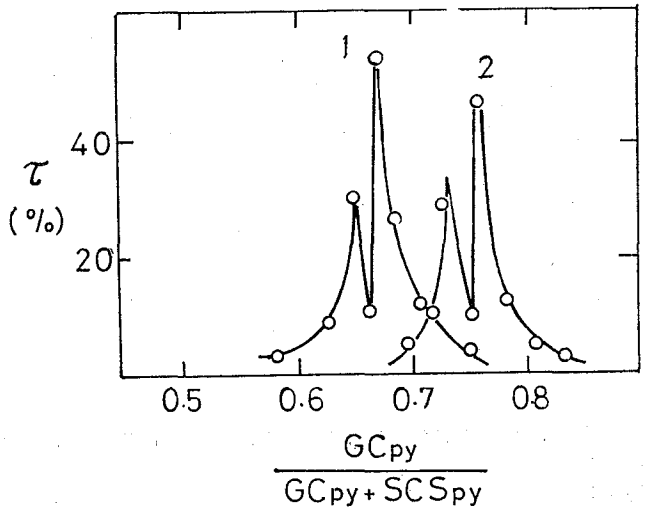

Fig. 3. Turbidity *vs.* mixing ratio for GC–SCS system at pH=4.05 (1) and 5.90 (2).

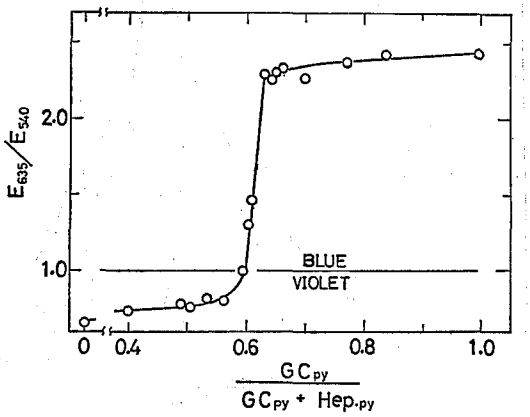

Fig. 4. Absorption ratio at 635 nm to 540 nm plotted against mixing ratio for GC-Hep system at pH=2.75. Initial GC concentration: 0.0004 mol/l. Toluidine Blue concentration: 0.0005 mol/l.

540 nm and the mixing ratio of GC and Hep at pH 2.75. The result is compared with the curve 2 in Fig. 2. The change of color was very sharp. The mixing ratio R of the point of color change, *i.e.*, $E_{635}/E_{540}=1.0$, was smaller than that of the minimum point of turbidity by about 0.02. The deviations were almost same extent on GC–Hep system. With respect to GC–SCS system, the deviations were less than 0.01. Thus, the mixing ratio R_{max} or R_{min} could be regarded as the composition of the polyelectrolyte complex formed at the given pH. This recognition was further confirmed by Hosono *et al.*[20] with elementary analysis of the precipitated complex.

When the interaction between polyanion and polycation is stoichiometric, a neutral complex is formed. In such a case, the following equations should hold for GC–Hep and GC–SCS system respectively.

$$C_{(HSO_3)}+\alpha C_{(COOH)}=\beta C_{(NH_2)} \quad (2)$$

$$C_{(HSO_3)}=\beta C_{(NH_2)} \quad (3)$$

where $C_{(HSO_3)}$, $C_{(COOH)}$, and $C_{(NH_2)}$ are the molar concentration of the ionizable groups designated in parentheses, and α and β are the degrees of dissociation of COOH and NH_2, respectively. The stoichiometric composition at given pH can be calculated from the results in Fig. 1 without any corrections on the presumption that the coexisting oppositely charged polyions have no influence on the value of α and β. Such stoichiometric composition, as well as experimental points obtained from Fig. 2 and Fig. 3 were plotted against pH in Fig. 5 and Fig. 6. With respect to experimental points, some turbidity curves in a pH range were overlapped, therefore, for convenience, only two curves were shown in Fig. 2 and Fig. 3. Naturally, whole experimental points were plotted in Figs. 5 and 6.

In the case of GC–Hep system, experimental points agree fairly with the calculated values, especially at a lower region, pH<4. On the other hand, the experimental points of the GC–SCS are remarkably apart from the calculated values. However, it should be noted that the experimental curve of GC–Hep system is quite similar to that of GC–SCS system. In both experimental curves, the numerical values of R are nearly equal to 0.65 at lower pH region, pH<5, and then increased with in-

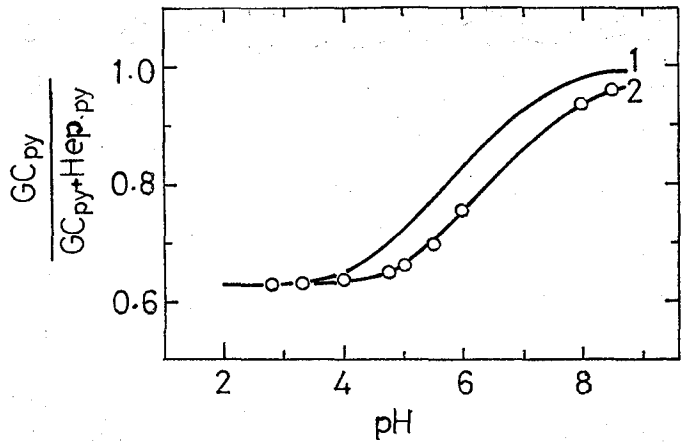

Fig. 5. Composition of complex plotted against pH for GC-Hep system.

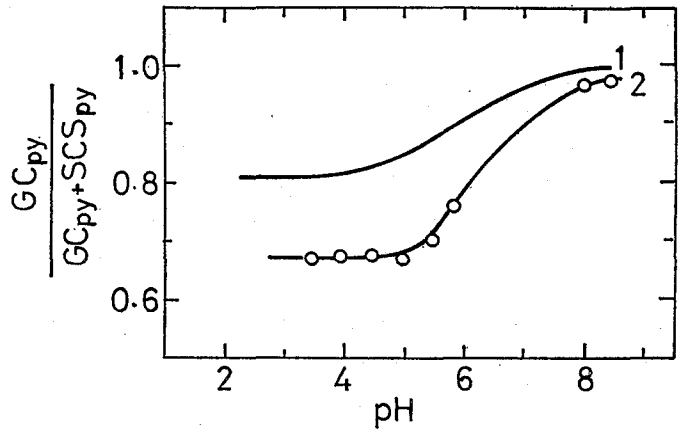

Fig. 6. Composition of complex plotted against pH for GC–SCS system.

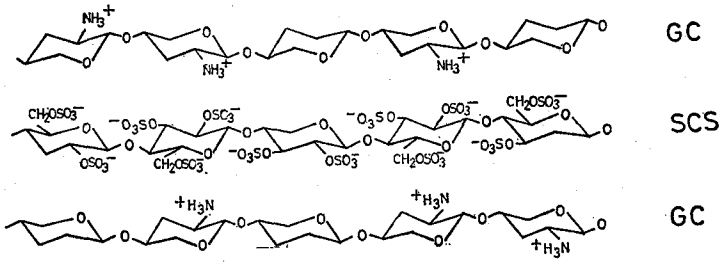

Fig. 7. Schematic model for a ladder complex composed of 1 SCS pyranose and 2 GC pyranose.

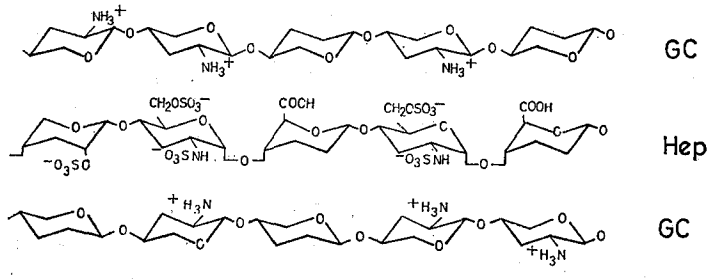

Fig. 8. Schematic model for a ladder complex composed of 1 Hep pyranose and 2 GC pyranose.

creasing pH value in similar fashion. This result indicates that the backbone chain conformation of component polymers plays an important role on the reactions. The complex composition R at lower pH is about 0.65, which may suggest that the complex is in a form of ladder composed of 2 polycation pyranose rings and 1 polyanion pyranose ring, as shown in Fig. 7 and Fig. 8, from the following considerations: (1) both components would adopt extended chain conformations under this pH region because of their high charge density; (2) both Hep and SCS have the pyranose rings, in which sulfonic groups are located on both sides of the molecules as conspicuous group of the molecules. In GC–SCS system, complex formation is far from stoichiometric

in particular at lower pH region at which both components are fully ionized. Accordingly, the obtained complex should be anionic. However, as is suggested from Fig. 7, the charge density of GC, *i.e.*, degree of deacetylation, should affect the net charge of the complex. Stoichiometric interaction may be possible if GC with high charge density is used.

In conclusion, we point out that the backbone chain conformation of component polymers together with the location of ionizable groups is important factor to discuss the formation of polyelectrolyte complexes.

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