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Formation and properties of polyelectrolyte complexes of chitosan hydrochloride and sodium dextransulfate

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Abstract

Methods of potentiometry, turbidimetry, colorimetry, IR spectroscopy, and element analysis were used to investigate the conditions of formation and the properties of non-stoichiometric polyelectrolyte complexes of chitosan hydrochloride (CHC) and sodium dextransulfate (the molecules of both polysaccharides appear as semirigid chains, but their charges are opposite). It was determined that the complexes' formation of polyelectrolytes studied is predominantly electrostatic in the presence of urea. As was also found turbidity and stability of the polycomplexes solutions depended markedly on pH value of CHC and a nature of the low-molecular-weight salts added. The complexes obtained were soluble in water, aqueous urea, and water-organic mixtures. The extent of solubility depended on the composition of the complexes and could be influenced by addition of appropriate concentrations of certain low-molecular-weight salts.

Keywords: Chitosan hydrochloride; Polyelectrolyte complexes; Sodium dextransulfate; Formation; Properties

1. Introduction

Main features and conditions of formation of non-stoichiometric soluble polyelectrolyte complexes (PEC) of flexible-chain polyelectrolytes with opposite charges were studied in detail by (Zezin and Kabanov, 1982). In contrast, non-stoichiometric PEC formed by rigid-chain polysaccharides with opposite charges, have thus far received only minor attention.

As shown in paper (Fukuda & Kikuchi, 1977), PEC of chitosan and sodium dextransulfate (SDS) are water-insoluble at molar ratios of the respective components within the range 0.8–2.5; moreover, the PEC differ in their antithrombogenic properties, depending on the concentration of the salt bonds formed. In another work (Chandy & Sharma, 1990), PEC of chitosan and carboxymethyldextran were shown to be likewise insoluble in water; the authors speculated that their antithrombogenic properties were determined by the molecular structure of these compounds.

Insolubility of such PEC in water and other solvents precludes both the study of their properties in solution and the search for applied use of these compounds. Therefore, obtaining of soluble PEC formed by chitosan with various biologically active anionic polysaccharides is a high-priority task.

In the present work, we studied the conditions of formation of soluble PEC by SDS and chitosan hydrochloride (CHC), as well as the behavior of these compounds in aqueous solutions of salts.

2. Experimental

The original preparation of chitosan, produced from chitin of Antarctic krill (*Euphausia superba*), had an average-viscosity molecular weight (M_v) of 150 kDa and a deacetylation degree of 85%. In order to obtain species with lesser molecular weight ($M_v = 18$ kDa), chitosan was subjected to acid hydrolysis in 1N HCl at 100 °C for 5 h (Gamzazade et al., 1985). The resulting preparation of chitosan was dissolved in 0.3N HCl and precipitated with anhydrous acetone; then, CHC thus formed was washed with dry acetone and dried in vacuum at 50 °C. The other component of the PEC was SDS with $M_v = 500$ kDa and sulfur content of 17.4% (Pharmacia, Sweden).

Soluble PEC of SDS and CHC (PEC_{SC}) were obtained by mixing aqueous solutions of the two components (differing in molarity) and stirring the mixture in the presence of urea and NaCl until the attainment of steady pH-value (the

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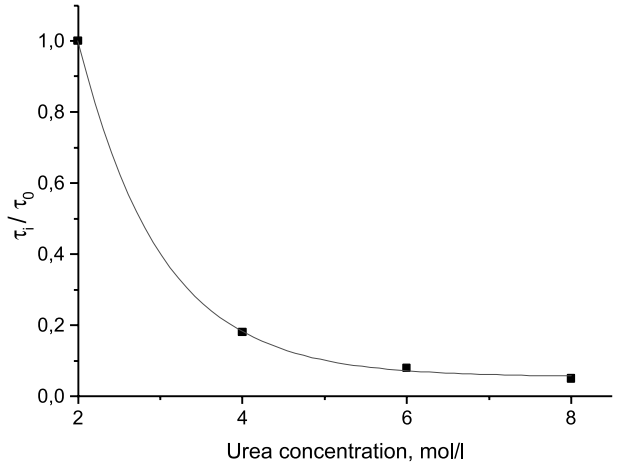

Fig. 1. Dependence of relative turbidity (τ_i/τ_0) of PEC_{SC} solutions (Z = [CHC]/[SDS]: 0.17; pH 7.6; C_{SDS}: 0.3 g/l) on urea concentration.

solution of CHC had a pH of 6.5). The product was isolated by precipitation with a 7-fold excess of absolute ethanol (Gamzazade & Nasibov, 1995). The composition (Z) of PEC_{SC} was described by the ratio of the initial molar concentrations of CHC and SDS.

The process of complexing was monitored by potentiometry and turbidimetry, using, respectively, type OP-208 pH-meter (Hungary) equipped with a combined glass electrode and a Specol spectrophotometer (Germany) with a titration cell (Ti) and a 30-ml cuvette. The results of turbidimetry were expressed as ratios of the observed turbidity (τ_i) to the maximum turbidity (τ_0) at 556 nm.

The character of the ionic interaction between CHC and SDS was monitored colorimetrically, using a DU-50 spectrophotometer (Beckman, USA) and the cationic dye, methylene blue (Fluka, Switzerland). The colorimetric data were used for estimating the degree of conversion, $\theta = C_i/C_0$, (where C_i is the concentration of salt bonds in PEC and C_0, the initial concentration of chitosan amino groups).

The composition and structure of PEC_{SC} were studied by element analysis, high-speed sedimentation (on a MOM 3170-5 analytical ultracentrifuge, Hungary), and IR spectroscopy in KBr tablets (on a Specord M 80 spectrophotometer, Germany).

In vitro anticoagulant activity (AA) of PEC_{SC} was assayed according to Russian Pharmacopoeia (Article FS-42-1526-79), using heparin (AA = 171 U/mg; Abbott, USA) as a standard. The tests were performed in 40-well polystyrene plates for passive hemagglutination. In brief, 25-μl aliquots of anticoagulant solutions, each containing a fixed amount of heparin or PEC (5–15 μg), were introduced into the wells of the plate. Thereafter, 1.3% $CaCl_2$ (75 μl), physiological saline (400 μl), and bovine plasma (500 μl) were added into each well, and the plate was incubated at 20 °C for 1 h; the extent of plasma coagulation was determined by visual examination.

The concentration–response relationship obtained with heparin was used as a reference for determining the AA of PEC_{SC}; for each PEC, we determined the concentration effecting a 50% inhibition of coagulation.

3. Results and discussion

Various non-ionic interactions, such as hydrogen bond formation, dispersion, and other forces make a significant contribution to process that take place when charged polymers form complexes with each other. To rule out effects of non-specific forces, but mostly of H-bonds, the interaction between CHC and SDS was carried out in comparatively dilute media in the presence of urea.

As it can be seen from Fig. 1, the turbidity of the system decreased sharply during the initial period after addition of urea; at urea concentrations in excess of 4 M, the turbidity varied within narrow limits. Therefore, further examination of PEC_{SC} was performed in the presence of 4 M of urea.

The starting pH value of the CHC solution influences considerably the character of the interaction of CHC and

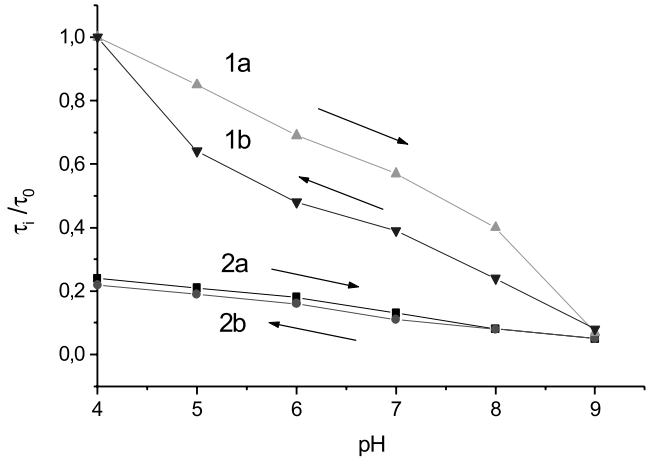

Fig. 2. Curves of forward and reverse turbidimetric titration for PEC_{SC} solutions (Z = 0.33) obtained using CHC solutions with pH 5.0 (1a and 1b) or pH 6.5 (2a and 2b).

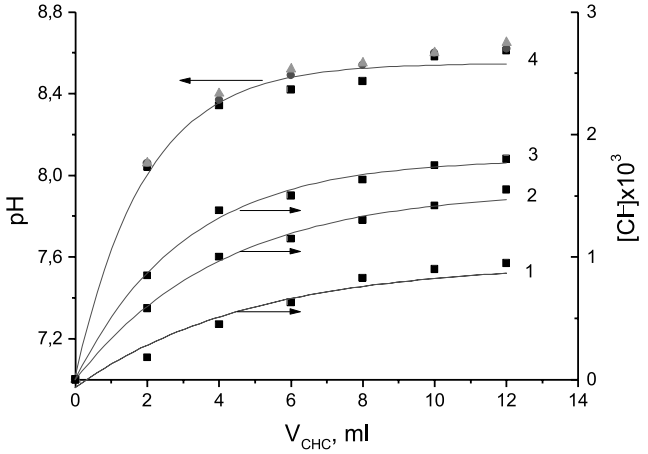

Fig. 3. Changes in pH (4) and the concentration of chloride ions (1–3) in the course of titration of SDS solutions with CHC solutions at different initial component ratios, Z = [CHC]/[SDS]: 0.11 (1), 0.17 (2), 0.33 (3); C_{PEC} = 0.3 g/dl.

SDS. Thus, the turbidity of a PEC_{SC} solution formed by mixing solutions of CHC with pH 5.0 and SDS is appreciably decreased by addition of alkali, and then it undergoes further increase after subsequent addition of acid. The titration curves obtained do not coincide with each other and form a hysteresis loop (Fig. 2, curves 1a and 1b). It is remarkable that, in the absence of alkali, the turbidity of the solution gradually decreased with the passage of time.

In contrast, the turbidity of a PEC_{SC} solution formed by mixing solutions of CHC with pH 6.5 and SDS is lower, and the curves of forward and reverse titrations fully coincide with each other (Fig. 2, curves 2a and 2b).

The process of ionic complexing of CHC and SDS is accompanied by either the formation of a low-molecular-weight byproduct, NaCl, or the accumulation of chloride ions. Indeed, SDS titration by a solution of CHC with pH

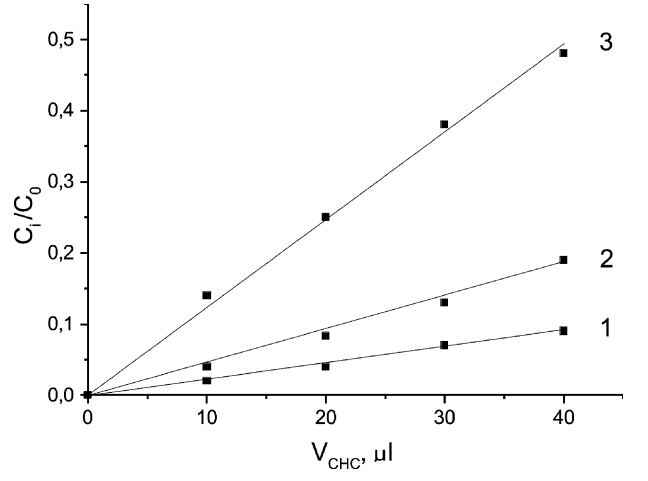

Fig. 4. Colorimetric titration of a solution of the labeled SDS by a CHC solution for Z equal to 0.11 (1), 0.17 (2), or 0.33 (3) at [SDS] = 0.008 mmol/l, C_i is the concentration of salt bonds; C_0, the initial concentration of CHC amino groups.

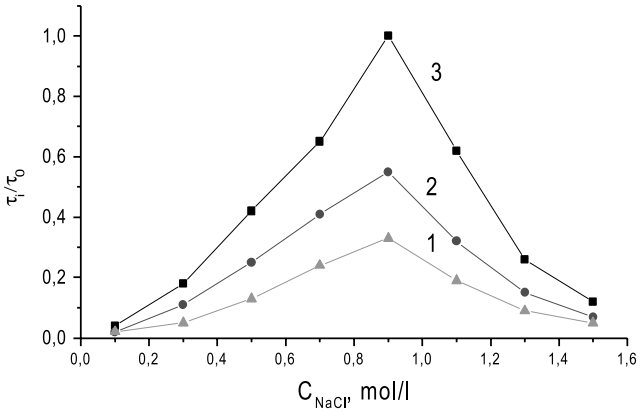

Fig. 5. Dependence of relative turbidity of PEC_{SC} solutions on NaCl concentration for Z equal to 0.11 (1), 0.17 (2), or 0.33 (3) at [PEC] = 0.3 g/dl, pH 7.6.

6.5 (in this case, the concentrations of protonated and non-protonated amino groups may be taken to be approximately equal to each other) results in the formation of complexes, which is accompanied by an increase in both the pH and the concentration of chloride ions (Fig. 3). Thus, depending on the proportion of CHC in the initial mixture, the curves of chloride ion accumulation take different appearance (matching the molar ratio of PEC components in each particular case; see curves 1, 2, and 3), whereas curves of pH changes for the same mixtures are virtually coincident (curve 4). Thus, according to our observations, the majority of those CHC amino groups that participate in the process of CHC and SDS complexing are protonated. It follows that the process of interaction of CHC with SDS is predominantly electrostatic, at least under the conditions found by us.

The nature of the interaction between CHC and SDS is confirmed by the results of titration of solutions of methylene blue-labeled SDS with CHC solutions. As it is seen from Fig. 4, the process of substitution of the cationic dye, which is bound to sulfate groups of SDS, with a stronger cation, CHC, is characterized by a linear dependence of the actual concentration of salt bonds on the ratio of interacting components of the PEC_{SC}.

The behavior of solutions of the obtained PEC_{SC} strongly depends on both the nature and the concentration of the low-molecular-weight salts added. As it is seen from Fig. 5, the dependences of the relative turbidity of PEC_{SC} with different composition on the concentration of NaCl are bell-shaped

Table 1
Sedimentation coefficients for PEC_{SC} solutions differing in the composition of SDS and CHC (4 M urea, 0.05 M NaCl, pH 7.6, C_{PEC} = 0.6 g/dl)

Z	$S_c \times 10^{13}$ (s)
0.33	3.9
0.17	3.2
0.11	2.8
SDS	2.1

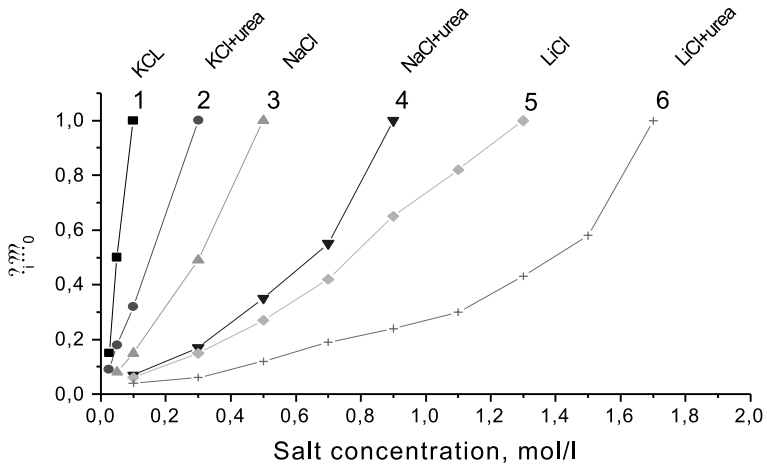

Fig. 6. Dependence of relative turbidity of PEC_{SC} solutions ($Z = 0.17$) on salt concentration in the absence (1, 3, 5) and presence (2, 4, 6) of urea; [PEC]with urea = 0.3 g/dl and [PEC]without urea = 0.05 g/dl.

(which is characteristic of other PEC) and have maxima at 0.9 mol/l for all the species investigated. It should be noted that turbidity maxima of PEC formed by flexible-chain polymers adopt values of similar order at appreciably lower NaCl concentrations, most likely, because of the poorer thermodynamic quality of the solvent (without urea) and the smaller ionic strength of such complexes.

The peculiar behavior of PEC_{SC} formed by CHC and SDS is further illustrated by the results of turbidimetric titration of these PEC (dissolved in water or aqueous urea) with various counterions (Fig. 6). As the figure shows, the counterions showed the following rank order of potency in terms of their ability to increase the area under the solubility curves: $K^+ > Na^+ > Li^+$. The effect seems to be related to differences in the affinity of these counterions to sulfate groups of the PEC_{SC}. Thus, the rank order of coupling potency of these cations matches that of their Coulomb interactions with other polysulfates (Armstrong & Strauss, 1969).

These findings allow us to assume that ionic coupling of CHC and SDS and the resulting formation of PEC_{SC} under the conditions found by us involve practically all interacting macromolecules, which is corroborated by the ability of the PEC_{SC} to give stable and transparent solutions in basic media. The possibility of controlling the composition of the PEC_{SC} by varying the initial ratio of CHC and SDS provides further evidence in support of the above assumption.

The results of element analysis and high-speed sedimentation of several PEC_{SC} produced by changing the initial ratios of CHC and SDS are shown in Table 1. All sedimentograms obtained for the PEC_{SC} contained one distinct peak, the sedimentation coefficient (S_c) of which differed markedly from S_c values of the individual components (Table 1). This suggests that all CHC entered into the reaction is likely distributed in regular intervals between the chains of SDS, which is present in excess. In other words, it can be concluded that the obtained PEC_{SC} solutions contained predominantly macromolecular complexes of CHC and SDS, the composition of which was close to the ratio of these components in the initial mixture.

The formation of macromolecular ionic complexes from CHC and SDS under the conditions found by us is also confirmed by the comparison of IR spectra of these complexes with IR spectra of their component mixtures. A new absorption band at about 1520 cm^{-1}, which is not present in IR spectra of the components, is responsible for the deformation fluctuations of NH_3^+ groups; the intensity of this band is increased with the proportion of CHC in the PEC_{SC}.

The study of the effect of the composition of PEC on their AA demonstrated that all samples tested had higher activities than SDS, in spite of the likely partial neutralization by CHC of the negative charge of the SDS moiety of PEC_{SC}. Moreover, the AA is maximum for the PEC_{SC} with $Z = 0.11$ (Fig. 7), which is similarly to the highest activity in lipoprotein sorption assays for the PEC_{SC} with $Z = 0.17$ (Gamzazade et al., 1997). It is tempting to speculate that

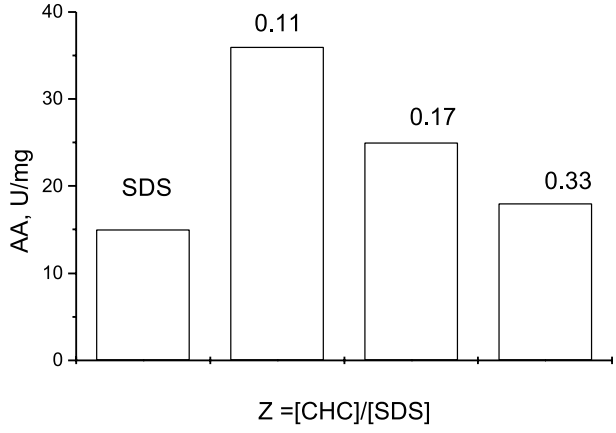

Fig. 7. AA of SDS and PEC_{SC} with different Z.

Table 2
PEC_{SC} solubility in organic solvents (i, insoluble; w/s, weakly soluble; s, soluble)

Z	Solubility									
	DMSO/water, w/w						Formalin		Formic acid	
	30:70		50:50		70:30		25 °C	70 °C	25 °C	70 °C
	25 °C	70 °C	25 °C	70 °C	25 °C	70 °C				
0.33	i	s	i	s	i	i	s	w/s	i	s
0.17	i	s	w/s	s	s	s	i	w/s	i	s
0.11	w/s	s	s	s	s	s	w/s	s	w/s	s

the high AA of the PEC is underlain by optimum density and distribution of surface charges in the PEC_{SC} chains.

Thus, the biological properties of the PEC_{SC} can be controlled by varying their composition, which is important for the compounds exhibiting several types of biological activity.

In contrast to what was observed in aqueous media, the solubility of the PEC_{SC} in certain organic solvents showed virtually no dependence on the composition of the PEC_{SC} (Table 2), but it could be increased by adding water and by increasing the temperature. The highest solubility of the PEC_{SC} was observed in aqueous dimethylsulfoxide (30:70 water/DMSO).

Thus, changes in the ratio of initial components (i.e. high-molecular-weight SDS and low-molecular-weight CHC), the degree of protonation of CHC, and in the nature of the reaction medium can lead to PEC_{SC} with different composition, soluble in aqueous media and water-organic mixtures.

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