ARTICLES

Interpolyelectrolyte Reactions in Solutions of Polycarboxybetaines†

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The utility of different polycarboxybetaines (PCB) as positively charged components of polyelectrolyte complexes was elucidated by potentiometry, turbidimetry, and fluorescence spectroscopy. PCB practically did not quench the fluorescence of either pyrenyl-tagged poly(methacrylic acid) or ethidium bromide intercalated in DNA. This indicates small, if any, interaction of PCB with the above polyanions due to formation of stable ion pairs between carboxylic and quaternary amino groups positioned in each repeated unit of PCB chains. Potentiometric titration curves of PCB solutions practically coincided with the curve of distilled water in the studied pH range 2−11. Addition of sodium poly(styrenesulfonate) (PSS) did not result in phase separation or noticeable change of the titration curve of PCB at pH > 7, whereas at pH < 7 phase separation occurred and the titration curve of the mixture significantly changed, being in close proximity with the curve of poly(acrylic acid). The pH value corresponding to the "cloud point" decreased gradually with the increase in PSS content, invariably of PSS molecular weight in the studied region $M_w = 4000-100\,000$. These findings suggest that sulfonate groups of PSS are not able to compete with carboxylate groups of PCB for binding with the amino groups in neutral and alkaline media, whereas the competitive binding becomes pronounced at pH < 7. The revealed ability of PCB to interact efficiently with PSS forming polyelectrolyte complex with pH-controlled solubility can be important for development of bioseparation techniques.

Introduction

Charged polymers due to their solubility in aqueous media are widely used in various fields of pure and applied science.[1,2] This family of polymers consists not only of polycations and polyanions but also polymers that possess both cationic and anionic groups, i.e., polyampholytes (statistical or block copolymers of cationic and anionic monomers) and polybetaines (polymers with cationic and anionic moieties in the same monomer unit). The latter find wide applications including desalination of water, sewage treatment, flocculation, coagulation, drilling fluids, enhanced oil recovery, drag reduction, etc.[3,4]

Although conventional polyelectrolytes and polyampholytes are well-studied (for reviews, see ref 2 and 3), polybetaines in bulk and in solutions are by far less described. The majority of the studies were performed on polybetaines with sulfobetaine functionalities[5−7] minimizing the pH control over the system. The efforts in research of polycarboxybetaines (PCB) solutions were mainly aimed on investigation of solubility and viscosity properties of the polymers.[8−10] Thus, inter- and intramolecular aggregation of PCB was shown to be affected by the presence of alkyl spacers in the repeat units.[8,9] Potentiometric titration of PCB revealed significant influence of external salt on the ionization behavior of the polymers.[11] This behavior and viscosity of the solutions are greatly affected by steric hindrance of the cationic moiety.[12] The relationship between structural differences of PCB and pH-dependent behavior of their solutions was established recently by capillary electrophoresis.[13]

Reactions of polybetaines with positively or negatively charged polymers, i.e., interpolyelectrolyte reactions, are poorly described. To the best of our knowledge, only one comprehensive study on the interpolyelectrolyte reaction was conducted by Chen et al.[7] in which polysulfobetaines were used. Herein we report the results of study on interpolyelectrolyte reactions in PCB solutions that suggest that mixtures of PCB with polyanions can possess pH-sensitive properties not inherent in starting polymers.

Experimental Section

Materials. NaOH, HCl, TRIS, and MES buffers were purchased from Sigma (USA). In all experiments twice distilled and additionally purified by Milli-Q (Millipore, USA) water was used.

Ethidium bromide (EB) was purchased from Sigma. The concentration of EB in solution was determined spectrophotometrically assuming a molar extinction coefficient 5600 L mol^{-1} cm^{-1} at 480 nm.[14]

Calf Thymus DNA. The Na salt of highly polymerized calf thymus DNA (∼10 000 base pairs) was purchased from Sigma and used without further treatment. Concentration of DNA

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CHART 1

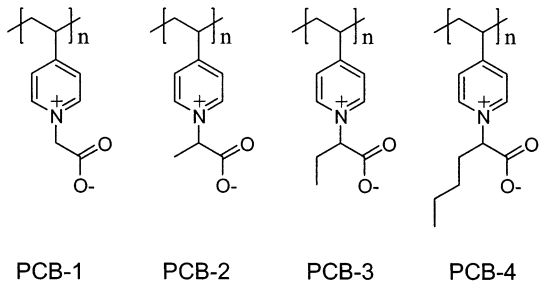

TABLE 1: Properties of Studied Polycarboxybetaines

polycarboxybetaines (PCB)	degree of substitution, %	$10^{-4}M_n$, g·mol^{-1}
PCB-1	>95	2.3
PCB-2	95	2.1
PCB-3	81	2.2
PCB-4	79	2.2

phosphate groups in the solutions was determined by UV absorbance measurements at 260 nm assuming molar extinction coefficient 6500 L·mol^{-1}cm^{-1}.[15]

Poly(acrylic acid) (PAA) was prepared by radical polymerization of the monomer and fractionally precipitated in methanol/ethyl acetate mixture.[16] The fraction with weight-average molecular weight $M_w = 170\,000$ was used.

Poly(methacrylic acid) (PMAA) was synthesized by radical polymerization and fractionally precipitated in methanol/ethanol mixture.[17] PMAA fraction with weight-average molecular weight $M_w = 340\,000$ was used. PMAA tagged by fluorescent pyrenyl groups (PMAA*) was synthesized by interaction of the PMAA fraction with pyrenildiazomethane as described elsewhere.[17] PMAA* sample contained 1 fluorescence label per 320 monomer units, as determined from the UV spectrum of PMAA* solution assuming a molar extinction coefficient 5×10^4 L mol^{-1} cm^{-1} at 342 nm.[17] A solution of tagged sodium poly(methacrylate) (PMA*) was prepared by neutralization of PMAA* aqueous solution with 1 equiv of NaOH with respect to carboxylic groups of the polyacid.

Sodium poly(styrensulfonate) (PSS) samples with weight-average molecular weights $M_w = 4000$ and 100 000 were purchased from Serva (Germany) and used without purification.

Poly(N-ethyl-4-vinylpyridinium) bromide (PEVP) with weight-average molecular weight $M_w = 20\,000$ was synthesized and characterized as described elsewhere.[18]

Polycarboxybetaines (PCB) were synthesized by controlled radical polymerization of 4-vinylpyridine to yield a polymeric precursor poly(4-vinylpyridine), which was further reacted via polymer analogous reaction with esters of corresponding bromocarboxylic acid to obtain four different polymers.[13] Therefore all the utilized samples had equal degrees of polymerization determined by size exclusion chromatography (SEC) performed on the precursor polymer. The utilized polymers possessed pyridiniocarboxylate structure with different length of an additional alkyl chain at α carbon (Chart 1). The degree of conversion in the polymer analogous reaction was established by ^{1}H NMR analysis. The determined values of the substitution degree together with molecular weight of precursor polymer were used to calculate the molecular weights of PCB samples. These characteristics are listed in Table 1.

Methods. *SEC* measurements were performed on a Spectra Physics chromatograph with multiangle light scattering (Wyatt) and refractive index (Viscotek) detectors on a column combination (TSKGEL 6, 17 μm, 6000, 5000, 3000 PW, PSSGEL HEMABIO 40, 10 μm; eluent: aqueous solution of 10 g/L acetic acid charged with 0.2M sodium sulfate).

Spectrophotometric measurements were conducted using a Hitachi 150−20 Spectrometer (Japan) in a water-thermostatic cell under permanent stirring at 25 °C.

Fluorescence intensity measurements of the solutions were made using a Jobin Yvon-3CS spectrofluorometer (France) with water-thermostatic stirred cell holder. The measurements were made in a capped quartz fluorescence cell upon permanent stirring at 25 °C. The excitation and emission wavelengths in experiments with EB were set at 535 and 595 nm, respectively, whereas in experiments with PMA* the wavelengths were 342 and 395 nm, respectively.

The DNA solution was directly mixed with EB in the fluorescence cell. The composition of the obtained complex DNA·EB was [EB]/[P] = 0.25, where [P] is the molar concentration of DNA phosphate groups. At this ratio corresponding to 1 molecule of intercalated EB per 2 pairs of DNA bases (four nucleotides), the maximum of EB fluorescence intensity was observed.[19] The concentration of EB was 1×10^{-5} mol·L^{-1} in all measurements, and the DNA concentration was 4×10^{-5} mol·L^{-1}.

Solutions of PMA* or complex DNA·EB were titrated by solutions of polycarboxybetaines or PEVP. The time interval between the titrant additions was 10 min.

Turbidimetric Titration. Solutions of PCB + PSS mixtures of different composition in distilled water were titrated with HCl solution. The time interval between the additions was 5 min. Both the values of absorbance at 400 nm and pH of the mixture were measured after addition of each portion of HCl to determine the pH corresponding to the onset of turbidity ("cloud point").

Potentiometric titration of aqueous solutions was conducted using a MultiLab 540 (WTW, Germany) potentiometer with a Metler Toledo glass calomel combination electrode. The measurements were performed in a water-thermostatic stirred cell holder under an argon atmosphere at 25 °C. A 7 mL aliquot of the solution was titrated with 0.1 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH solutions using the Hamilton microsyringe. The portions of the titrant were added under vigorous stirring with 5 min time intervals, sufficient for achieving constancy in measured pH values.

Results and Discussion

Solubility. Polyampholytes are known to exhibit low solubility at pH close to their isoelectric point, a property that is exploited for creation of polyampholyte systems with pH-controlled phase separation.[3] As might be anticipated, the studied polymeric zwitterions are also prone to precipitate at the isoelectric points due to compensation of the positive and negative charges. Therefore, a solubility of PCB merits consideration.

Inasmuch as quaternary amino groups of PCB are charged at any pH, one could expect precipitation of the samples in neutral and alkaline media where the complete ionization of the carboxylic groups occurs. However, all samples proved to be water-soluble in the whole studied range $2.0 < pH < 11.0$. The reason for this discrepancy might be the presence of a certain amount of nonquaternized pyridine rings in the utilized samples (see Table 1), which can endow PCB molecules with the solubility. Also, it was found[13] that the utilized PCB macromolecules show only a limited and in any case intramolecular interaction of the anionic and the cationic part of the molecule. The degree of interaction depends on the chemical structure of the PCB and on the pH. In any case, there is a

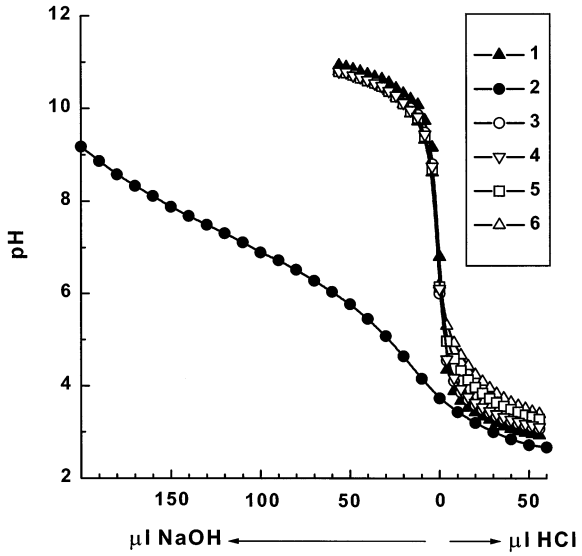

Figure 1. Potentiometric titration curves of distilled water (1) and solutions of PAA (2), PCB-1 (3), PCB-2 (4), PCB-3 (5), and PCB-4 (6) by 0.1 mol·L^{-1} HCl and 0.1 mol·L^{-1} NaOH solutions. $V_0 = 7$ mL. $T = 25$ °C. Concentration of carboxylic groups in solutions of PAA and PCB was 3×10^{-3} mol·L^{-1}

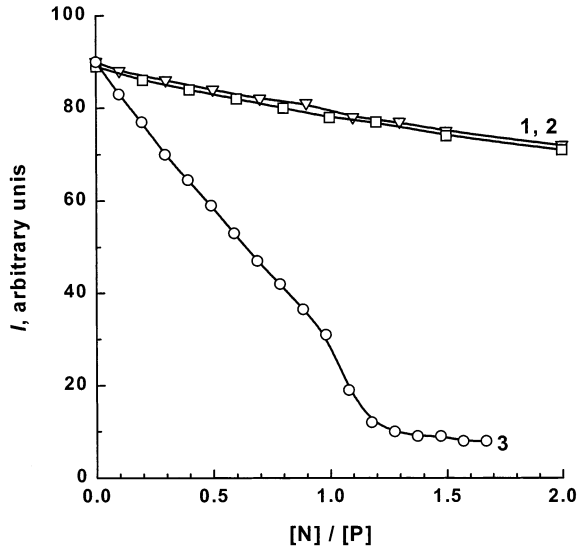

Figure 2. Dependencies of fluorescence intensity I on the ratio of polyelectrolyte concentrations [amino groups]/[phosphate groups] = [N]/[P] in the solutions of mixtures of DNA·EB complex and PCB-1 (1), PCB-4 (2), and PEVP (3). Conditions: 0.02 mol·L^{-1} MES, pH 4.0, 25 °C, [P] = 4×10^{-5} mol·L^{-1}, [EB]/[P] = 0.25.

remaining amount of charges in the macromolecule, due to the limitation of the interaction. We assume these charges to be responsible for the complete solubility of PCB in the whole studied range of pH.

The established inability of the polybetaine samples to undergo pH-induced phase transition restricts possible applications of PCB, in particular for bioseparation based on utilization of polyampholytes with pH-controlled solubility.[3] On the other hand, the unexpectedly high solubility allows studying of the polybetaines by methods used for investigation of polymer solutions.

Acid−Base Equilibrium. To elucidate the influence of zwitterionic structure of the repeat units on acid−base equilibrium, potentiometric titration of PCB solutions has been performed. As was already mentioned, quaternary amino groups of PCB are charged at any pH. If they strongly affect ionization of the carboxylic groups, then the titration curves should be similar to the curve of water; otherwise, PCB solutions should be titrated like poly(carboxylic acid)s. Accordingly, the titration curves of PCB solutions as well as the curves of titration of distilled water and poly(acrylic acid) were obtained (Figure 1).

It is seen that in neutral and alkaline media the curves practically coincide with the curve of distilled water (curve 1) and differ markedly from the curve of poly(acrylic acid) (curve 2). The run of the curves suggests practically complete ionization of PCB carboxylic groups in the above pH region. In acidic media, the curves remain in close proximity with the curve of water titration and far apart from the curve of poly(acrylic acid). It implies that protonation of carboxylate group of PCB is hindered by the adjacent quaternary amino group, forming a rather stable ion pair with the carboxylate group. In other words, pyridinium rings play the role of strong competitors for the protons.

The moderate shift of the titration curves from acidic to neutral media in PCB-3 and PCB-4 solutions (Figure 1, curves 5 and 6) can be explained by protonation of nonquaternized pyridine groups in this pH region for the amount of these groups in the samples is noticeable.

However, it might be not the sole reason of the shift. Despite the distance between carboxylic and amino groups in the repeated units of all PCB samples being the same (see structural formula), the introduction of a bulky side chain consisted of 2 and 4 methylene groups in the case of PCB-3 and PCB-4, respectively, can encourage protonation of the carboxylate groups reflected by the shift of the curves. This assumption agrees well with data from studying the polybetaines by capillary electrophoresis.[13] Lengthening the methylene sequence could also result is a gradual lowering of dielectric constant in the vicinity of the ion pairs. According to Coulombs' law, this change of the environment should strengthen the ionic interactions, e.g., between the proton and the carboxylate group, encouraging the protonation. However, the decrease in the dielectric constant is favorable to the interaction between charges in the ion pairs as well. To explain above shift of the curves, we are thus led to suggest that for PCB with a sufficiently long hydrophobic spacer the protonation dominates the charge−charge interaction, which hardly is the case. So, the gain of this factor to the shift of the curves can be ignored.

Thus, the amino group and carboxylate group located in the same repeat unit of the polybetaines form a rather stable ionic pair "inactivating" each other. This may significantly weaken the electrostatic interaction of PCB with polycations or polyanions and even make it impossible. A good case in point is the revealed poor ability of polybetaines to bind with native DNA.

Interactions of PCB with DNA and PMA. Monitoring the interaction was performed by fluorescence quenching technique using the approach[18] based on competitive displacement of intercalated cationic dye ethidium bromide by the added polycation. The addition of all studied polybetaines (Figure 2, data for PCB-2 and PCB-3 are not shown) to the solution of complex DNA·EB at pH 4 yielded only in a slight change in the fluorescence intensity, which was much weaker as compared with the quenching capacity of poly(*N*-ethyl-4-vinylpyridinium) bromide (Figure 2, curve 3). In the latter case, the addition of 1 equiv of positively charged pyridinium groups of PEVP per negatively charged phosphate groups of DNA proved to be sufficient for virtually complete transfer of the dye into solution where the fluorescence of ethidium bromide is fully quenched. The same regularity was observed in the whole operative region

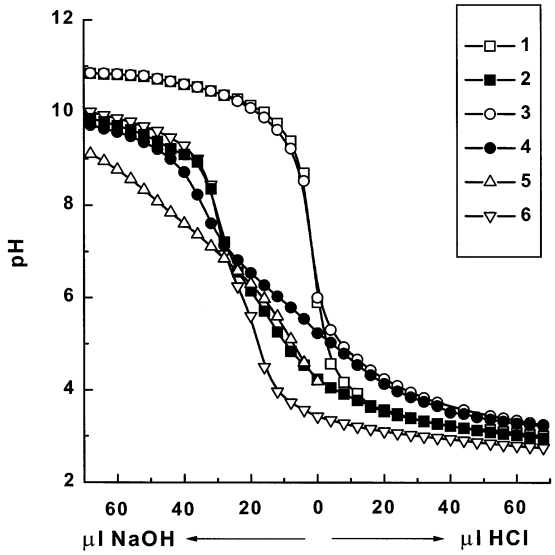

Figure 3. Potentiometric titration curves of solutions of PCB-2 (1), equimolar mixture PCB-2 + PSS (2), PCB-4 (3), equimolar mixture PCB-4 + PSS (4), PAA (5), and PSS (6) by 0.1 mol·L^{-1} HCl and 0.1 mol·L^{-1} NaOH solutions. $V_0 = 7$ mL. $T = 25$ °C. The concentration of carboxylic groups in solutions of PAA and PCB and sulfonate groups in solution of PSS was 1.5×10^{-3} mol·L^{-1}

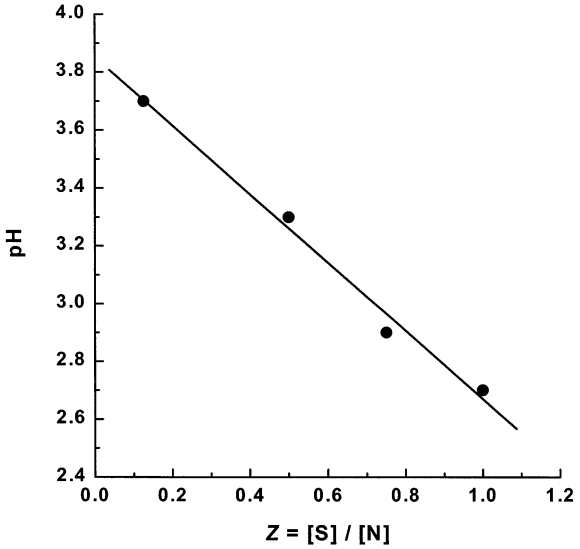

Figure 4. Dependence of "cloud point" pH values in solution of mixtures PSS + PCB-2 on composition of mixture, Z = [sulfonate groups]/[amino groups] = [S]/[N]. [N] = 3×10^{-3} mol·L^{-1}, $T = 25$ °C.

$4.0 \leq$ pH ≤ 10.0 where DNA remains in native state (data not shown). Thus, the polybetaines are not able to bind with DNA strongly enough to squeeze out the dye from the double helix, which supports the above view on the inactivating influence of the carboxylate groups on the adjacent amino groups of PCB.

Data obtained on studying PCB interaction with poly(methacrylate) anion also demonstrated poor ability of the polybetaines to interact with polyanions. Fluorescence titration of labeled PMA* with PCB-1 solution resulted in gradual decrease of the fluorescence intensity, but efficiency of the quenching was much lower (ca. 5%) as compared with the efficiency of quenching of PMA* fluorescence with poly(*N*-ethyl-4-vinylpyridinium) bromide. This significant difference suggests rather low affinity of PMA* to pyridinium groups of the polybetaine that reflects "inactivation" of these groups by the neighbored carboxylate groups.

Nevertheless, we managed "to activate" polybetaines using sodium poly(styrenesulfonate) as an "activator". PSS bares the sulfonate group in each repeat unit of the chain and, hence, strongly binds with various polycations being an efficient competitor to polyanions with other acidic moieties.[20,21]

Complexation of PCB with PSS. Electrostatic interaction of PSS with amino groups of polybetaines resulted in destroying PCB ion pairs and liberating carboxylate groups. Inasmuch as the carboxylate groups were protonated in neutral and acidic media, the competitive binding was monitored by potentiometry (Figure 3).

Potentiometric titration curve of PSS mixture with PCB-2 (Figure 3, curve 2) consisted of two parts, reflecting the different behavior of the system. At pH > 7.0, the curve practically coincided with the curve of PSS (curve 6) and markedly differed from the titration curve of poly(acrylic acid) (curve 5). At pH < 7.0, a run of the curve significantly changed being quite different from the titration curves of both polybetaine (curve 1) and PSS (curve 6) and very close to the titration curve of PAA (curve 5). These findings suggest that at pH > 7.0 sulfonate groups of PSS are not able to compete efficiently with carboxylate groups of PCB for binding with the amino groups, whereas at pH < 7.0 the competitive binding of PSS not only takes place but is very strong for it results in protonation of a majority of the carboxylate groups.

Run of the titration curve of PSS mixture with PCB-4 (Figure 3, curve 4) follows the same trend with even more pronounced binding of PSS. Thus, at pH > 7.0 curve 4 differs from the curve of free polybetaine titration (curve 3) with the marked shift toward curve 5 of PAA titration. At pH < 7.0, curve 4 was also drastically distinct from the curve of PCB-4 titration and arranged even above curve 5 of PAA titration. This apparent contradiction seems to be the result of protonation of pyridine groups contained in the polybetaines that are titrated in the same pH region (curve 3). The revealed increase in efficiency of PSS binding is in favor of the assumption of the growing accessibility of amino groups of PCB with the lengthening of methylene sequence in their repeated units. So, an equilibrium of the competitive reaction is effectively controlled by pH and can be shifted completely either to formation of a PSS−PCB polyelectrolyte complex or its destruction.

Data obtained on turbidimetric titration of the mixtures support this statement. At pH > 7.0, mixture of PSS and PCB-2 was transparent at all studied composition Z = [sulfonate groups]/[amino groups], whereas in acidic media the system became turbid. The latter evidences the complex formation. Value of pH corresponding to the onset of the phase separation ("cloud point") was dependent on composition Z of the mixture decreasing gradually and linearly with increase in PSS content (Figure 4). At $Z > 2$, the mixture remained transparent even in strong acidic media, at pH < 2. This finding suggests that PSS might produce a dual effect on solubility of the complex acting in opposite directions. On one hand, binding of PSS sulfonate groups with amino groups of PCB results in formation of hydrophobic sequences encouraging phase separation. On the other hand, PSS included in the complex in an excess amount enhances its solubility owing to the unbound negatively charged sulfonate groups. It is evident that the former effect predominates in the mixtures with relatively small amounts of PSS, whereas the latter one becomes more and more pronounced with increases in PSS content. Noteworthy, the phase separation in mixtures of PCB-2 and PSS proved to be nonsensitive to molecular weight of PSS in the studied region $M_w = 4000-100\,000$.

Conclusions

Solutions of polycarboxybetaines and mixtures thereof with polyelectrolytes were studied by potentiometry, turbidimetry, and fluorescence quenching techniques. Close proximity of the oppositely charged groups in the repeat units of the polybetaines hindered accessibility of the amino groups for DNA and poly(methacrylic acid). The competitive displacement of the carboxylic moieties was feasible only in acidic media in the presence of strong competitor sodium poly(styrenesulfonate). The polyelectrolyte complex formed underwent pH-dependent phase transition. The results provide means of converting inactive PCB into negatively charged polymers with pH-controlled solubility, promising for application in biotechnology, e.g., for development of bioseparation techniques.

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