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Dynamic Properties of Block Ionomer Complexes with Polyion Complex Cores

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ABSTRACT: Reactions of polyion coupling and interchange were investigated using fluorescence quenching techniques in three systems: (1) soluble nonstoichiometric interpolyelectrolyte complex (IPEC) of poly(methacrylic acid) (PMA) and poly(N-ethyl-4-vinylpyridinium bromide) (PEVP), (2) block ionomer complex (BIC) of poly(ethylene oxide)-*b*-poly(methacrylic acid) copolymer (PEO-*b*-PMA) and PEVP, and (3) BIC of cross-linked ionic core PEO-*b*-PMA (#PEO-*b*-PMA) and PEVP. All measurements were done at the excess of the polyanion (base-molar ratio [PEVP]/[PMA] = 0.2). Rapid polyion coupling was observed following mixing the components in all three systems. However, unexpectedly, the rate of the polyion exchange and substitution reactions increased in the following order: IPEC < BIC < core cross-linked BIC. We posit that the free polyanion chain approaching and interpenetrating the polyion complex experiences greater electrostatic repulsion of the excess PMA units exposed at the IPEC surface compared to the PMA units hidden in the core of BIC. This may slow down the transfer of the PEVP chain from the IPEC to the free polyanion. Evidently, the steric repulsion of PEO chains in the BIC shell does not hinder the association of the reacting species or interpenetration and electrostatic coupling of the polyion chains.

Introduction

The area of block ionomer complexes (BICs) has emerged a decade ago as a result of intersection of the studies on self-assembly of (i) interpolyelectrolyte complexes (IPECs) and (ii) ionic block copolymers in selective solvents.[1–6] One type of BICs is formed as a result of electrostatic interactions between double-hydrophilic block- or graft copolymers with oppositely charged polyelectrolytes or low molecular-weight ions.[7–10] Examples include (1) complexes of single-, double-, and triple-tail cationic surfactants or metal cations with anionic block copolymers;[11,12] as well as (2) DNA or alkyl sulfate surfactants with cationic block copolymers.[13–15] Such BICs spontaneously form nanosized structures with the polyion complex core and water soluble polymer shell, which is often made of poly(ethylene oxide) (PEO). They are also known as "*polyion complex micelles*".[6,14] A similar type of core–shell structures can be formed by PEO-*b*-poly(ethyleneimine) (PEO-*b*-PEI), as a result of coordination interactions of PEI block with the transition metal compounds.[16–19] In addition, hydrogen bonds and hydrophobic interactions can also contribute to the formation of BICs.[20,21] All together, the core–shell BICs can incorporate a variety of compounds or even particles into a core through a combination of electrostatic, hydrophobic and/or hydrogen bonding interactions. The water-soluble polymer shell stabilizes the particles in aqueous dispersion. Numerous applications of these BICs in the delivery of DNA,[22–25] therapeutic proteins,[26–28] low molecular weight drugs,[29–31] and imaging agents[32,33] are being developed, with hundreds of papers published.[34] Furthermore, using BIC as templates, the polymeric micelles with cross-linked ionic cores and nonionic corona have been synthesized and are now being evaluated as carriers for drugs and imaging agents.[35]

Although the formation and properties of BICs are extensively investigated, relatively little is known about the dynamic behavior of their dispersions. It is well-known that regular soluble IPECs can participate in highly cooperative polyion interchange reactions with other polyelectrolyte components present in the solution.[36,37] For example, formation of the IPECs after mixing of oppositely charged polyions in aqueous solutions involve a consecutive series of polyion coupling and exchange reactions. Furthermore, a polyion incorporated into soluble IPEC can be displaced by another polyion of the same charge sign, which is known as a reaction of polyion substitution. The rate and equilibrium of these reactions are strongly affected by the chemical structure and lengths of the reacting polyions as well as the chemical nature and concentration of the low molecular mass electrolytes.[36,37] Initial studies indicate that polyion exchange and substitution can also proceed in the dispersions of the core–shell BICs.[7,10,13,38–40] However, no systematic studies comparing IPECs and BICs have been conducted so far to determine whether these reactions can be affected by the core–shell architecture of BICs. To address this deficiency this study compares polyion coupling and interchange involving (1) the regular IPEC of poly(methacrylic acid) (PMA) and poly(N-ethyl-4-vinylpyridinium bromide) (PEVP), (2) the BIC of poly(ethylene oxide)-*b*-poly(methacrylic acid) copolymer (PEO-*b*-PMA) and PEVP, and (3) the BIC of cross-linked ionic core PEO-*b*-PMA (#PEO-*b*-PMA) and PEVP. The fluorescence quenching technique was used to determine directions and rates of these reactions. This study suggests that there is little if any effect of PEG corona on the reaction directions. However, unexpectedly, we discovered that polyion interchange in PEO-*b*-PMA/PEVP and especially in #PEO-*b*-PMA/PEVP BIC systems proceed much faster than similar reactions in the regular PMA/PEVP IPEC. These findings are important for the practical use of BIC in drug delivery, because such reactions are essential for loading of BICs with macromolecular components as well as for release of these components in the surrounding media.

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Experimental Section

Materials. The syntheses of PMA and poly (4-vinylpyridine) (PVP) samples were carried out by radical polymerization followed by fractionation by dropwise precipitation according to literature methods.[41] PEO-*b*-PMA (Polymer Source Inc.) was used as supplied. The molecular mass of PMA homopolymer is $\overline{M}_w$ =134,000, which corresponds to the average degree of polymerization ($\overline{DP}_w$) of 1,560. The molecular masses of PEO-*b*-PMA blocks are $\overline{M}_{n\ PEO} = 7500$ and $\overline{M}_{n\ PMA} = 15\ 500$ ($DP_n = 170$ for PEO and 180 for PMA) and the M_w/M_n for PEO-*b*-PMA is 1.45. The molecular mass of PVP is $\overline{M}_w = 5700$ ($DP_w = 55$). PVP was quaternized by ethyl bromide to obtain PEVP with 95% substitution. The samples of PMA* or PEO-*b*-PMA* labeled with fluorescein groups were synthesized by interaction of 4′-(aminomethyl)fluorescein (Molecular Probes) with PMA segments. PEO-*b*-PMA* as an example, briefly, 69.2 g (0.003 mmol) of PEO-*b*-PMA and 1.2 mg N-hydroxysuccinimide were dissolved in 2 mL N,N′-dimethylformamide (DMF) and then 2.2 mg 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) in 1 mL CH_2Cl_2 was added. The mixture was stirred for overnight. A 5 μL aliquot of triethylamine and 1.8 mg of fluorescein in 100 μL of DMF were added to the mixture and reacted overnight. CH_2Cl_2 was evaporated and then the mixture was dialyzed against water for at least two days. The solution was freeze-dried to give a yellow powder. The degrees of labeling of each sample determined by absorption spectroscopy and expressed as numbers of fluorescein moieties per COO^- groups were 1:780 for PMA* and 1:720 for PEO-*b*-PMA*. The polyion core cross-linked micelles, #PEO-*b*-PMA were synthesized as reported before and described in the Supporting Information.[35] Briefly, a two-step process was used. The first step involved self-assembly of PEO-*b*-PMA copolymers into BICs in the presence of Ca^{2+} ions. At the second step, the carboxylic groups of PMA in the core of the PEO-*b*-PMA/Ca^{2+} micelles were cross-linked using 1,2-ethylenediamine in the presence of EDC. The cross-linking degree of #PEO-*b*-PMA was estimated to be about 20%. The fluorescein labeled #PEO-*b*-PMA (#PEO-*b*-PMA*) was prepared from PEO-*b*-PMA*. The method was the same as for #PEO-*b*-PMA.

Sample Preparation. The complexes were prepared by mixing solutions of PEVP with each of PMA, PEO-*b*-PMA or #PEO-*b*-PMA in deionized water with the subsequent addition of NaOH up to pH = 10. The base-molar concentrations of polyanions were 2.7 mM, as determined by potentiometric titration. The concentration of PMA, PEO-*b*-PMA, and PEVP in the complexes was 0.237, 0.741, and 0.114 mg/mL respectively. The base-molar concentrations of polycations were 0.54 mM. The compositions of the mixture were kept constant at $\Phi = 0.2$ (where Φ is the ratio of base-molar concentrations of polycation to polyanion chain units). All measurements were carried out at room temperature.

Light Scattering. All solutions were filtered through a Millipore GS 0.45 μm filter. Static light scattering (SLS) measurements were carried out using scattered laser light goniometer "Photocor Complex" (Photocor Instruments) and a 25-mW He−Ne laser operating at 633 nm wavelength as the light source. Refractive index increments used to determine the optical constant K were measured by a KMX-16 differential refractometer (Milton Roy) with a 0.5-mW He−Ne laser as a light source. Polymer concentrations were varied from 0.17 to 0.32 g/dL and from 0.06 to 0.43 g/dL for PEO-*b*-PMA and PEO-*b*-PMA/PEVP complex with $\Phi = 0.2$, correspondingly. M_w measurements were performed in 0.1 M NaCl solution. Dynamic light scattering (DLS) and ζ-potential measurements were carried out with a ZetaPlus Zeta Potential Analyzer (Brookhaven Instruments Corp.) operating at scattering angle $\theta = 90°$. Hydrodynamic radii R_h and ζ-potential were measured at a base-molar concentration of 1.3 mM anionic groups and are presented as means ± SD ($n = 5$). The angular dependence of effective diffusion coefficients were measured using the scattered laser light goniometer Photocor Complex and the data were extrapolated to the zero angle to determine $(R_h)_0$.

Fluorescence Spectroscopy. The polyion coupling and interchange reactions were initiated by the fast feeding of 0.7 mL of PEVP, IPEC or BICs (0.54 mM cationic base-molar concentration and/or 2.7 mM anionic base-molar concentration) into 0.7 mL of solutions of the fluorescein-labeled polyanions (2.7 mM base-molar concentration). The final base-molar concentrations of polyanions (free and IPEC- or BIC-incorporated) were 2.7 mM whereas final base-molar concentrations of polycations were 0.27 mM, providing an overall mixture composition $\Phi = 0.1$. The same concentrations were used for the recording of the fluorescence spectra. The fluorescence steady-state intensity measurements were carried out using Cary Eclipse spectrofluorometer (Varian Inc.). The kinetic curves were recorded using $\lambda_{ex} = 488$ nm and $\lambda_{em} = 520$ nm.

Results and Discussion

Solution Behavior of IPEC and BICs. Mixing of solutions of oppositely charged polyions is accompanied with the polyion coupling and formation of IPECs or BICs stabilized by a system of cooperative electrostatic bonds. The driving force for this reaction is the gain in entropy due to the release of the small counterions initially condensed with the reacting polyion chains.[37,42] For polyions with sufficiently high charge density, such as PEVP and entirely ionized PMA (pH 10), the equilibrium of the coupling reaction is shifted totally toward quantitative formation of the complex. As a result of neutralization of the charges the solubility of the complexes decreases compared to the initial polyions. This leads to precipitation of stoichiometric complexes of regular IPECs above certain critical compositions. Therefore, we conducted these studies at $\Phi = 0.2$ when PEVP and PMA form soluble nonstoichiometric complexes. The $(R_h)_0$ of these complexes was 16 ± 3 nm. Notably, the counter length of PMA used in these studies is almost 30-times longer than that of the PEVP. In this case a longer PMA chain is called the "host" while the shorter PEVP chain is called the "guest".[36,37] We did not observe self-assembly of the PMA/PEVP IPECs into larger aggregates, which is consistent with previous reports that such complexes represent a single lyophilizing PMA chain coupled with several PEVP chains.[36,37] Contrary to regular IPECs, the BICs do not precipitate even upon complete neutralization of the polyion charges. However, such complexes self-assemble into micelle-like aggregates with neutralized polyion cores, and remain stable in aqueous dispersion due to the lyophilizing effect of PEO chains. Such aggregates were also formed in the mixtures of PEO-*b*-PMA and PEVP at $\Phi = 0.2$. On the basis of the angular dependence of the apparent diffusion coefficient the particle size of the complexes extrapolated to the zero angle was $(R_h)_0 = 25 \pm 3$ nm. The ζ-potential of the BIC particles was negative and equal to −10 ± 2.4 mV. The aggregates represented approximately 19 PEO-*b*-PMA chains neutralized with 11 PEVP chains as estimated by SLS.[43] Finally, the polyion core cross-linked #PEO-*b*-PMA micelles were composed of approximately 100 PEO-*b*-PMA chains.[35] They were highly swollen in water and exhibited a radius of 145 ± 3.9 nm with a polydispersity index of 0.11 and negative ζ-potential of − 19 ± 3.5 mV. Such a large size is probably explained by the low degree of cross-linking, which previously was estimated as approximately two cross-links per PEO-*b*-PMA chain.[35] Upon addition of the PEVP, the particle radius decreased to 130 ± 2.7 nm, while the ζ-potential remained practically the same. This suggests that at the studied compositions of the PMA chains, the resulting complexes remained charged and the complexes were swollen.

Kinetics of the Polyion Coupling Reactions. To follow the reactions of polyion coupling the PMA*, PEO-*b*-PMA*, and #PEO-*b*-PMA* were each labeled with fluorescein. Polyion coupling results in fluorescence quenching by PEVP. The coupling reactions of the regular IPEC and BIC can be presented by the following schemes:

$$\mathrm{PMA}^* + \mathrm{PEVP} \rightarrow \{\mathrm{PMA}^* {\cdot} \mathrm{PEVP}\} \quad (1a)$$

$$\text{PEO-}b\text{-PMA}^* \text{ (or \#PEO-}b\text{-PMA}^*\text{)} + \mathrm{PEVP} \rightarrow \{\text{PEO-}b\text{-PMA}^* {\cdot} \mathrm{PEVP}\} \text{(or } \{\text{\#PEO-}b\text{-PMA}^* {\cdot} \mathrm{PEVP}\}\text{)} \quad (1b)$$

(Here and elsewhere the brackets { } indicate IPEC or BIC.)

Figure 1 shows the decreases in the normalized fluorescence intensity, I/I_0, of the labeled polyanions (PMA*, PEO-*b*-PMA*, or #PEO-*b*-PMA*) after addition of PEVP. The values of I_0 and I correspond to the labeled polyanions alone and the reaction mixture, respectively. A dramatic decrease in I/I_0 immediately after addition of the polycation suggests that the initial phase of the coupling reactions occurs very fast (nanoscond or microsecond order) and is followed by the slower phase. This phenomenon, previously described for regular IPECs[36] is due to a rapid formation of nonequilibrium polyion complexes as a result of a random binding of the neighboring polyion chains in the reacting mixture. Subsequent slower fluorescence quenching involves both intra- and intermolecular rearrangements of primary polyion complexes formed during the fast stage. The rates of these secondary processes increase as the NaCl concentration increases due to the destabilization of the salt bonds between the polyions in the presence of the elementary salt.[36,37]

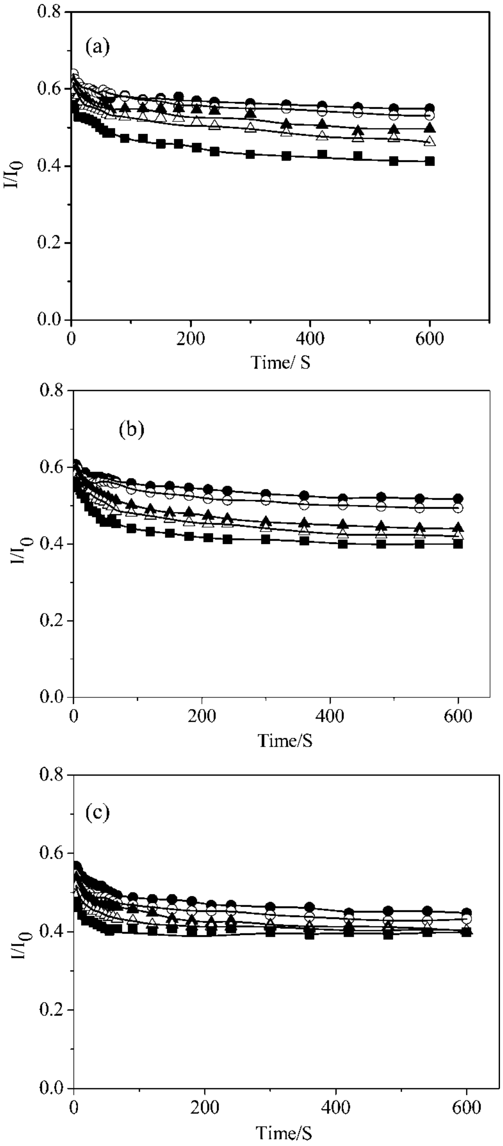

Figure 1. Changes in the fluorescence intensity in solutions of (a) PMA*, (b) PEO-*b*-PMA* and (c) #PEO-*b*-PMA* after addition of PEVP. [NaCl] = 0 (•), 0.002 M (○), 0.005 M (▲), 0.007 M (△), and 0.01 M (■).

It is noteworthy that the coupling reactions involving cross-linked #PEO-*b*-PMA micelles are practically as fast as those involving PEO-*b*-PMA copolymer. This means that the fluorescent labels attached to the cross-linked cores of the #PEO-*b*-PMA micelles are readily accessible to the PEVP chains, possibly due to localization of these labels at the periphery of the cores. Furthermore, it appears that the PEO chains in the corona of the cross-linked BIC micelles do not impede the binding of PEVP chains. This was further illustrated by reacting #PEO-*b*-PMA with long PEVP ($\mathrm{DP_w} = 4890$) exhibiting, in the presence of salt, practically same rates as the short PEVP (data not shown).

Equilibrium of the Polyion Interchange Reactions. Soluble nonstoichiometric IPECs are known to participate in cooperative reactions of polyion interchange.[36,37] These reactions involve transfer of guest polyion chains (GP) from the IPECs to the host polyion chains (HP) fed into the solution. The general scheme of interchange reaction can be represented as

$$\{\mathrm{HP_1}{\cdot}\mathrm{GP}\} + \mathrm{HP_2} \rightleftharpoons \{\mathrm{HP_2}{\cdot}\mathrm{GP}\} + \mathrm{HP_1} \quad (2)$$

If HP_1 is identical to HP_2, reaction 2 represents the polyion exchange reaction. If HP_1 and HP_2 are chemically different, reaction 2 is a polyion substitution reaction.

The principal possibility of the polyion interchange reaction (eq 2) in the PEO-*b*-PMA/PEVP system was first demonstrated by us.[3] More recently Hollappa et al. examined polyion exchange in the BIC formed by PEO-*b*-PMA and poly(methacryloyloxyethyl)trimethylammonium chloride), but no comparison with regular IPEC or #PEO-*b*-PMA was made.[40] In the present work we used fluorescence quenching technique to examine the equilibrium in the exchange reactions between PEO-*b*-PMA/PEVP or #PEO-*b*-PMA/PEVP BICs with the free PEO-*b*-PMA* or #PEO-*b*-PMA*:

$$\text{PEO-}b\text{-PMA}^* + \{\text{PEO-}b\text{-PMA}{\cdot}\mathrm{PEVP}\} \rightleftharpoons \{\text{PEO-}b\text{-PMA}^*{\cdot}\mathrm{PEVP}\} + \text{PEO-}b\text{-PMA} \quad (3a)$$

$$\text{\#PEO-}b\text{-PMA}^* + \{\text{\#PEO-}b\text{-PMA}{\cdot}\mathrm{PEVP}\} \rightleftharpoons \{\text{\#PEO-}b\text{-PMA}^*{\cdot}\mathrm{PEVP}\} + \text{\#PEO-}b\text{-PMA} \quad (3b)$$

The analogous reaction involving the IPEC was also characterized for comparison. After completion of the polyion interchange reaction, the sizes of IPEC and BIC of PEO-*b*-PMA decreased by ca. 20%, whereas the sizes of BIC of #PEO-*b*-PMA practically did not change.

Reaction 3a proceeding from left to right results in the formation of BIC*, in which some part of fluorescein groups are quenched by PEVP. The same reaction from right to left represents the transfer of PEVP to nonlabeled PEO-*b*-PMA, in which fluorescein groups are released and fluorescence intensity is increased. The equilibrium of these two reactions can be determined by emission spectra of fluorescein as shown in Figure 2 (using reaction 3a as an example). Spectra 1 and 4 correspond to the free PEO-*b*-PMA* and BIC*, respectively. Spectra 2 and 3 correspond to the products of reaction 3a in each direction. Clearly, the reaction proceeds in each direction and reaches the same equilibrium state (same fluorescence). Assuming that the difference in the fluorescence between spectra 1 and 4 is 100%, the equilibrium in the reaction is shifted toward formation of BIC* (86% of fluorescence decreased upon mixing BIC and PEO-*b*-PMA* vs 14% of fluorescence increased upon mixing BIC* and PEO-*b*-PMA). This can be explained by several factors that favor formation of the PEVP complex with the labeled copolymer. First are the hydrophobic interactions

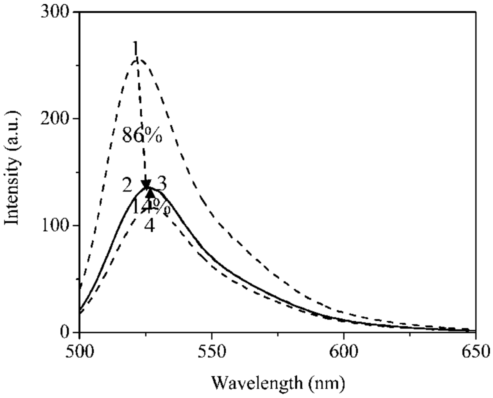

Figure 2. Fluorescence spectra of (1) PEO-*b*-PMA*, (2) {PEO-*b*-PMA·PEVP} + PEO-*b*-PMA*, (3) {PEO-*b*-PMA*·PEVP} + PEO-*b*-PMA, and (4) {PEO-*b*-PMA*·PEVP}. (1−4) The base-molar concentrations of the PEO-*b*-PMA* and PEO-*b*-PMA were 2.7 mM; [NaCl] = 0.01 M. λ_{ex} = 488 nm. (2, 3) $\Phi = 0.2$.

of the fluorescein label, which plunges into the hydrophobic sites formed by polyion complex. Second is the formation of charge transfer complex between the fluorescein and PEVP. Third is the direct ion coupling of a carboxylate group of fluorescein and pyridinium group of PEVP. Notably, this observation is similar to the previous report[36] that the pyrenyl group serves as an "anchor" stabilizing regular IPECs and resulting in the shift of equilibrium in the polyion exchange reaction toward an IPEC of labeled PMA vs unlabeled PMA. Similar behavior was also observed for the #PEO-*b*-PMA micelles (data not shown) as well as for all substitution reactions such as the following:

$$\text{PMA*} + \{\text{PEO-}b\text{-PMA}\cdot\text{PEVP}\} \rightleftharpoons \{\text{PMA*}\cdot\text{PEVP}\} + \text{PEO-}b\text{-PMA} \tag{4}$$

In particular, reaction 4 is shifted to right nearly completely (95%). A complete list of exchange and substitution reactions studied in this work is available in the Supporting Information.

Kinetics of the Polyion Exchange Reactions. Typical kinetic curves of the polyion exchange reactions are shown in Figure 3 in the coordinates of relative fluorescent intensity (I/I_0) *vs* time. The I/I_0 sharply decreases within several minutes due to the transfer of PEVP chains to the labeled polyanion chains. For each system the rate of the reaction increases as the ionic strength elevates, which evidently is due to the destabilization of the salt bonds between the polyions.[44]

The kinetic data were analyzed using equation for the second-order irreversible reaction:[36]

$$\frac{q}{1-q} = k_{\text{II}} c_0 t \tag{I}$$

where the conversion degree $q = (I_0 - I)/(I_0 - I_e)$ is calculated from the kinetic curves, k_{II} is the rate constant of the bimolecular second-order reaction, c_0 is the initial molar concentration of the labeled reacting species, t is the time, and I_e/I_0 is the equilibrium value of fluorescence determined after the completion of the reaction at about 24 h. To account for the concentration of the reacting species in eq I, the aggregation numbers for the BICs were taken into account.

The kinetic curves for all studied exchange reactions linearized in the coordinates of $q/(1 - q)$ vs time (Figure 4), suggesting that these reactions followed the second order kinetics. However the rate constants k_{II} and half-conversion times ($\tau_{1/2}$) were different for each of the three systems (Table 1). Unexpectedly, the BIC of #PEO-*b*-PMA and PEVP exhibits the highest rate constant and shortest $\tau_{1/2}$. In contrast, the IPEC system shows the lowest rate constant and longest $\tau_{1/2}$. The parameters for the reactions involving BIC are intermediate.

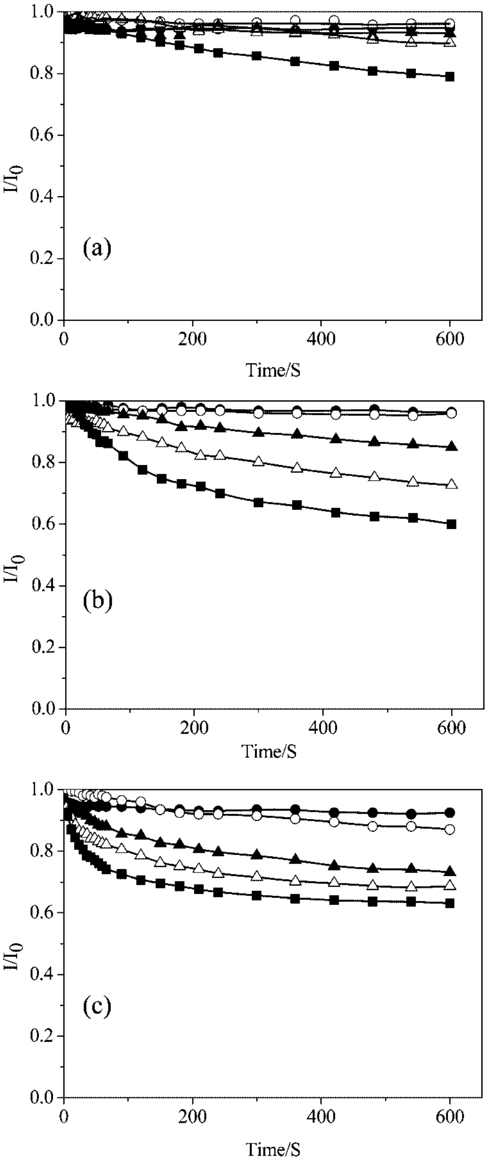

Figure 3. Kinetic curves of exchange reactions: (a) {PMA·PEVP} + PMA* → {PMA*·PEVP} + PMA; (b) reaction 3a left to right and (c) reaction 3b left to right. [NaCl] = 0 M (•), 0.002 M (○), 0.005 M (▲), 0.007 M (△), and 0.01 M (■).

Our previous study reported that PEO-*b*-PMA and PEVP BIC display a CMC type behavior.[1,3] Therefore, higher reaction rates for this system could in principle be explained by disassembling of micelle-like BIC into "unimeric" complexes and subsequent rapid polyion interchange. However, cross-liked BIC cannot disassemble and have the highest molecular mass and size of all the complexes studied. Therefore, the rapid polyion interchange in these systems represents a novel finding.

One may expect that the polyion exchange reaction could be affected by diffusion. However, at least in the case of IPEC in the studied range of ionic strengths it was shown that diffusion is not rate limiting.[36,37,45] In fact the free PMA chain and IPEC particle rapidly diffuse toward each other, the polyion chains interpenetrate and form an intermediate nonequilibrium tertiary aggregate.[36,37,45] The rate limiting step in this reaction is the subsequent transfer of the PEVP chain from one PMA chain to another in the formed aggregate. The reaction rates increase as the system of salt bonds between polyions loosens, which explains acceleration of these reactions upon increase of the

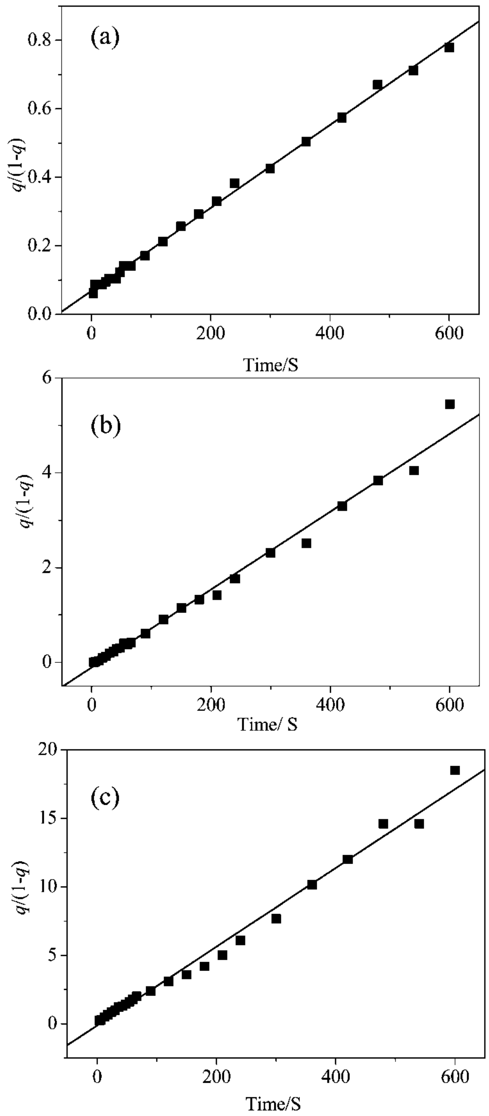

Figure 4. Dependence of $q/(1 - q)$ on the time of Figure 3 reactions in (a) IPEC, (b) BIC, and (c) core cross-linked BIC systems. [NaCl] = 0.01 M.

Table 1. Rate Constant K_{II} as Well as Half-Conversion Time ($\tau_{1/2}$) of Exchange Reactions

	systems (0.01 M NaCl)	$K_{II}/10^3$ M^{-1} S^{-1}	$\tau_{1/2}$/s
exchange	PMA* + {PMA·PEVP}	0.70	>600
	PEO-*b*-PMA*+ {PEO-*b*-PMA·PEVP}	10.0	138
	# PEO-*b*-PMA * + {#PEO-*b*-PMA·PEVP}	181.0	32

ionic strength. Since the sizes of the BIC and especially cross-linked BIC are greater than that of IPEC one may expect slower diffusion rates in the BIC systems. This could in principle decrease the rates of the exchange reaction. To the contrary, a different pattern of the reaction rates was observed in our study. In fact based on DLS the diffusion coefficients for BIC and cross-linked BIC are ca. 4.8×10^{-8} cm^2/s and 1.8×10^{-8} cm^2/s, which still is the same order as the diffusion coefficient for the IPEC (7.9×10^{-8} cm^2/s). On the basis of this, one can estimate the rate constants of diffusion collisions for the reacting species as 10^7–10^8 M^{-1} s^{-1}. This estimate is higher than the observed kinetic constants for polyion exchange reactions suggesting that diffusion is not a rate limiting step for each studied system.

On the basis of this we posit that the likely reason for the observed kinetic behavior is the difference in the processes of PEVP chain interpenetration and transfer within the intermediate aggregates. Specifically, to explain slower reactions in regular IPEC compared to BICs, one should assume that there is a greater energy barrier for interpenetration of the chains in IPEC compared to BICs.[44] The soluble nonstoichiometric IPEC contains an excess of the PMA chains. These charged chains surround the areas of neutralized polyions and are necessary to maintain the solubility of this complex in water. Clearly, the migrating free PMA chain should overcome the electrostatic repulsion of the PMA chains in the complex to form an intermediate aggregate. In contrast, in BIC the solubility is provided by the PEO chains while the PMA is located in the core. On the basis of this prediction, we hypothesize that the free polyanion (HP_2 in reaction 2) experiences greater electrostatic repulsion of the excess PMA units exposed at the IPEC surface compared to the PMA units in the core of the BIC or cross-linked BIC. This may slow down the transfer of the PEVP chain from the IPEC to the free polyanion compared to the BICs. In the case of the cross-linked BIC the core localization of the PMA chains is determined by the chemical cross-links, which may explain the greatest reaction rates observed in these systems. Furthermore, in such cross-linked BIC at composition of 0.2 the PEVP chains may be located at the periphery of the cross-linked PMA core, which can further accelerate the reactions. Evidently, the steric repulsion of PEO chains in the shell of the BICs does not hinder the interpenetration of the reacting species and transfer of PEVP chains within the polyion micelle core. All together, this analysis is also consistent with the kinetics of the polyion coupling reactions discussed above.

Table 2. Rate constant K_{II} as Well as Half-Conversion Time ($\tau_{1/2}$) of Substitution Reactions

	systems (0.01 M NaCl)	$K_{II}/10^3$ M^{-1} S^{-1}	$\tau_{1/2}$/s
substitution	PMA* + {PEO-*b*-PMA·PEVP}	9.0	149
	PMA* + {#PEO-*b*-PMA·PEVP}	32.0	116
	PEO-*b*-PMA* + {PMA·PEVP}	1.4	419
	PEO-*b*-PMA* + {#PEO-*b*-PMA·PEVP}	68.0	87
	#PEO-*b*-PMA* + {PMA·PEVP}	2.3	160
	# PEO-*b*-PMA* + {PEO-*b*-PMA·PEVP}	16.0	72

Kinetics of the Polyion Substitution Reactions. The kinetic equation of the second-order irreversible reaction of polyion substitution is derived in the Supporting Information:

$$\frac{q}{1-q} = k_{II}\frac{DP^* \times N^*}{DP \times N}c_0 t \tag{II}$$

where q, k_{II}, c_0, and t have the same meaning as in eq I, DP* and DP are the degrees of polymerization of labeled and unlabeled polyanions, and N^* and N are the aggregation numbers of labeled and unlabeled particles.

Similarly, to the cases of the exchange reactions the data linearized in the coordinates of q/(1−q) vs time. The data are presented in Table 2. The second rate constants k_{II} and half -conversion times ($\tau_{1/2}$) suggest that in any substitution reaction, IPEC systems were characterized by the slowest kinetics. In contrast the #PEO-*b*-PMA/PEVP systems exhibit the fastest kinetics. This result is generally consistent with the polyion exchange kinetics.

Conclusion

This work focuses on the dynamics of polyion coupling and interchange reactions in IPEC, BIC and core cross-linked BIC systems. Rapid polyion coupling was observed following mixing the components in all three systems, suggesting that PEO shell in BICs does not impede the binding of PEVP chains with the PEO-*b*-PMA or #PEO-*b*-PMA. However, the kinetics of polyion interchange reactions in IPEC, BIC, and core cross-linked BIC strongly depended on the type of the system. Unexpectedly, the rate of the polyion exchange and substitution reactions increased in the following order: IPEC < BIC < core cross-linked BIC. This effect is explained based on the assumption that free polyanion host chain experiences greater electrostatic repulsion of the excess PMA units exposed at the IPEC surface compared to the repulsion of the PMA units hidden in the core of BIC or cross-linked BIC. The excessive electrostatic repulsion may slow down the reactions in the case of IPEC compared to BIC or the cross-linked BIC. As expected, added elementary salt strongly increased the rates of the polyion interchange reactions. Such reactions play major role in structural transitions in polyion complexes essential for their applications for delivery of proteins and DNA using IPECs and BICs.[46,47] Furthermore, demonstration that reactions of coupling and interchange can rapidly proceed in the case of cross-linked BIC is a novel and unexpected finding. It is of principal significance for loading of such systems with biological polyions (e.g., oligonucleotides or polypeptides) as well as for release of such polyions in the external environment. The observation that these reactions rapidly proceed even at relatively low ionic strengths reassures that they will also take place at higher ionic strength at physiological conditions (0.15 equiv of NaCl). Therefore, the results are of fundamental and practical significance for the development of drug and gene delivery compositions in pharmaceutics and other fields.

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Supporting Information Available: Text giving solution preparation and synthesis information for the cross-linked micelles and derivation of kinetic equations, figures showing Zimm plots, text and a table giving a summary of SLS data, and a figure showing the kinetic curves of exchange reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(43) The molecular mass of PEO-*b*-PMA/PEVP BIC at $\Phi = 0.2$ was 475 kDa (Zimm plot presented in the Supporting Information). The aggregation number was estimated (i) under assumption that the composition of the complexes was equal to the composition of the mixture and (ii) using the available molecular masses for PEO-*b*-PMA and PEVP. This is an approximation because BIC had rather high polydispersity index.

(44) Reaction of the polyion interchange proceeds in two stages—first, diffusion of the free polyion chain towards the formed complex resulting in three component aggregate and, second, transfer of oppositely charged polyion (PEVP) from one chain to another in this aggregate.[37] The latter stage is believed to be rate-limiting and its rate increases as the ionic strength decreases because the added salt promotes formation of defects in polyion coupling thus facilitating the polyion transfer. We posit here that PEVP chain transfer requires interpenetration of the chains or IPEC (or BIC) and the free polyion, which may be affected by the electrostatic repulsion.

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