FOCUS REVIEW

Polyion complex micelle formation from double-hydrophilic block copolymers composed of charged and non-charged segments in aqueous media

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Polymeric micelles are representative self-assembly structures of block copolymers and have widely been investigated from both fundamental and applied aspects. In 1995, we discovered polyion complex (PIC) micelles formed from a pair of oppositely charged block copolymers with poly(ethylene glycol) segments through electrostatic interactions in aqueous media, which expanded the concept of polymeric micelle formation in selective solvents. Hereafter, extensive studies have been carried out on PIC micelles, for example, fundamental characterizations as a novel class of self-assembly systems and applications as nanocarrier systems for the delivery of charged molecules with therapeutic efficacies, including nucleic acids and proteins. This review mainly focuses on physicochemical studies on the formation of PIC micelles, particularly the critical molecular factors that have a role to determine the self-assembly scheme of charged block copolymers for micelle structures.
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INTRODUCTION

Recently, supramolecular structures prepared via the self-assembly of polymers with multiple functionality units have attracted progressive interest for the development of multifunctional nanosystems, including supramolecular catalysts, nanocarrier systems for drugs and imaging probes, and nanodevices for data storage and processing. Polymeric micelles formed via the self-assembly of block copolymers are a typical example of multifunctional nanosystems.[1–5] The micelle formation is induced by the difference in the solubility of each segment in the solvent. It is well-documented that, in an aqueous medium, block copolymers that are composed of hydrophilic and hydrophobic segments undergo multimolecular aggregation due to hydrophobic interactions to form a polymeric micelle structure with a self-aggregated hydrophobic core surrounded by a shell layer of hydrophilic polymer strands.[6–8]

Because the segregation of water-incompatible segments of block copolymers is a main driving force for polymeric micelle formation, it is natural to assume that intermolecular interactions other than hydrophobic interactions can drive the micelle formation of block copolymers in aqueous media. In this regard, we focused on the polyion complex (PIC) formation from a pair of oppositely charged polyelectrolytes in an aqueous medium as the driving force of polymeric micelle formation. PICs under charge-neutralized conditions usually segregate from aqueous media as precipitates. However,

we discovered in 1995 that mixing of a pair of oppositely charged block copolymers spontaneously resulted in narrowly distributed PIC micelle structures with a size lower than 100 nm instead of the large precipitates.[9] This behavior can be explained by the segregation of the PIC structure from the aqueous exterior to form the core of the micelles that are surrounded by hydrophilic and non-ionic shell layers composed of other block copolymer segments, in this case poly (ethylene glycol). This finding opened a new avenue in drug delivery research, that is, the use of PIC micelles as nanocarrier systems because they can feasibly stably incorporate various biologically active macromolecules, including nucleic acids and proteins, into their core.[10–14] Because the application of PIC micelles to drug delivery systems has already been described in many review articles,[15–18] we would like to focus mainly on the fundamental aspects of the unique physicochemical behaviors of PIC micelles assembled from various block copolymers.

FORMATION OF PIC MICELLES FROM A PAIR OF OPPOSITELY CHARGED BLOCK COPOLYMERS

To explore the possibility of PIC micelle formation from a pair of oppositely charged block copolymers, a first study was performed using a pair of poly(ethylene glycol) (PEG)-poly(L-lysine) and PEG-poly(α, β-aspartic acid) block copolymers (PEG-PLys and PEG-PAsp) (Figure 1).[9] Upon mixing aqueous solutions of PEG-PLys and PEG-

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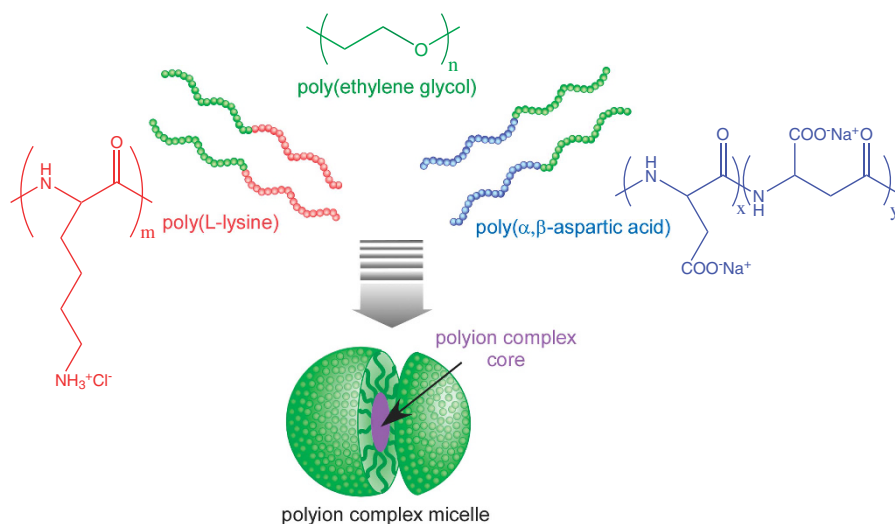
Figure 1 Polyion complex micelle formation from a pair of oppositely charged block copolymers.
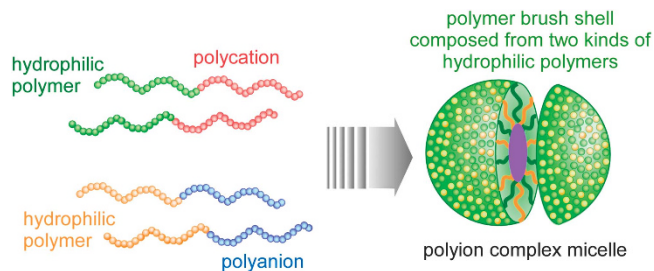

Figure 2 Formation of polyion complex micelles with a mixed hydrophilic shell layer.

PAsp to neutralize their charge, the spontaneous formation of PIC micelles with an extremely narrow size distribution occurred, as confirmed by dynamic light scattering measurements. The preparation of polymeric micelles with a narrow size distribution from hydrophilic–hydrophobic block copolymers often requires the block copolymer to be dissolved in an organic solvent that is a good solvent for both segments of the block copolymer and is followed by solvent exchange with water. In contrast, one of the advantages of PIC micelles is that they form spontaneously via the simple mixing of aqueous solutions of polymers without exchanging solvent properties, which means that the whole process is under thermodynamic equilibrium conditions.

It is obvious that a wide variety of polyelectrolytes are feasible for the PIC core formation.[19–24] Alternatively, there are also several reports on PIC micelle formation from block ionomers with hydrophilic segments other than PEG.[25–27] In this way, one can even place two different hydrophilic polymer strands into the same shell structure of a PIC micelle by selecting block aniomers and catiomers with different hydrophilic and non-ionic segments, as schematically shown in Figure 2. However, this situation is difficult if the hydrophilic polymer strands are inherently immiscible with each other, and this would be an interesting research subject to explore. There may be the possibility of phase separation in the outer shell structure of PIC micelles to induce unique surface properties and morphologies in the micelles. From a functionality point of view, a temperature-responsive PIC micelle with a mixed shell structure was prepared from a block aniomer of temperature-responsive poly(N-isopropylacrylamide)

(PNIPAAm) and poly(acrylic acid) with a block catiomer of PEG and poly(N-methyl-2-vinylpyridinium iodide).[28,29] Because charged segments must be uniformly distributed within the inner core so that their charge is neutralized, the PEG and PNIPAAm chains are also expected to uniformly distribute in the outer shell of the PIC micelles. To confirm the uniform mixing of the PEG and PNIPAAm chains, a two-dimensional ^{1}H nuclear Overhauser effect spectroscopy nuclear magnetic resonance experiment was performed, and it was found that the proton from the PEG chain is close to the PNIPAAm chain (0.5 nm).[28] This result indicated that the combinatorial entropy to maximize the distribution of the ion pairs in the core drives the PEG and PNIPAAm strands to become miscible in the shell structure of the micelles. Interestingly, even in the PIC micelle that formed with a mixed shell structure, dehydration of the PNIPAAm chains occurred above the lower critical solution temperature of the PNIPAAm, which led to the reversible aggregation of the micelles.[28,29]

EFFECT OF THE BLOCK COPOLYMER COMPOSITION ON THE PIC MICELLES

Although many reports addressing the formation of PIC micelles from various block ionomers are available, there are only a limited number of reports on the effect of the block ionomer composition on the physicochemical properties of the micelles, such as their average particle size and association number. Voets et al.[30] prepared PIC micelles from a polyacrylamide-poly(acrylic acid) block aniomer (PAAm-PAA) and the cationic poly(2-methylvinylpyridinium iodide). They synthesized PAAm-PAA bearing a PAA segment with a fixed degree of polymerization (DP) and an outer shell-forming PAAm segment with different degrees of polymerization, and they evaluated the effect of the PAAm chain length on the micelle characteristics using light scattering and small-angle X-ray scattering measurements. They confirmed that the average size of the micelles increased in proportion to the DP of PAAm, whereas the aggregation number decreased.

Using PEG-PAsp as the block aniomer in combination with PEG-PLys or PLys, we gained further insight into the physicochemical properties of PIC micelles by means of light scattering measurements.[31] PIC micelles were prepared using PAsp with a DP of 18, 37 or 78 in combination with PEG with a molecular weight of 5000 (referred to hereafter as PEG-PAsp18, PEG-PAsp37 and PEG-
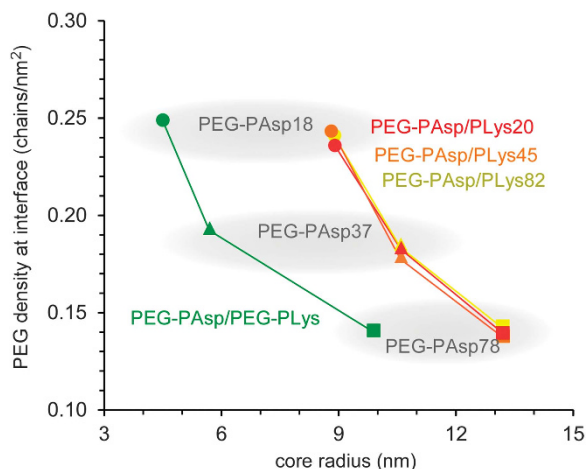
Figure 3 Relationship between the core radius and poly(ethylene glycol) (PEG) density at the interface for polyion complex micelles prepared from various combinations. The data points obtained for the polyion complex micelles using PEG-PAsp18, PEG-PAsp37 and PEG-PAsp78 as the block aniomers are shown as circle, triangle and square symbols, respectively. The green solid line corresponds to PIC micelles made from the PEG-PAsp/PEG-PLys pair with matched chain lengths. Red, orange and yellow solid lines correspond to the PIC micelles made from PEG-PAsp with different compositions and PLys20, PLys45 and PLys82, respectively.

PAsp78), PLys with a DP of 18, 35 or 78 in combination with PEG 5000 (referred to hereafter as PEG-PLys18, PEG-PLys35 and PEG-PLys78) and PLys with a DP of 20, 45, or 82 (referred to hereafter as PLys20, PLys45 and PLys82). For the PEG-PAsp/PEG-PLys micelles prepared from a pair of matched length (i.e., matched polymerization degree) of charged segments and all the combinations of the PEG-PAsp/PLys micelles, the formation of monodispersed PIC micelles with a size of several tens of nanometers was confirmed. In the PEG-PAsp/PEG-PLys micelles, a longer charged chain length resulted in a larger hydrodynamic radius. In contrast, the hydrodynamic radius of the PEG-PAsp/PLys micelles was almost constant at ~ 25 nm and was independent of the chain length of the charged segments. The molecular weights of the PIC micelles substantially varied depending on their composition. The weights ranged from 500 000 for PEG-PAsp18/PEG-PLys18 to 7 500 000 for the PEG-PAsp78/PLys micelles, as determined by static light scattering measurements.

Then, the PIC inner core radius was calculated from the molecular weight of the PIC micelles based on the assumption that a PIC micelle is a hard sphere, and the radii are plotted in Figure 3 against the PEG density at the interface. Here, the PEG density was calculated as the ratio of the association number of the PEG chains versus the surface area of the PIC inner core. The PIC core radius increased with an increase in the length of the PAsp segment in PEG-PAsp for both the PEG-PAsp/PEG-PLys and PEG-PAsp/PLys micelles. Note that, in the PEG-PAsp/PEG-PLys micelles (green-colored line in Figure 3a), the chain lengths of both the PAsp and PLys segments were equal (closed green circle: PEG-PAsp18/PEG-PLys18; closed green triangle: PEG-PAsp37/PEG-PLys35; closed green square: PEG-PAsp78/PEG-PLys78) to satisfy the chain length matching conditions for micellization, which will be discussed in the next section. Alternatively, in the PEG-PAsp/PLys micelles, chain length matching is not necessary for micelle formation, and thus the data obtained for each PEG/PAsp (closed circle, closed triangle and closed square) pairing with PLys with different DPs are shown in distinctive colored lines (red: PLys20; orange: PLys45; and yellow: PLys82). It is obvious that the block/homo
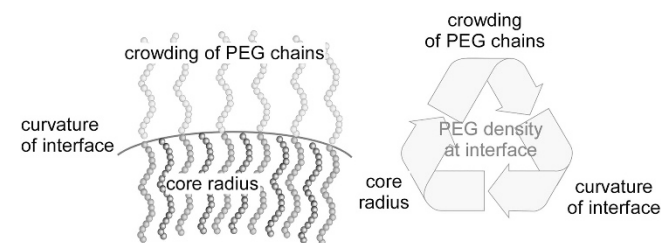
pair always has a larger core radius in the micelles than that of the block/block pair with the same composition of PEG-PAsp. This may be because the arrangement of the block/block pairs in the micelle is restricted; the junction connecting the non-charged and charged segments is situated at the interface of the inner core and the outer shell. In contrast, the homoionomers are neutralized in the inner core, and there are no restriction in their arrangement on either end. PIC micelles from a block/homo pair have more freedom to increase their association number. This may be responsible for the larger inner core size of the PEG-PAsp/PLys micelles compared with that of the PEG-PAsp/PEG-PLys micelles.

It is interesting to note that, in terms of the PEG density at the interface, there was no difference between the block/block pair and block/homo pair if the composition of the PEG-PAsp was the same. The PEG density at the interface seems to be solely determined by the length of the charged segments of the block ionomer. Given that the junction of the non-charged and charged segments of the block ionomers aligns at the core/shell interface, the radius of the inner core depends on the length of the charged segment of the block ionomers. A shorter charged-segment results in a smaller radius of the inner core, which is obviously seen in Figure 3. However, when the radius of the inner core decreases, the curvature of the interface increases, and the crowding of the outer shell-forming segments eventually relaxes in the outward direction. Accordingly, there is more room to increase the PEG density at the interface. However, if the length of the charged segment increases, the radius of the inner core also increases, causing the curvature of the PIC inner core to decrease. This facilitates crowding of the shell-forming segments, and they compete with the density increase in the PEG strands at the interface. In this way, the association number and, eventually, the size of the PIC micelles can be reasonably explained by considering the balance between the length of the non-ionic and charged segments of the block ionomers (Figure 4). It should be noted that the decreased fraction of non-charged hydrophilic segments in block ionomers over a certain extent favors the stabilization of the PIC lamellae structure to induce the self-assembly transition from micelles to vesicles, as described elsewhere.[32,33]

RECOGNITION PHENOMENON IN PIC MICELLE FORMATION

The PIC micelle formation described above occurs under thermodynamic equilibrium conditions, and recognition of the charged-segment length between oppositely charged block copolymers in the PIC micelle formation has been confirmed.[34] When PEG-PAsp and PEG-PLys, which have matched charged-segment lengths, were mixed, the formation of PEG-PAsp/PEG-PLys micelles was observed, as described in the preceding section. However, when PEG-PAsp and PEG-PLys with charged segments of different lengths were mixed, PIC micelles with a diameter of several tens of nanometers were not
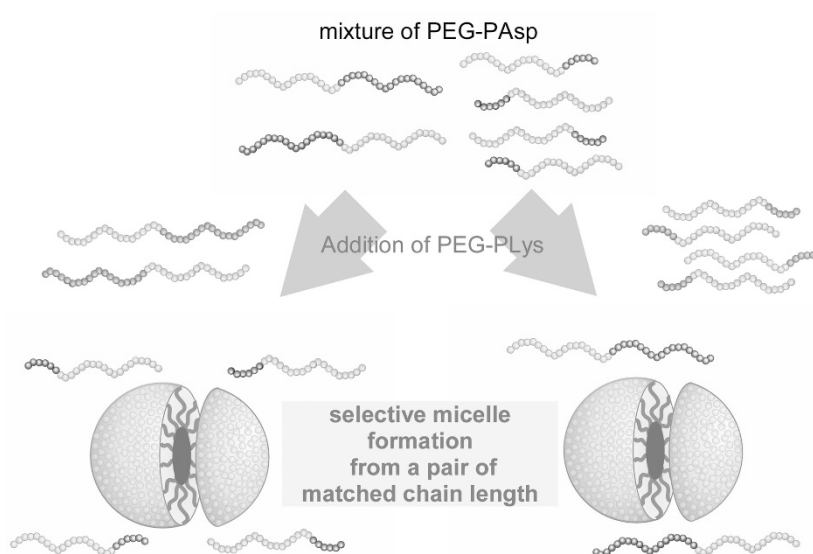

Figure 4 Parameters determining the block copolymer association into polyion complex micelles. A full color version of this figure is available at the *Polymer Journal* journal online.

Figure 5 Chain length recognition in the formation of polyion complex micelles. A full color version of this figure is available at the *Polymer Journal* journal online.

detected by dynamic light scattering. Alternatively, in static light scattering measurements, the presence of PICs with an average molecular weight one order greater than the molecular weight of the used block copolymers was confirmed. When the association number was calculated from the molecular weights of these PICs, the number of the block copolymer with the longer charged chain was always unity in each combination, which indicated the formation of a PIC with the smallest neutralizing unit (unit PIC (uPIC)). In other words, different types of PICs were formed by the matched and unmatched combinations of charged-segment lengths, namely, PIC micelles and uPICs.

Interestingly, strict recognition of the chain length occurred when adding a block ionomer with a particular charged-segment length to a mixture of oppositely charged block ionomers with a different charged-segment length. Block ionomers exclusively select the oppositely charged partner with a matched segment length to form PIC micelles, and the unmatched partner remains in solution in its free form (Figure 5). This phenomenon was confirmed through gel permeation chromatography measurements and ^{1}H nuclear magnetic resonance analysis of the micelle fraction.[31,34] Both the matched and unmatched pairs form uPIC. However, only the uPIC from the matched pair can grow into PIC micelles via a secondary association because the ion pairs can homogeneously distribute in the core with the alignment of the junction of the non-charged (PEG) and charged segments at the core/shell interface. This homogeneous distribution of ion pairs in the core is difficult for unmatched pairs without phase mixing of PEG and ionomer segments at the interface.[34] Apparently, PIC micelles are more thermodynamically stable than uPICs above a critical concentration to decrease the interfacial free energy and increase the entropy of the charged segments in the segregated PIC core. As exchange of ionomer strands among uPIC freely occurs in the solution, equilibrium shifts to the selective formation of PIC micelles from matched pair in the solution.

UNIT PICS AND MICELLES

Recently, an interesting phenomenon related to the equilibrium between uPICs and PIC micelles was found in the PIC formation
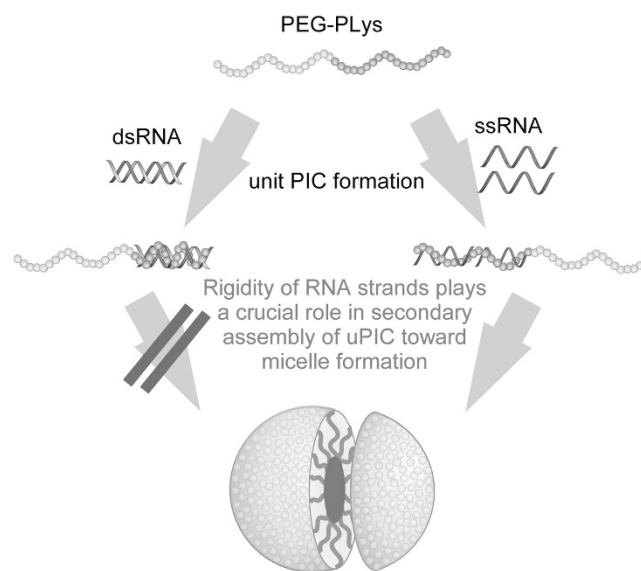

Figure 6 Effect of the rigidity of the ionomer strands on polyion complex (PIC) micelle formation from uPIC. A full color version of this figure is available at the *Polymer Journal* journal online.

process from PEG-PLys and RNA.[35] Either 21-mer single-strand RNA or double-strand RNA (dsRNA) was mixed in an aqueous solution with PEG-PLys. The DP of the PLys segment was fixed at 40, and the molecular weight of the PEG segment was systematically varied in the range from 2k to 42k. The single-strand RNA/PEG-PLys combination formed uPICs under dilute conditions, and then, as the concentration increased above a critical value, the combination underwent a secondary association to form the PIC micelle structure. Interestingly, dsRNA/PEG-PLys showed no such PIC micelle formation even at a very high concentration approaching the C* of the PEG in aqueous media. This critical difference in the PIC association behavior between single-strand RNA and dsRNA may originate from the significant difference in the rigidity of the oligonucleotide strands (Figure 6). As mentioned previously, the factors that enhance the secondary

association of uPICs into PIC micelles are a reduction in the interfacial free energy between the aqueous phase and the PIC and an increase in the positioning freedom of the charged segments in the multi-molecular assembly of the PIC core that is segregated from the aqueous exterior compared with that of the uPIC structure. A factor that inhibits the secondary association is the steric repulsion between the PEG strands in the shell layer of the micelles. Single-strand RNA is very flexible in nature and has the advantage of more positioning freedom for association into PIC micelles. Alternatively, dsRNA is rigid because of its double-stranded structure, and it has inherently less conformational freedom. Thus, no significant increase in the positioning freedom in the PIC phase is expected to compensate for the increased steric repulsion of the PEG strands in the PIC micelle structure, and dsRNA/PEG-PLys remains as uPICs in aqueous media.[35] The formation of a stable uPIC structure from dsRNA is used to construct a palisade of block ionomers to pair with dsRNA (small interfering RNA) onto gold nanoparticles to create systemically injectable nanocarriers to deliver small interfering RNA therapeutics to target organs to treat cancer.[36,37]

CONCLUSION

The current status of research on PICs formed from block ionomers was described here from a physicochemical viewpoint with a focus on the structural parameters of the block copolymers. PIC micelles prepared in this way usually have excellent dispersivity in aqueous media and have been found to be useful as nanocarrier systems, particularly for charged compounds. Furthermore, as focused on this review paper, the intensive study on the process of PIC micelle formation provides new insights on the self-assembly of block copolymers in thermodynamic equilibrium conditions in water, such as the chain length recognition and transition from uPIC to PIC micelle structures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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