Micellization

# Chaotropic-Anion-Induced Supramolecular Self-Assembly of Ionic Polymeric Micelles**

Yang Li, Yiguang Wang, Gang Huang, Xinpeng Ma, Kejin Zhou, and Jinming Gao

Dedicated to Professor George M. Whitesides on the occasion of his 75th birthday

Abstract: Traditional micelle self-assembly is driven by the association of hydrophobic segments of amphiphilic molecules forming distinctive core–shell nanostructures in water. Here we report a surprising chaotropic-anion-induced micellization of cationic ammonium-containing block copolymers. The resulting micelle nanoparticle consists of a large number of ion pairs ($\approx 60\,000$) in each hydrophobic core. Unlike chaotropic anions (e.g. ClO_4^-), kosmotropic anions (e.g. SO_4^{2-}) were not able to induce micelle formation. A positive cooperativity was observed during micellization, for which only a three-fold increase in ClO_4^- concentration was necessary for micelle formation, similar to our previously reported ultra-pH-responsive behavior. This unique ion-pair-containing micelle provides a useful model system to study the complex interplay of noncovalent interactions (e.g. electrostatic, van der Waals, and hydrophobic forces) during micelle self-assembly.

Responsive materials have received considerable attention for the construction of nanosystems that allow highly selective recognition, catalysis, and transfer operations in a wide range of photonic, electronic, and biological applications.[1] Various nanosystems that respond to changes in pH value,[2] enzymatic expression,[3] redox potential,[4] temperature,[5] and light[6] have been developed successfully. The underlying science in the development of many of these responsive systems resides in the supramolecular self-assembly principles conceptualized over two decades ago by Whitesides and Lehn.[7] In contrast to covalent chemistry, supramolecular self-assembly engages a multitude of weak and reversible noncovalent interactions (e.g. electrostatic and hydrophobic interactions, hydrogen bonds, etc.) to achieve a thermodynamically stable nanostructure.[7a,c] This strategy has the advantage of reaching sizes (10^4–10^{10} Da) that are not easily achievable by covalent chemistry, and the resulting system often displays positive cooperativity over the behavior of single molecules in solution.

Recently, our lab has established a series of tunable, ultra-pH-sensitive micelle nanoparticles from different block copolymers (PEO-*b*-PR, where PEO is poly(ethylene oxide) and PR is the ionizable tertiary amine block).[2d,e,8] At pH values below the transition pH (pH_t), micelles dissociate into unimers with protonated ammonium groups. At $pH > pH_t$, the neutralized PR segments become hydrophobic and self-assemble into the micelle cores (left panel in Figure 1).[9] Hydrophobic micellization dramatically sharpens the pH transition, so that the fluorescence activation (on/off states) is narrowed to less than 0.25 pH units, compared to 2 pH units for small-molecular pH sensors.
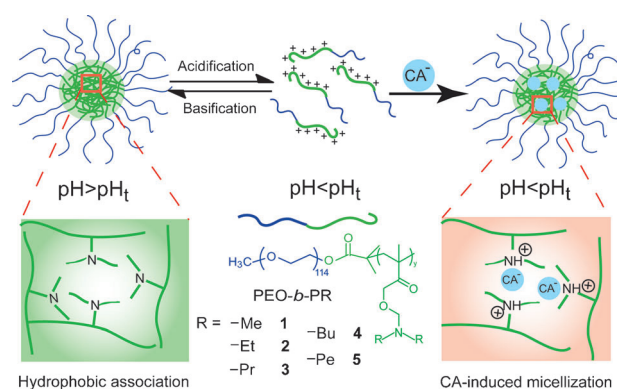
Figure 1. Self-assembly of ionizable polymeric micelles by two independent mechanisms. The left panel shows the induction of micellization by an increase in the pH value, resulting in the PR segments becoming neutralized and hydrophobic to drive micelle formation. Surprisingly, addition of chaotropic ions (CA, such as ClO_4^-) at a low pH value also leads to micellization with ammonium PR segments (right panel). Structures of a series of PEO-*b*-PR copolymers (**1**–**5**) with different hydrophobic side chains are shown in the inset. CA = chaotropic anion, pH_t = transition pH value.
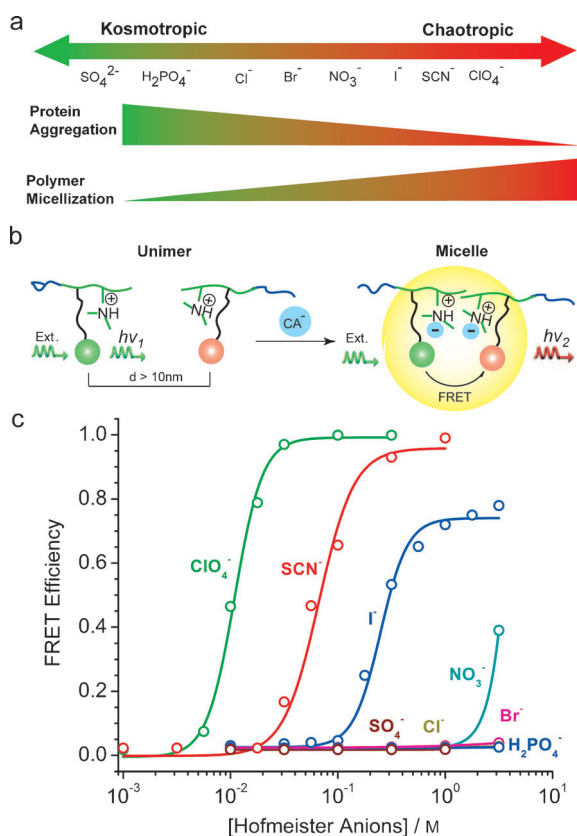
Herein, we report the discovery of chaotropic-anion-induced micellization of protonated PEO-*b*-PR copolymers at pH values below the pH_t (right panel in Figure 1). Surprisingly, an anti-Hofmeister trend was observed, in which the presence of chaotropic anions but not kosmotropic anions resulted in micellization,[10] in contrast to their effects in protein aggregation (Figure 2a).

We first established a fluorescence resonance energy transfer (FRET) method to investigate the micelle self-assembly process. FRET is highly sensitive for the detection of conformational and phase transitions of polymers/proteins

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Figure 2. a) Chaotropic anions induce micelle self-assembly of PEO-*b*-PR copolymers with protonated PR segment, which is a reversed effect (salt-out) with respect to their ability to solubilize proteins (salt-in). b) Illustration of FRET design to investigate CA-induced micelle self-assembly. Addition of CA results in micelle formation and efficient energy transfer from donor (TMR) to acceptor (Cy5) dyes. c) Chaotropic anion-induced micelle self-assembly showing the anti-Hofmeister trend.
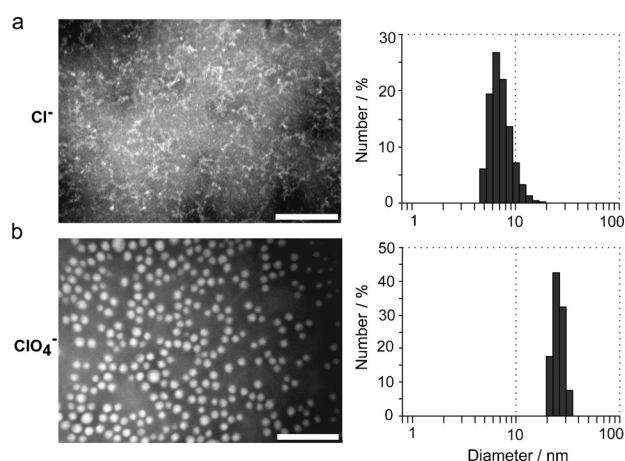
The FRET effects were quantified to compare different anions in their abilities to induce micellization (Figure 2c). FRET efficiency was normalized as $(F_A/F_D)/(F_A/F_D)_{max}$, where F_A and F_D were the fluorescence intensity of TMR and Cy5 at different anion concentrations, respectively; $(F_A/F_D)_{max}$ was the maximum value of F_A/F_D (3.3) at high ClO_4^- concentrations. FRET efficiency was plotted as a function of concentration for different anions. Results displayed an anti-Hofmeister trend, in which chaotropic anions were able to induce unimer association (i.e. micellization), whereas the kosmotropic anions were unable to do so (Figure 2c). This result is in contrast to the classical Hofmeister effect in protein solubilization, in which kosmotropic ions are known to induce protein aggregation in water but not the chaotropic ions.[14]

Copolymer **3** displayed different detection sensitivity toward the chaotropic anions. Data show that the FRET sensitivity followed the order of $ClO_4^- > SCN^- > I^- > NO_3^-$. We define FC_{50} as the anion concentration at which the FRET efficiency was at 50%. The values of FC_{50} were 11, 68, and 304 mM for ClO_4^-, SCN^-, and I^-, respectively. For NO_3^-, only a weak FRET effect was observed at its saturation concentration (≈ 3 M). A more detailed examination showed that only a three-fold increase in ClO_4^- concentration (i.e. from 6 to 18 mM, Figure 2c) was necessary to increase FRET efficiency from 10% to 90%. This narrowed concentration dependence suggests an increased cooperative response similar to the ultra-pH response as reported previously.[2d,e,8]

To further confirm chaotropic-anion-induced micellization, we employed transmission electron microscopy (TEM) and dynamic light scattering (DLS) to investigate the changes in morphology and hydrodynamic diameter during micelle transition, respectively. We used chloride anions (Cl^-) as a negative control. In the presence of 50 mM Cl^-, copolymer **3** remained as unimers at pH 5.0 (below its pH_t at 6.1, Figure 3a). In contrast, copolymer **3** self-assembled into spherical micelles when Cl^- was replaced with ClO_4^- (Figure 3b).

because the energy-transfer efficiency is inversely proportional to the sixth power of the donor–acceptor distance.[11] In our method, we conjugated block copolymers (**1–5** in Figure 1 inset, see also Table S1 in the Supporting Information)[12] with either a donor or acceptor dye. We chose PEO-*b*-poly(di-*n*-propylaminoethyl methacrylate) (**3**, $pH_t = 6.1$) as a model copolymer, and tetramethyl rhodamine (TMR, $\lambda_{ex}/\lambda_{em} = 545/580$ nm)/Cy5 ($\lambda_{ex}/\lambda_{em} = 647/666$ nm) as donor/acceptor, respectively.[13]

At pH 4, the tertiary amines in **3** ($pH_t = 6.1$) were protonated and the resulting copolymers were soluble in water as dispersed cationic unimers. No FRET effect was observed because of the large distance between the unimers (i.e. TMR and Cy5) in solution. The addition of chaotropic anions (e.g. ClO_4^-, SCN^-, or I^-) resulted in the decrease of fluorescence intensity from TMR and increase of emission intensity of Cy5 (Figure S1), thus indicating the formation of polymeric micelles. Micelle formation was hypothesized to bring TMR and Cy5 to close proximity within the micelle core, thereby dramatically increasing FRET efficiency (Figure 2b). In contrast, kosmotropic anions (e.g. SO_4^{2-}, $H_2PO_4^-$) did not lead to any FRET transfer (Figure S2), even at concentrations close to their solubility limits (Table S2).

Figure 3. TEM and DLS analyses of micelle transition of copolymer **3** in the presence of Cl^- (a) and ClO_4^- anions (b). Concentrations of both anions were controlled at 50 mM (pH 5.0). The scale bars are 100 nm in the TEM images.

DLS analyses showed an increase in the hydrodynamic diameter from 7 ± 2 to 26 ± 3 nm when the anions were changed from Cl^- to ClO_4^- (Figure 3). This increase in size reflects the transition of copolymer **3** from the unimer state to the micelle state, consistent with the FRET and TEM data. At pH 7.4, copolymer **3** was present as spherical micelles with hydrodynamic diameters of 27 ± 2 and 28 ± 3 nm in the presence of Cl^- and ClO_4^- anions, respectively (Figures S3 and S4). For nonionizable amphiphilic block copolymers such as PEO-*b*-poly(D,L-lactic acid) (PEO-*b*-PLA), neither a change in pH value nor the addition of ClO_4^- had any effects on the micelle state (Figure S5).

We then studied the chaotropic-anion-induced self-assembly in the presence of competing kosmotropic or borderline anions. Copolymer **3** was dissolved at pH 4 with different initial concentrations of competing SO_4^{2-} or Cl^- anions. Then chaotropic anions ClO_4^- were added to induce micellization (Figures S6–S9). Figure 4a shows the representative example
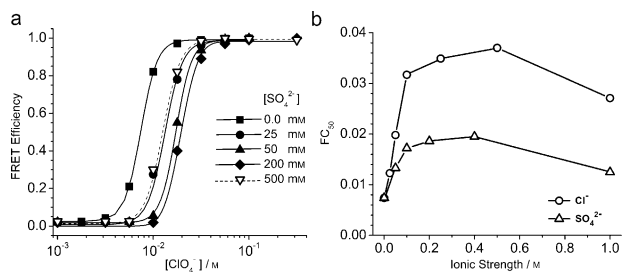
Figure 4. a) ClO_4^--induced self-assembly of copolymer **3** in the presence of different concentrations of competing SO_4^{2-} anions. b) The FRET efficiency (FC_{50}) from ClO_4^--induced self-assembly as a function of the ionic strength of the competing Cl^- and SO_4^{2-} anions. The pH value of the solution was controlled at pH 4 in these studies.

of FRET efficiency as a function of ClO_4^- concentration. Addition of SO_4^{2-} anions was able to decrease the sensitivity of ClO_4^- in micelle induction. We quantified the FC_{50} values to evaluate the effect of competing anions (Figure 4b). We observed an interesting bell curve as a function of the ionic strength of the competing anions. At low ionic strength (<0.1M), addition of competing anions decreased the ability of ClO_4^- to induce micelle formation, consistent with their competition with the ammonium groups of the PR segment. At high ionic strength (>0.5M) of SO_4^{2-} or Cl^-, however, we observed an enhancement of ClO_4^--induced self-assembly. We attribute this effect to the more ordered bulk water structures at high kosmotropic ion concentrations, which makes the hydrophobic association during micelle self-assembly more favorable.

Finally, we investigated the effect of the hydrophobic strength of the PR segment on the chaotropic-anion-induced micellization (Figure 5a). We synthesized a series of PEO-*b*-PR copolymers (**1**–**5** in Figure 1, inset) that bear alkyl chains of different lengths from methyl to pentyl groups on the tertiary amines. Results showed a clear dependence of ClO_4^--induced self-assembly on the hydrophobicity of the PR segment (Figure S10). With the least hydrophobic side
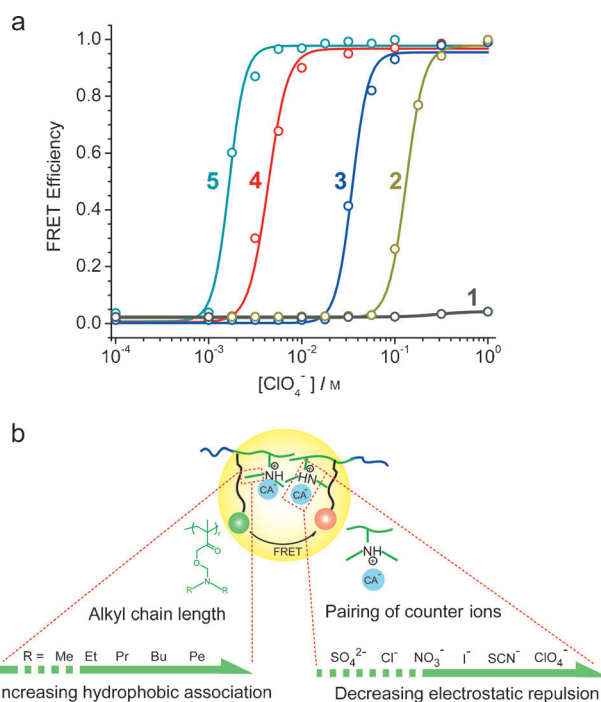
Figure 5. a) The hydrophobic strength of the PR segment affects the ability of ClO_4^- to induce micellization. The more hydrophobic PR segment (e.g. pentyl groups in **5**) increases the ClO_4^- sensitivity to induce micelle formation. b) An empirical model depicting two important contributing factors (length of hydrophobic alkyl chain and chaotropic anions) on the self-assembly of ionic polymeric micelles.

chains (i.e. methyl in **1**), no micellization was observed, even at the highest ClO_4^- concentrations (1M). In contrast, the most hydrophobic side chains (pentyl in **5**) resulted in the most sensitive induction of micellization by ClO_4^- anions. The FC_{50} values were 2, 4, 35, 134 mM when the side chains were pentyl, butyl, propyl, and ethyl groups, respectively (Figure 5a).

The results from this study illustrate a highly unusual micelle self-assembly process of block copolymers with tertiary ammonium groups induced by chaotropic anions. The current nanosystem is characterized by several unique features: first, chaotropic anions were able to form stable ion pairs with positively charged ammonium groups in the hydrophobic micelle core environment. Assuming that the majority of the ammonium groups are present in the ionized state, this translates into approximately 60 000 ion pairs per micelle with an estimated core size of 14 nm (calculation based on 800 polymer chains per micelle,[8b] 70–80 repeating units of monomers containing amino groups per polymer chain, and a PEO shell size of 6 nm[15]). Second, only chaotropic anions were able to induce micelle formation, whereas the kosmotropic (SO_4^{2-}) and borderline (Cl^-) anions did not pertain this ability. This trend appears to be contrary to that observed in classical protein solubilization studies. Third, the ability of chaotropic anions to induce micellization appears to show positive cooperativity similar to an ultra-pH-sensitive response. Our previous study showed fluorescence activation (10% to 90% response) that occurred within a pH value change of 0.25 units ($<$ 2-fold in $[H^+]$). The current

study shows that the FRET transfer was induced by a three-fold increase of $[ClO_4^-]$. Last, competition experiments with kosmotropic and borderline anions illustrated a bell-curve behavior, which points to the complexity and subtle nature of the micelle self-assembly process in the current system.

We built an empirical model (Figure 5b) to depict the factors that contribute to the micelle self-assembly process. We hypothesize that the hydrophobic interactions from alkyl chains of increasing lengths provide the dominant driving force for micelle formation. This is supported by the lack of micelle formation when the side chain of the tertiary amines is a methyl group (as indicated by the dashed line on the left side of Figure 5b). Similarly, neutralized copolymer 1 did not form micelles at pH values above its pH_t.[2d] Meanwhile, anions also play a critical role in micellization. Kosmotropic anions, which are known to have strong hydration shells and weak polarization characteristics,[16] are energetically less favorable in the formation of ion pairs[17] and stabilization of ion pairs in the hydrophobic core. Chaotropic anions, with their strong polarizability and low energy cost at removing the hydration sheath,[18] allows formation of stable ion pairs in the hydrophobic micelle core. Further studies are necessary to elucidate the thermodynamic contributions in enthalpy and entropy to the overall free energy of micelle phase transitions by the chaotropic anions.

In conclusion, we report a surprising micelle self-assembly process enabled by chaotropic anions with block copolymers containing hydrophobic, cationic ammonium groups. Unlike conventional micelles with simple hydrophobic cores, the current ionic micelles contain a large number of ion pairs in the core environment. The resulting micelles provide a good model system to study the fundamental process of supramolecular self-assembly through the interplay of noncovalent forces (e.g. electrostatic, van der Waals, and hydrophobic interactions) in aqueous environments. From the application standpoint, results from this study may also open up new opportunities to tailor micelle systems with stabilized ion pairs for the delivery of charged drug molecules.

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