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

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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 (~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, where only a 3-fold change in ClO_4^- concentration was necessary for micelle formation, similar to our previously reported ultra-pH responsive behaviour. This unique ion pair-containing micelle provides a useful model system to study the complex interplay of non-covalent interactions (e.g. electrostatic, van der Waals and hydrophobic forces) during micelle self-assembly. This may lead to new fundamental insights and strategies for the future design of cooperative systems in molecular sensing and drug delivery applications.

Keywords

micelle self-assembly; anti-Hofmeister effect; amphiphilic block copolymers; ion pairs

Responsive materials have received considerable attention for the construction of nanosystems that allow for 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,^[2] enzymatic expression,^[3] redox,^[4] temperature,^[5] and light^[6] have been successfully reported. 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 non-covalent interactions (e.g. electrostatic and hydrophobic interactions, hydrogen bonds, etc.) to achieve a thermodynamically stable nanostructure.^[7a, 7c] This strategy has the advantage of reaching sizes (10^4 – 10^{10} Da) that are not easily achievable by covalent chemistry, and the

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resulting system often displays positive cooperativity compared to single molecular behaviours 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, 2e, 8] At pH 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 transitions, where the fluorescence activation (on/off states) is narrowed to <0.25 pH unit, compared to 2 pH units for small molecular pH sensors.

In this study, we report a serendipitous discovery of chaotropic anion-induced micellization of protonated PEO-*b*-PR copolymers at pH below pH_t (right panel in Figure 1). Surprisingly, an anti-Hofmeister trend was observed, where chaotropic anions resulted in micellization but not the kosmotropic anions^[10], in contrary to their effects in protein aggregation (Figure 2a).

We first established a fluorescence energy resonance transfer (FRET) method to investigate the micelle self-assembly process. FRET is highly sensitive in detecting conformational and phase transitions of polymers/proteins 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, Table S1)^[12] with either a donor or acceptor dye. We chose PEO-*b*-poly(dipropylaminoethyl 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 due to the large distance between the unimers (therefore TMR and Cy5) in solution. 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), 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 contrary, 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).

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 where chaotropic anions were able to induce unimer association (i.e. micellization) whereas the kosmotropic anions were not (Figure 2c). This is in contrary to the classical Hofmeister effect in protein

solubilisation, where 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 FRET sensitivity followed the order of $\text{ClO}_4^- > \text{SCN}^- > \text{I}^- > \text{NO}_3^-$. We define FC_{50} as the anion concentration that 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 weak FRET effect was observed at its saturation concentration (~ 3 M). More detailed examination shows that only 3-fold ClO_4^- concentration change (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, 2e, 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 anion (Cl^-) as a negative control. In the presence of 50 mM Cl^- , copolymer **3** stayed as a unimer 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). DLS analyses showed increase of hydrodynamic diameters from 7 ± 2 to 26 ± 3 nm when the anions were changed from Cl^- to ClO_4^- , respectively (Figure 3). This size increase reflects the transition of copolymer **3** from 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 at 27 ± 2 and 28 ± 3 nm in the presence of Cl^- and ClO_4^- anions, respectively (Figures S3–S4). For non-ionizable amphiphilic block copolymers such as PEO-*b*-poly(D,L-lactic acid) (PEO-*b*-PLA), neither pH change nor ClO_4^- addition 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^- . Then chaotropic anions ClO_4^- were added to induce micellization (Figures S6–S9). Figure 4a shows the representative example 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 (< 0.1 M), 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 (> 0.5 M) 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 hydrophobic strength of PR segment on chaotropic anion-induced micelleization (Figure 5a). We synthesized a series of PEO-*b*-PR copolymers (**1–5** in Figure 1 inset) bearing different alkyl chain 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 chains (i.e. methyl in **1**), no micellization was observed even at the highest ClO_4^- concentrations (1 M). In contrary, the most hydrophobic side chains (pentyl in **5**) resulted in the most sensitive micellization induction by ClO_4^- . The FC_{50} values were 2, 4, 35, 134 mM when the side chains were pentyl, butyl, propyl and ethyl groups, respectively (Figure 5a).

Results from the above studies illustrate a highly unusual micelle self-assembly process from block copolymers with tertiary ammonium groups induced by chaotropic anions. There are several unique features in the current nanosystem: first, chaotropic anions were able to form stable ion pairs with positively charged ammonium groups in the hydrophobic micelle core environment. Assuming majority of the ammonium groups are in the ionized state, this translates into ~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 amino group-containing monomers per polymer chain and 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 counter that in classical protein solubilization studies. Third, the ability of chaotropic anions to induce micellization appears to show positive cooperativity similar to ultra-pH sensitive response. Our previous study showed fluorescence activation (10% to 90% response) occurred within 0.25 pH unit (< 2 -fold in $[\text{H}^+]$). Current study show FRET transfer happened in a span of 3-fold $[\text{ClO}_4^-]$ change. Lastly, 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 increasing alkyl chain 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 methyl group (as indicated by the dashed line on the left arm of Figure 5b). Similarly, neutralized copolymer **1** did not form micelles at pH 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 hydration sheath^[18], allows for 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 non-covalent forces (e.g. electrostatic, van der Waals and hydrophobic interactions) in the aqueous environment. From the application standpoint, results from this

study may also open up new opportunities of using stabilized ion-pair interactions for the delivery of charged drug molecules using tailored micelle systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Self-assembly of ionizable polymeric micelles by two independent mechanisms. The left panel shows the induction of micellization by pH increase, where the PR segments become neutralized and hydrophobic to drive micelle formation. Surprisingly, addition of chaotropic ions (CA, such as ClO_4^-) at low pH 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.

Figure 2.

(a) Chaotropic anions induce micelle self-assembly from PEO-*b*-PR copolymers with protonated PR segment, a reversed “salt-out” effect from their abilities 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.

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.

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 ionic strength of competing Cl^- and SO_4^{2-} anions. The solution pH was controlled at 4 in these studies.

Figure 5.

(a) Hydrophobic strength of PR segment affects the ability of ClO_4^- in micelle induction. 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 (hydrophobic alkyl chain length and chaotropic anions) on the self-assembly of ionic polymeric micelles.