Polyion Complex Micelles with Core–Shell Structure: Their Physicochemical Properties and Utilities as Functional Materials

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SUMMARY: Polyion complex (PIC) micelles were found to form from mixtures of a charged block copolymer with oppositely charged compounds including synthetic ionomers, surfactants, enzymes and DNA. This paper highlights unique physicochemical properties of PIC micelles, including their extremely narrow distribution and the strict chain length recognition. The features of PIC micelles made from a pair of oppositely charged block copolymers and enzyme were described focusing their utilities as functional materials including vehicles for enzyme delivery and sensoring devices for diagnosis.

Introduction

Multimolecular association of block copolymers in selective solvents, leading to block copolymer micelles, is one of the substantial research topics in the field of supramolecular chemistry. It has been studied extensively so far by many research groups not only for fundamental aspects[1-11] but also from the viewpoint of specific applications. The latter include drug delivery systems[12-17], separation technology[18-20] and surface modification[21,22]. In these applications, the core–shell architecture of block copolymer micelles in a mesoscopic size range (10–100 nm) often plays an important role, including the steric stabilization propensity of the shell and the reservoiring ability of the core for various substances. Indeed, a stable micelle system of amphiphilic block copolymers with a core charged with an anti-cancer drug (doxorubicin) was developed to demonstrate its excellent utility in targeting therapy of solid tumors[15].

Block copolymer micelles in aqueous medium are sterically stabilized by the shell of hydrophilic blocks surrounding the core of water-incompatible blocks to prevent them from aggregation. The most extensively studied block copolymer micelle system in aqueous

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medium is the one utilizing hydrophobic interaction as the driving force of the core segregation. Very recently, a new type of block copolymer micelles in aqueous medium has emerged based on an association force other than hydrophobic interaction: the formation of polyion complex (PIC) micelle through electrostatic interaction between a pair of oppositely charged block copolymers with poly(ethylene glycol) (PEG) segments[23,24]. PIC micelles were also found to form by mixing a charged block copolymer with a variety of oppositely charged compounds including vinyl polymers, surfactants, poly(amino acid)s, DNA and enzymes as summarized in Table 1.

Table 1. PIC micelle system formed from a charged block copolymer and various kinds of oppositely charged compounds

Combination	Ref.
Charged block copolymer / cationic polymer	
PEG-*block*-poly(α,β-aspartic acid) / PEG-*block*-poly(L-lysine)	23, 24
PEG-*block*-poly(α,β-aspartic acid) / poly(L-lysine)	25
PEG-*block*-poly(sodium methacrylate) / poly(l-ethyl-4-vinylpyridinium)	26
Charged block copolymer / surfactant	
PEG-*block*-polymethacrylate / cetylpyridinium, didodecyl(dimethyl)ammonium, dimethyl(dioctadecyl)ammonium or methyl(trioctyl)ammonium	27, 28
Charged block copolymer / biologically active compound	
PEG-*block*-polyspermine / oligonucleotide	29-31
PEG-*block*-poly(L-lysine) / oligonucleotide, DNA	32-34
PEG-*block*-poly[2-(dimethylamino)ethyl methacrylate)] / DNA	35
PHPMA[a]-*block*-poly[2-(trimethylammonio)ethyl methacrylate] / DNA	33
PEG-*block*-poly(α,β-aspartic acid) / egg white lysozyme	36

[a] poly[*N*-(2-hydroxypropyl)methacrylamide]

Worth mentioning is that PIC micelles entrapping biologically active compounds, including enzyme and DNA, have potential utilities in the field of drug delivery and biomedical engineering.

We wish to deal here with physicochemical properties of PIC micelles formed by mixing a charged block copolymer with synthetic polymers or enzyme with a special focus on the

utilities of these PIC micelles as functional materials such as in a nanometric-scale intelligent enzymatic reactor.

PIC micelle system formed from charged block copolymer and synthetic polymers[23-25)]

It is well known that an aqueous mixture of a pair of oppositely charged polyelectrolytes immediately forms a visible aggregate, e.g. a precipitate or coacervate, under electrically neutral conditions. In a sharp contrast with this well-known phenomenon, a mixture of two oppositely charged block copolymers (block ionomers) with PEG segments exhibits no precipitation, even at an electrically neutral mixing ratio, and keeps permanent transparency. Mixing PEG-*block*-poly(α,β-aspartic acid) [PEG–P(Asp)] with PEG-*block*-poly(L-lysine) [PEG–P(Lys)] in the 1 : 1 mole ratio of charged units results in the formation of associates with a size of several tens of nanometers as confirmed by dynamic light scattering (DLS) measurements. The size of the formed associates, which are now called polyion complex (PIC) micelles, is constant in a wide range of concentrations, up to 1.0 wt%. The zeta potential of PIC micelles has a very small absolute value, suggesting that PEG blocks effectively stabilize the PIC core by forming a hydrophilic environment. It was indicated that the water-insoluble PIC of P(Asp) and P(Lys) segments was sharply segregated as a core from aqueous medium by a shell composed of hydrophilic PEG segments. Extremely narrow size distribution was another interesting characteristic of PIC micelles made from charged block copolymers.

The preparation of PIC micelles is simple and reproducible, mere mixing of aqueous solutions containing oppositely charged components. It should be noted that in the case of a block copolymer micelle system in a selective solvent, the exchange of a good solvent for both blocks for a selective solvent by dialysis or dropwise addition of a selective solvent is needed to prepare the micelles[8)]. The involvement of the kinetically-driven process of solvent exchange often results in the ambiguity of the obtained micelles in terms of size and polydispersity, especially in the case of kinetically-frozen core segregation observed in the micellization of block copolymers with glassy segments. PIC micelle formation proceeds simply, in a single solvent (buffer) with a constant thermodynamic property. Consequently, the whole process of PIC micellization is thermodynamically regulated to give a monodisperse micelle with a constant size.

PIC micelles were compared in polydispersity with polystyrene latex and natural viruses on the basis of the cumulative analysis of dynamic light scattering. As shown in Table 2, PIC micelles had size distribution as narrow as naturally occurring supramolecular assemblies, viruses.

Table 2. Polydispersity of PIC micelles, polystyrene latex and natural viruses

	Average diameter[a)]	Polydispersity index (μ_2/Γ^{-2})[a)]
PIC micelles		
PEG–P(Asp) / PEG–P(Lys)	30 – 40 nm[b)]	0.03 – 0.05[b)]
PEG–P(Asp) / P(Lys)	50 nm	0.01 – 0.05[b)]
Polystyrene latex	285 nm	0.12
Natural viruses		
sendai virus	292 nm	0.08
λ phage	79 nm	0.06

[a)] Obtained by cumulative analysis of dynamic light scattering.
[b)] Depends on the composition of used polymers.

This narrow size distribution was also confirmed by atomic force microscopy. Monodisperse particles, ca. 40 nm in diameter, on mica in distilled water can be clearly observed (Fig. 1).

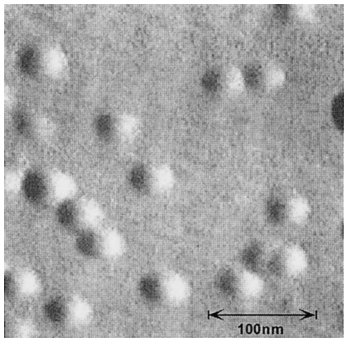

Fig. 1: AFM view of PIC micelles on mica formed from PEG (MW 5000) - P(Asp) (DP 78) and P(Lys) (DP 45) in distilled water

A unique phenomenon, chain length recognition, was found to occur in the process of PIC micelle formation from PEG–P(Asp) and PEG–P(Lys). Adding PEG–P(Lys) to PEG–P(Asp), having matched or unmatched chain lengths of P(Asp) segments and P(Lys) segments of PEG–P(Lys), the two copolymers selectively formed PIC micelles having matched chain length of charged segments, leaving PEG–P(Asp) with unmatched segment free. It was suggested on the basis of light scattering measurements that the key determining factor in this recognition process is the strict phase separation between the PEG shell and PIC core domains, requiring regular alignment of junctions between PEG and charged segments at the

interface of the two domains. This type of chain length recognition is a novel example in the molecular recognition system using an assembly of coiled block copolymers.

Formation of PIC micelles entrapping enzyme in the core[36,37)]

PIC micelles were also formed from a mixture of a charged block copolymer with biologically active molecules including enzymes and DNA. The formation of PIC micelles entrapping enzyme molecules might be interesting not only from fundamental viewpoints but also for development of functional materials. Egg white lysozyme as a cationic enzyme was selected as a partner of anionic PEG–P(Asp) to form PIC micelles.

The relation between the mixing ratio (r, the ratio of the number of aspartic acid residues in PEG–P(Asp) to the total number of lysine and arginine residues in lysozyme) and physicochemical properties including the association number, core radius and shell thickness were evaluated using light scattering techniques. The block copolymer used in this study contained a PEG block of M_w 12 000 and polymerization degree of the P(Asp) block was 15. The stoichiometric mixing ratio r was 1.00. The micelle formation proceeded in cooperative manner at $r < 1.00$, and at $r = 1.00$ (stoichiometric condition), all the lysozymes and PEG–P(Asp) in the system participated in the PIC micelle formation. Thus, at $r < 1.00$, all the PEG-P(Asp) in solution forms a stoichiometric micelle ($r = 1.00$) with lysozyme (average diameter ca. 50 nm), leaving excess lysozyme in solution in the free form. On the other hand, the micellization had non-cooperative characteristics at $r > 1.00$ and PIC micelles in this region contained excess PEG–P(Asp), yet keeping a narrow size distribution with their average diameter increasing linearly from 50 to 80 nm with increasing r values. Even in this region of $r > 1.00$, while showing an increase in average diameter, the core radius kept a constant value (7 nm), and only the shell thickness increased from 16 to 27 nm (Fig. 2). The association number of PEG–P(Asp) also increased at $r > 1.00$ with increasing r values, although the number of lysozyme molecules in the core was calculated to be a constant (ca. 50) independent of the r values.

Assuming PIC micelles to have a single core surrounded by a shell of tethered PEG chains, the distance from the core center to the interface between the core and corona (core radius) should be mainly determined by the chain length of the P(Asp) segment. Consequently, unless P(Asp) segments undergo a substantial change in their conformation, the core radius should be independent of r, which is in line with the results shown in Fig. 2. An increase in

the shell thickness with increasing r can be explained by an increase in the shell density with increasing association number imparting to tethered PEG more elongated conformation.

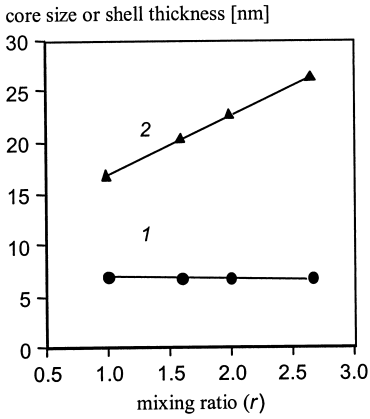

Fig. 2: Change in the core radius (*1*) and the shell thickness (*2*) with the mixing ratio for PIC micelles formed from lysozyme and PEG-P(Asp).
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Utilities of enzyme-entrapping PIC micelles entrapping enzymes as functional materials[38,39)]

Enzymatic activity of micelle-incorporated lysozyme was evaluated using two types of substrates, *Micrococcus luteus* cells and 4-nitrophenyl-penta-*N*-acetyl-β-chitopentaoside (NP–$(GlcNAc)_5$). Using *Micrococcus luteus* cells as substrate, the micelle-entrapped lysozyme showed no enzymatic activity. The direct interaction of cell with lysozyme was totally inhibited by the PEG layer surrounding the lysozyme core of the micelle. On the other hand, when NP–$(GlcNAc)_5$ was used as substrate, lysozyme showed appreciable enzymatic activity. This is because NP–$(GlcNAc)_5$ can diffuse into the core of PIC micelles, undergoing hydrolysis by lysozyme in the core. It is worth noticing that the micelle-incorporated lysozyme showed twice higher apparent enzymatic activity than free lysozyme as summarized in Table 3.

Table 3. The apparent enzymatic activity of micelle-incorporated lysozyme at various mixing ratios (r) [a)]

	1.000	1.600	2.000	2.667
Apparent activity[a)]	1.68	1.87	1.96	2.06

[a)] The ratio of the initial reaction rate of the micelle-incorporated lysozyme to that of free lysozyme.

The kinetic constants, maximum velocity (V_{max}) and Michaelis constant (K_m), for hydrolysis of NP–$(GlcNAc)_5$ by lysozyme were then determined to get insight into the enhanced

enzymatic reaction of lysozyme through the incorporation into the micelle. By entrapping lysozyme in the core of PIC micelles, the K_m values drastically decreased while the V_{max} values changed only slightly. Thus, an increase in apparent enzymatic activity might be related to the apparent change in K_m values. It should be noted that CD spectra were identical for micelle-incorporated and free lysozyme, indicating that the incorporation into the micelle core induced no detectable change in the secondary structure of lysozyme. Consequently, it is unlikely that a considerable change in the K_m value through micellization is derived from an essential change in the kinetic constants. A possible reason for a decreased K_m values in PIC micelles is the substrate condensation in the micelle. The partition coefficient (K_p) of NP–$(GlcNAc)_5$ in the micelle phase was calculated in order to evaluate the condensation effect. Figure 3 shows the relationship between the mixing ratio (r) and the partition coefficient (K_p).

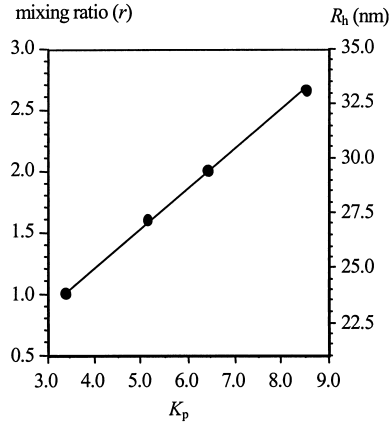

Fig. 3: Change in the partition coefficient (K_p) of NP-$(GlcNAc)_5$ in the micelles with the mixing ratio (r) or hydrodynamic ratio (R_h)

Apparently, the K_p values strongly correlate with the r values. As mentioned, the hydrodynamic radius of the micelle also depends on the mixing ratio r. Thus, as clearly demonstrated in Fig. 3, the K_p values are a function of the average size of micelles and can be controlled by changing the mixing ratio r. Such control of enzymatic reaction through the formation of supramolecular assembly might provide a new approach in designing novel nano-fabricated enzymatic reactors.

Conclusion

This paper briefly described physicochemical properties and biochemical functions of PIC micelles of charged block copolymers with oppositely charged natural and synthetic polyelectrolytes. In the formation of PIC micelles, the length of the charged segment in the

block copolymer plays a key role, physicochemical properties including the association number and core size of the micelle strongly depending on it. Activity of enzymes was shown to be controlled through entrapment into the core of PIC micelles. The micelles entrapping enzyme in the core might be useful as vehicles in enzyme therapy and novel formulation of diagnostics.

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