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Fluorescence and biological stabilization of phosphorous-functionalized mesoporous silica nanospheres modified with a bis(8-hydroxyquinoline) Zn complex

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Greenish-emitting phosphorous-functionalized mesoporous silica (PMPS) nanospheres were fabricated by modifying their surfaces with (8-hydroxyquinoline) zinc (Znq₂). Simulated body fluid soaking test and subsequent gas-adsorption measurement revealed that Znq₂-modification could dramatically suppress the biodegradation of the nanospheres. This study establishes Znq₂ as a novel and potential surface modifier of mesoporous silicas and demonstrates that effective surface design of the hosts will allow to exploit potential functionalities of the modifier.

Ordered porous materials such as metal organic frameworks (MOFs), zeolites, and mesoporous silicas (MPSs) have been widely investigated as unique hosts that can incorporate organic molecules and inorganic clusters into well-defined nanospaces [1]. Particularly, MPSs are promising nanoporous hosts with a large surface area and a uniform and adjustable pore structure, allowing facile modification on their internal and external surfaces with metal ions and functional molecules [2]. They have been utilized for biomedical applications [3], catalytic processes [4], and adsorption-based applications [5].

Metal 8-hydroxyquinolinolate complexes such as tris(8-hydroxyquinoline)aluminum (Alq₃) and their related derivatives are known to be typical optoelectronic building blocks [6]. This class of molecules effectively interacts with nanoporous materials and is easily immobilized on their surfaces through chemical adsorption [7]. It is worth noting that molecular assembly in such small nanospaces restricts the conformation of the guest molecule and induces unique guest–guest and/or host–guest interactions. This, in turn, leads to the emergence of optoelectronic properties that are not exhibited in solutions and bulk crystals. For instance, Fazaeli et al. demonstrated that compared to the case of bulk α-Alq₃ crystal, covalent grafting of Alq₃ and its substituent molecules on the MPS surface resulted in a significant blue shift of the fluorescence due to changes in the coordination environment of the Al³⁺ center [8]. Similarly, Du et al. successfully synthesized (8-hydroxyquinoline) zinc (Znq₂)-modified MPS and observed a significant blue shift of its greenish fluorescence when the host surface was functionalized with mercapto and sulfonic acid groups [9]. These studies suggest that the optical properties of Alq₃ and Znq₂ can be modulated through the assembly in the nanospaces of MPSs and through their interface functionalization.

In this study, we have prepared phosphorous-containing MPS (PMPS) nanospheres using diethyl(2-bromoethyl)phosphonate as a phosphorous source [10]. The mesoporous channels of PMPS were modified with Znq₂. Adsorption experiments revealed that Znq₂ was more favorably adsorbed on the PMPS surface than the case on the MPS surface. Interestingly, Znq₂ modification of the PMPS surface efficiently prevented its anionic component dissolution from the surface of the mesopores into the simulated body fluid (SBF). Additionally, it was revealed that Znq₂ could be utilized as a surface modifier that could induce fluorescence as well as biological stability as the host functionalities.
The MPS and PMPS nanospheres were synthesized according to our previously reported method [10]. For synthesizing PMPS, the phosphorous to silica (P/Si) molar ratio was set to 6 (i.e., P/Si = 6) by adjusting the amounts of the silicate and phosphorous sources. Adsorption of Znq₂ into the prepared nanospheres was induced by admixing the nanospheres and an ethanol solution of Znq₂ of several initial concentrations (n) ranging from 0.15 to 0.75 mM. The resulting particles were washed with ultrapure water and dried under reduced pressure for 24 h. The Znq₂-modified MPS and PMPS were designated as nZ/MPS and nZ/PMPS, respectively, where n indicates the concentration of Znq₂ used for the chemisorption experiments. For the synthesis of PMPS, the phosphorous source diethyl(2-bromoethyl)phosphonate was hydrolyzed together with the hydrolysis reaction of diethylamine. The hydrolyzed phosphorous source, C₄H₇BrO₂P, interacts with the surface of the MPS precursor via the hydrogen bonding of the hydroxyl groups, followed by Si-O-P bond formation via the dehydrated condensation between their hydroxyl groups. Subsequent calcination led to decomposition of the bromoethyl moiety of the phosphorous source [11], and thus, the phosphate group was retained on the surface of the silicate framework. As discussed later, these functional groups strongly enhanced Znq₂ adsorption on the PMPS surface. Details of the synthesis procedures are described in the ESI.

Fourier transform infrared (FT-IR) spectroscopy of MPS and PMPS was performed to ascertain if phosphorous was introduced into the polysiloxane covalent framework. Figure 1 shows the FT-IR spectra of MPS and PMPS in the range of the siliceous bonding region. Results of the peak deconvolution analysis are also shown in this figure. Total difference between the calculated and observed spectra was 5.0% for MPS and 6.3% for PMPS, suggesting that sum of the deconvoluted peaks well reproduced the observed data. The spectra of MPS (Fig. 1a) shows three shoulder-like peaks at 1204, 1092, and 969 cm⁻¹, and the peak deconvolution analysis revealed that these peaks are assignable to a couple of Si-O-Si asymmetric and symmetric stretching vibrations and Si-OH stretching vibrations [12]. On the other hand, in the case of PMPS (Fig. 1b), a new hump was observed at 1174 cm⁻¹, corresponding to the Si-O-P stretching vibration [13]. This indicated that a significant amount of phosphorous was successfully introduced into PMPS.

The prepared MPS and PMPS were modified with Znq₂ through chemisorption, and their gas-adsorption property was examined using N₂ adsorption/desorption experiments. The adsorption/desorption isotherms suggested apparently different isotherm characteristics of nZ/MPS and nZ/PMPS (Fig. S1). For all n values, nZ/MPS exhibited a type IV isotherm [Fig. S1a-e] according to the IUPAC classification [14], and their hysteresis loops exhibited complete reversibility. This suggested that nZ/MPS had cylindrical mesopores that were smaller than ≈4 nm [15]. Indeed, low-angle X-ray diffraction (PXRD) measurement suggested that the wall thickness was approximately 3 nm for nZ/MPS (Fig. S2a). In contrast, the adsorption/desorption isotherms of nZ/PMPS (Fig. S1f-j) showed characteristic desorption shoulders with lower closure points at 0.42P₀, additionally, a plateau at high P/P₀ was absent. These features suggested H₃-type hysteresis [15], and possibly, an assembly of slit-shaped mesopores for nZ/PMPS. The PXRD profiles of nZ/PMPS indicated that the wall thickness of the slit-shaped mesopores was ≈4 nm (Fig. S2b).

Surface area analyses of nZ/MPS and nZ/PMPS (Fig. 2a) indicate that the latter had a smaller Brunauer-Emmett-Teller (BET) surface area (SBET) [16] than the former due to the disordered mesopore distribution induced upon phosphorous incorporation into the polysiloxane framework [10]. Additionally, the SBET value decreased slightly with increase in the equilibrium adsorption concentration of Znq₂, suggesting that Znq₂ adsorption on the host surface closed a portion of their mesopore channels.

Figure 2. (a) Variation in the SBET values of nZ/MPS and nZ/PMPS and the equilibrium adsorption concentration measured in the chemisorption experiments. (b) Equilibrium adsorption isotherm of Znq₂ obtained by the chemisorption experiments of MPS and PMPS. Znq₂ was immobilized on the host surfaces by monolayered adsorption. (c) Scatchard plots of MPS and PMPS.

The amount of Znq₂ loaded in the hosts was determined from the change in the visible absorbance of the Znq₂ solution before and after the chemisorption experiment. The relation between the equilibrium concentration and the amount of
more pronounced in the samples with less loaded Znq₂ (Fig. 3a), it can be assumed that the interactions between Znq₂ and the host surface were responsible for the optical modulation.

As mentioned before, the phosphate groups on the PMPS surface play an important role during adsorption to ensure the efficient immobilization of Znq₂. It should also be noted that the strong host-guest interaction in PMPS is likely to affect the optical properties of Znq₂. Indeed, Figure 3b suggests that the emission of nZ/PMPS was centered at almost a fixed wavelength in the range 492–496 nm, while nZ/PMPS exhibited a red shift from 492 to 504 nm with increase in the amount of loaded Znq₂. The fluorescence maxima of metal hydroxyquinoline complexes vary depending on their conformation and aggregation states, and particularly, the red shift in fluorescence can be attributed to the overlapping of π orbitals of each quinoline ring [20]. Generally, the nanospaces in the hosts limit the conformational freedom of the guest and inhibit their aggregation [21]. On the other hand, for the case of nZ/MPMS, a red shift in fluorescence was observed with increasing concentration of loaded Znq₂, indicating local molecular aggregation of Znq₂ possibly during the drying process. In contrast, the nearly constant fluorescence of nZ/PMPS suggests the immobilization of the Znq₂ monomer. It is speculated that the PMPS surface mainly has two adsorption sites, i.e., the negatively charged silanol (Si-O−) groups and the protonated phosphate (O=P-OH) groups [22], and that Znq₂ interacts with these adsorption sites via electrostatic and hydrogen bonding interactions, respectively. We assume that the multi-point adsorption characteristic of the PMPS surface would inhibit the mobility and re-organization of Znq₂, thereby leading to fluorescence stabilization.

We examined the biological stability of the Znq₂-modified mesoporous hosts. For this experiment, we soaked MPS, PMPS, 0.75Z/MPS, and 0.75Z/PMPS in SBF. The fluorescence spectra of (b) 0.75Z/MPMS and (c) 0.75Z/PMPS before and after SBF soaking.

Figure 3. Fluorescence spectra of (a) nZ/MPS and (b) nZ/PMPS. The inset shows the fluorescence microscopic images for n = 0.75 (λex = 356 nm).

Figure 4. (a) Time evolution of SiO₂⁻ concentration after dissolution from MPS, PMPS, 0.75Z/MPS, and 0.75Z/PMPS in SBF. Fluorescence spectra of (b) 0.75Z/MPMS and (c) 0.75Z/PMPS before and after SBF soaking.

adsorbate in the hosts suggested that Znq₂ adsorption was consistent with Langmuir type I isotherm [17]. Thus, Znq₂ was immobilized in the nanospaces of the hosts via monolayer adsorption. Nevertheless, it must be noted that the surface of the mesoporous channels was not fully covered with Znq₂; rather the surface coverage was remarkably low (discussed below) because the molecular size of Znq₂ (~1.5 nm) [18] is comparable to the size of the nanospaces in the hosts. The Scatchard plot (Fig. 2c) indicates that the maximum amounts of Znq₂ (Wmax) loaded in MPS and PMPS were 0.087 and 0.069 mmol g⁻¹, respectively. The difference could be attributed to the higher S BET value of nZ/MPS. The plot also suggests that the adsorption equilibrium constants (K eq) for nZ/MPS and nZ/PMPS were 2.3 and 4.9 M⁻¹, respectively. This implies that Znq₂ was more strongly immobilized on the PMPS surface than on the MPS surface. The favorable Znq₂ immobilization on the PMPS surface was also confirmed by surface occupation analyses (Fig. S3). Here, the surface occupation of Znq₂ was estimated from the equilibrium adsorption amount of the adsorbate and the S BET values of the hosts. Consequently, the maximum surface occupation was calculated to be 2.2% for nZ/MPS and 3.4% for nZ/PMPS, implying a small but significant difference between their molecular immobilization capacities. The above results suggest that Znq₂ should be more efficiently immobilized on PMPS surface than on normal silicate surfaces.

Figure 3 shows the fluorescence spectra of nZ/MPS and nZ/PMPS upon excitation at 356 nm. A broad, greenish emission centered around 500 nm, which is characteristic Znq₂ fluorescence, was observed [19]. Compared to the fluorescence spectra of Znq₂ recorded in the solid state or in ethanol (Fig. S4), the spectra of nZ/MPS and nZ/PMPS exhibited apparently broadened emission and a large blue shift of more than 50 nm, clearly suggesting that the adsorption of the guest chromophore into the hosts could modulate its optical properties. Because the degree of the blue shift seemed to be...
degradation of silicate compounds. The size of the MPS and PMPS particles used in this experiment was 207 nm (cv. 9.1%) and 204 nm (cv. 8.6%), respectively [10]. Figure 4a shows the time evolution of the SiO$_2^-$ concentration in the SBF; it is evident that the degradation of the host was significantly dependent on phosphorous incorporation and Znq$_2$ modification. As seen in the figure, MPS shows rapid dissolution until 12 h, following which an equilibrium state is attained. The total amount of the SiO$_2^-$ anions dissolved in 48 h from MPS was 2.8 wt%. The relatively low degradation rate was attributed to the condensation of the polysiloxane networks upon calcination [23]. PMPS also rapidly released the SiO$_2^-$ anions for the first 12 h; however, a plateau was not observed even after 48 h. The total amount of the SiO$_2^-$ anions dissolved from PMPS was 4.2 wt%. Hence, PMPS is likely to be more prone to degradation in a biological environment. The rapid degradation of PMPS perhaps originates from the strong affinity between the PMPS surface and divalent metal cations. This hypothesis is rationalized by the fact that calcium phosphates were mineralized on the host surface after SBF soaking [10]; that is to say, adsorption of the Ca$^{2+}$ cations on the host surface should be a triggering factor for SiO$_2^-$ dissolution and subsequent anionic compensation with ambient HPO$_4^{2-}$. In this reaction, the oxygen atoms of the hydroxyl and phosphate groups are assumed to predominantly behave as the reaction sites due to their strong ability to coordinate with the metal cations [24]. Indeed, we already reported that the PMPS surface forms a hydration layer having strong affinity with hydrophilic protein molecules [25]. In addition, the functional groups on the PMPS surface are negatively charged at a neutral pH [25,26]. Hence, PMPS should have more active reaction sites with the metal cations than MPS, leading to the rapid degradation of the former host.

Figure 4a also shows the SiO$_2^-$ dissolution behavior of the Znq$_2$-modified hosts. It is evident that surface modification dramatically prevented the amount of dissolved SiO$_2^-$ anions, possibly because of the enhanced surface hydrophobicity [27] and covering of the adsorption sites. One may concern that the Znq$_2$ modification would deteriorate the solubility of MPS and PMPS in aqueous solutions. However, the Znq$_2$ modification minimally affected surface charge and solubility of the hosts, because Znq$_2$ is preferentially immobilized in the nanospaces of the inner core due to high density of the adsorptive groups in the nanospaces [28], and the surface state of the outer shell is substantially identical to unmodified ones. Since the total amount of the dissolved SiO$_2^-$ anions was 0.45 and 0.21 wt% for 0.75Z/MPMS and 0.75Z/PMPS, respectively, it can be concluded that the Znq$_2$ modification rather stabilized the PMPS surface. The remarkable improvement in the biological stability of 0.75Z/PMPS was also confirmed from the N$_2$ adsorption/desorption isotherms of the samples obtained after SBF soaking. The isotherms and their $S_{BET}$ values (Fig. S5) indicate that the adsorption properties of 0.75Z/PMPS did not change remarkably even after SBF soaking, which was in contrast to the significant decline of the adsorption volume observed for 0.75Z/MPSS. The mesopore stability was also reflected in their fluorescence spectra (Fig. 4b,c), as the fluorescence emission maximum of 0.75Z/PMPS remained unchanged. Thus, we conclude that the Znq$_2$ modification significantly improved the biological stability of the mesopores in PMPS because of the strong interface affinity between Znq$_2$ and the PMPS surface.

In summary, we demonstrate a new concept of using Znq$_2$ as a surface modifier that can induce greenish fluorescence and biological stability to MPSS. We particularly highlight that the incorporation of phosphorous to the SBF surface allows the immobilization of monomeric Znq$_2$ due to the strong affinity between Znq$_2$ and the phosphates groups, further improving the biological stability of the host. Although there are reports on the modification of Alq$_3$, Znq$_2$, and other complex molecules in the nanospaces of mesoporous hosts [8,9], the studies were mainly focused on the optical and catalytic properties of the hybrid alone. In contrast, the present study focused on how the guest modification altered the properties of the host surface and demonstrates how effective interface design might be utilized to exploit the guest functions as well. We believe that the present concept will be especially useful in the development of biostable fluorescence markers and nanomedicines.

Conflict of Interest
There are no conflicts to declare.

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