In situ fabrication of pyrene derivative nanorods inside polyelectrolytes microcapsules with tunable fluorescent properties†

Zhipeng Wang, Mengying Liu, Yang Xie and Changyou Gao*

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The novel micro–nano composite poly(sodium styrene sulfonate)/poly(allylamine hydrochloride) multilayer microcapsules containing in situ-formed 1-pyrenecarboxaldehyde nanorods were fabricated and characterized. Fluorescence properties and functions of the 1-pyrenecarboxaldehyde nanorods could be effectively manipulated by electron donor/acceptor interaction within the permeable polyelectrolyte shells. The microcapsule integrated with the nanorods may shed creative light on the design of novel micro–nano structures and materials for nanoscience, and biological and other advanced technologies.

The development of nanostructure materials has aroused great interest from broad areas, including physics, chemistry, biology and materials science. Their excellent properties and functions such as fluorescence, catalysis, quantum effect and bioactivity not only represent important academic research value but also allow the development of innovative industrial applications. However, the highly active nanomaterials easily lose their functions in application. For instance, the excellent fluorescence properties and quantum yield of quantum dots can decrease sharply if the nanoparticles aggregate due to their high surface energy. In order to protect nanomaterials against environmental influences, different kinds of technologies are being developed by covering their surfaces with surfactants, polymers, or biomacromolecules. In addition, the in situ fabrication of nanomaterials paves an alternative way to protect their functions from disadvantages of transfer and storage. For example, metal nanoparticles are easily fabricated in situ on the shells of hollow structures to form composite functional devices. Nanomaterials can also use the in situ self-assembly advantage to simplify the fabrication process and create innovative functions. In this study, we introduce a novel way to fabricate the 1-pyrene carboxaldehyde nanorods (Py-CHO NRs) in situ inside polyelectrolyte multilayer microcapsules (MCs).

Very recently, we discovered an intriguing phenomenon of protrusion of one-dimensional nanorods (NRs) from poly(allylamine hydrochloride) (PAH)-graft-pyrene (Py) microcapsules. The NRs are composed of Py-CHO NRs and can generally be obtained through π–π stacking self-assembly during hydrolysis of Schiff base bonds between PAH and Py-CHO in weak acid solution. Although the as-prepared Py-CHO NRs show excellent fluorescence property due to the intrinsic nature of Py, the NRs are difficult to isolate from the fabrication environment, and thereby hardly to realize the function as advanced fluorescent devices. To self-assemble the Py-CHO NRs in a restricted space such as inside the MCs would be a promising solution for this question.

The hollow multilayer microcapsules are well developed and have been used as microcarriers and microreactors for drugs and biomacromolecules. On the other hand, the PAH-Py MCs are obtained from a CaCO3 microparticle template method before protrusion of the Py-CHO NRs, as shown in our previous study. Integration of these techniques is then possible to obtain Py-CHO NRs inside the MCs, as shown in Scheme 1. Here, poly(sodium styrene sulfonate) (PSS) and PAH, a typical pair of polyanion and polycation, are alternately assembled on the PAH-Py doped CaCO3 particles before template removal.

First, the PAH-doped CaCO3 particles with a diameter of 9.5 ± 1.1 μm were suspended in Py-CHO ethanol solution, so that the Schiff base reaction between Py-CHO and PAH in/on the CaCO3 particles could proceed sufficiently. After removal of the excess Py-CHO, the PAH-Py doped CaCO3 particles were further covered stepwise with (PSS/PAH)12 multilayers, resulting in a smoother surface compared to the pristine one (Fig. S1†). Treatment by ethylenediaminetetraacetic acid (EDTA) solution yielded the PAH-Py/(PSS/PAH)12 double-shell MCs with a diameter of 9.7 ± 1.3 μm. They collapsed only in the middle parts due to the very thick shell structure, leading to a concave morphology (Fig. 1a) which is similar to that of the bare PAH-Py MCs. When the MCs suspension was incubated in pH 2 HCl solution, the protons could penetrate the polyelectrolyte multilayers and protonate the Schiff base bond and amino groups of the inner PAH-Py shell. Therefore, hydrolysis of the Schiff base was initiated, and was followed by self-assembly of the Py-CHO. The NRs were formed and entrapped within the polyelectrolyte multilayer MCs. Within 2 h dense Py-CHO NRs could be observed inside the (PSS/PAH)12 MCs (Fig. 1b). The polyelectrolyte multilayer MCs expanded to a larger size with an average diameter of 13.4 ± 2.5 μm after the NRs formation. This phenomenon was triggered by an osmotic pressure increase as a result of release of charged PAH molecules during destruction of the

MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou, 310027, China. E-mail: cygao@mail.hz.zj.cn; Fax: +86-571-87951108; Tel: +86-571-87951108
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hydrophobic crosslinking points, i.e. the cleavage of the Py molecules. The $z$ direction scanning by confocal laser scanning microscopy (CLSM) demonstrated that the Py-CHO NRs were dispersed in three dimensions inside the MCs (Fig. S2†). After drying, the Py-CHO NRs were condensed with strong fluorescence on the same focal plane (Fig. 1c) due to collapse of the polyelectrolyte multilayer MCs of thin shell thickness (typically the thickness of a PSS/PAH bilayer is about 3 nm). Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) characterization further substantiated the structure change of the MCs and NRs formation inside the MCs after acid treatment (Fig. 1d,e). The packed Py-CHO NRs were covered by thin shells of MCs, indicating the decomposition of the inner PAH-Py shells and reduction of shell thickness. The magnified SEM image showed the completeness of the polyelectrolyte shell without noticeable holes and cracks (Fig. 1f). Moreover, no NRs could be found outside the MCs. Therefore, one can safely conclude that the Py-CHO NRs are assembled and kept inside the polyelectrolyte multilayer MCs.

The growth process of the Py-CHO NRs within the (PSS/PAH)$_{12}$ MCs was monitored by CLSM when the MCs were incubated in pH 2 HCl solution (Fig. 2). The Py moiety emitted average but relatively weak fluorescence due to the quenching effect of protonation of Schiff base in PAH-Py at the beginning of acid treatment (0 min). After 10 min, some green spots appeared close to the periphery of the inner PAH-Py shells and reduction of shell thickness. The magnified SEM image showed the completeness of the polyelectrolyte shell without noticeable holes and cracks (Fig. 1f). Moreover, no NRs could be found outside the MCs. Therefore, one can safely conclude that the Py-CHO NRs are assembled and kept inside the polyelectrolyte multilayer MCs.

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MCs, indicating the budding of NRs (Fig. 2b). With incubation time prolongation, more and more NRs grew inside the MCs (Fig. 2c–e), and eventually filled the whole MCs (Fig. 2f). During this process, no NRs were found protruding outside the MC shells, indicating the restriction effect of the polyelectrolyte multilayers under these conditions.

Now that the PAH-Py NRs could be fabricated and stored inside the MCs, they were also easily released by destruction of the MCs with ultrasonication, an often-used method to break the polyelectrolyte multilayer MCs. The released NRs (Fig. S3a–c†) had a similar morphology and dimensions (~8 μm in length and ~150 nm in diameter) (Fig. S3c†) to those fabricated directly from the solution (Fig. S3d, length of NRs 7–10 μm). The FTIR spectrum (Fig. S4†) shows typical peaks at 3039 cm⁻¹ (C–H in pyrene), 1680 cm⁻¹ (C=O in aldehyde), 840 cm⁻¹ and 711 cm⁻¹ (C=C in pyrene), confirming that the NRs are composed of only Py-CHO and not PAH. The strong fluorescence of the NRs was well maintained at 508 nm before and after release from the MCs (Fig. S5†).

It was then considered how the Py-CHO NRs would grow if spatial restriction came into play. To clarify this question, the PAH-Py/(PSS/PAH)n double-shell MCs with diameters of 5.5 ± 0.7 μm and 10.2 ± 1.1 μm and variable bilayer numbers of 4, 8, and 12 were prepared. In the 10.2 μm MCs with 8 bilayers of PSS/PAH, all the NRs were trapped inside the MCs (Fig. 3a). By contrast, in the 5.5 μm MCs of the same bilayer number, some of the NRs pierced through the MC shells (Fig. 3b). Fluorescence microscopy images (Fig. S6†) show further that this phenomenon could be applied to MCs of other bilayer numbers too. In order to quantify the Py-CHO percentage entrapped inside the MCs under different conditions, all the samples were washed by water to remove the NRs in the suspension and then ruptured by ultrasonication. The released NRs were dissolved by ethanol to quantify their amount using UV-vis spectroscopy. Fig. 3c shows that the entrapped NRs amount inside the 5.5 μm MCs was increased from 53.5% to 82.2% when the multilayer bilayer numbers increased from 4 to 12. However, the amount of NRs inside the 10.2 μm MCs (over 90%) was independent on the bilayer numbers. It is worth mentioning that the length of the NRs remained at ~7–10 μm under different conditions. Therefore, one can conclude that growth of the NRs on the capsule surface may also happen, and the growing force of the NRs is large enough to penetrate the MC with a thinner shell. Nevertheless, the covering PSS/PAH multilayers can function as a physical barrier that restricts outward growth of the nanorods to some extent.

With the protection of polyelectrolyte multilayer MCs, the fluorescent property of Py-CHO NRs could be easily tuned due to the good permeability of the MC shells. It is known that the Py molecule is a typical electron donor, which can form a charge transfer interaction with an electron acceptor such as methyl viologen (MV, chemical structure shown in Fig. 4a) to quench the fluorescence emission intensity. When the diluted MV solution...
(0.25 mL × 4, 0.5 mM) was added to the suspension of MCs (1 mL, 10^7 microcapsules per mL) in aliquots every 1 min, the fluorescence emission peak at 508 nm decreased to 54% of original intensity after 4 min (Fig. 4b, black square, original spectra in Fig. S7†), revealing that the charge transfer complex of Py-CHO and MV is formed in the MCs. However, the half quenching suggests the MV molecule can only bind with those Py-CHO molecules on the NRs surface but not with those within the NRs. Moreover, the multilayer shells, especially after capturing the positively charged PAH released from the inner PAH-Py shells, may show a repelling effect to the MV molecules of same charge, reducing the chance of direct molecular interaction between MV and Py-CHO. After the solution was washed sufficiently with water, the fluorescence could hardly be recovered (Fig. 4b, green triangles), indicating the stable interaction between Py-CHO and MV. However, when the 1,3,6,8-pyrenetetraysulfonic acid tetrasodium salt (PyTs) molecules, which have stronger interaction with MV, were added in aliquots as electron donor (chemical structure in Fig. 4a, 0.25 mL × 4, 0.5 mM), the fluorescence emission intensity of Py-CHO NRs was recovered in a stepwise manner to 90% of original value eventually (Fig. 4b, red circles). The weakening and recovery of the fluorescence emission was directly observed by CLSM (Fig. 4c–e), which also confirmed that the structure of Py-CHO NRs was not damaged during the whole process.

In conclusion, PAH-Py/(PSS/PAH)_n double-shell MCs were prepared and Py-CHO NRs were assembled inside the (PSS/PAH)_n MCs in pH 2 HCl solution. The growing force of the NRs is large enough to penetrate the MCs, in particular those with a thinner shell, and their entrapped amount is mediated by the MC size and bilayer number. The fluorescence emission intensity of Py-CHO NRs could be tuned by a charge transfer pair of MV and PyTs molecules. This novel composite structure with PAH-Py NRs inside polyelectrolyte multilayer MCs provides a creative strategy for in situ nanomaterials fabrication, and demonstrates the trend for controllable properties and functions of smart nanodevices.

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