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Investigation on Ligand Exchange Kinetics on CdSe/ZnS Quantum Dot Surface Utilizing Pyrene as Flourescent Probe

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Abstract Direct ligand exchange kinetics between hydrophilic molecules and quantum dots(QDs) was investigated. Meanwhile, pyrene was exploited as probe to detect the efficiency of the ligand exchange reaction between octadecylamine-coated QDs(ODA-QDs) and different ligands[ligand **1**: NH_2G3-OH, ligand **2**: G4.5-PEG5-FA5, ligand **3**: $(COOH)_2$G3-OH or ligand **4**: G4.5-PEG1-FA1]. It was indicated that water-soluble QDs exhibit the same fluorescence and absorption spectra as ODA-QDs when they were dissolved in chloroform. Furthermore, the cellular experiments demonstrated that the folic acid(FA) targeting poly(amidoamine)(PAMAM) modified QD conjugates could be used as molecular targeting sensing systems for nanoparticle probes.

Keywords Fluorescent probe; Ligand exchange kinetic; Poly(amidoamine) dendrimer; Pyrene; Quantum dot

1 Introduction

Quantum dots(QDs) have attracted intense research interest in biomedical applications in the past two decades, due to their unique electronic and a very useful set of optical properties. QDs possess narrow and symmetrical emission, broad excitation range, high fluorescence quantum yield(QY) as compared to those upconversion nanocrystals and anti-photobleaching feature as compared to organic dyes. All these properties make QDs visualized and long-term tracked in biomedical processes, including drug delivery, bio-imaging and bioassays[1—4]. However, the fluorescence intensity of the QDs coated with hydrophobic molecules, such as thiol ligands and octadecylamine, will be decreased due to surface defect, making them unsuitable for physiological environments[5,6]. Biomedical applications require stable, water-soluble nanoparticles with a narrow distribution of particle sizes. Among various methods including SiO_2-coated, micelle-coated and ligand exchange methods for phase transfer from oil to water soluble, surface ligand exchange with appropriate capping ligand becomes one preferred strategy because of the rich functional ligands[7—9].

Multidentate hydrophilic molecules-branched polyethylenimine(PEI) and dendrimer/dendron[poly(amidoamine), PAMAM][10—13] are promising ligands due to their high avidity, high affinity and exceptional stability compared with those thiol ligands which are prone to aggregation. However, the ligand exchange kinetics or the efficiency of exchanging reaction between hydrophilic dendron ligands or dendrimer ligands and hydrophobic capping on the surface of QDs has not been reported yet.

Pyrene is a polarity-sensitive flourescent probe, which is widely used to measure the hydrophobicity of microenvironments, such as micelles[14,15]. The present researches have systematically examined the hydrophobicity of localized domains of QDs capped with hydrophilic dendron ligands or dendrimer ligands to detect the efficiency of exchange reaction between different hydrophilic ligands and hydrophobic octadecylamine (ODA) of QDs with pyrene as a reporting probe. Furthermore, the ligand exchange kinetics of other ligand has also been researched.

In this work, the binding kinetics of multivalent ligands on QDs was investigated. We prepared FA-PEG-PAMAM@QDs (FA=folic acid, PEG=polyethylene glycol) nanocomplexes as potential targeting nanoprobes for bio-imaging *in vivo* and the binding ability to FA-receptor was examined *in vitro*. The results indicated that these QDs conjugates would benefit to the development of other nanoparticle probes and encapsulation technologies.

2 Experimental

2.1 Synthesis of Ligands

Tetra-dendron PAMAM dendrimers were synthesized

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using a modified procedure that was first developed by Tomalia *et al*.[16,17]. Synthesis of tetra-dendron dendrimers was derived from ethylenediamine cores(ligands **2** and **4**), and synthesis of di-dendron dendrimers was derived from mono-Boc-protected ethylenediamine cores(ligands **1** and **3**). The generation of dendrimers was calculated from G0, rather than the usual sense of G1, in order to keep corresponce with generation of dendron. Four different ligands have been synthesized in this study and the detailed experiments are described in the Electronic Supplementary Material of this paper.

2.2 Kinetics and Efficiency of Ligand Exchange

Before ligand exchange, the free octadecylamine(ODA) of QDs was precipitated with acetone twice and redispersed in chloroform. The core-shell CdSe/ZnS QDs were obtained from Ocean NanoTech Company[18]. Briefly, 10 mL of purified ODA-QDs in chloroform solution[optical density(OD)=1.0, Shimadzu 2450 spectrophotometer], 10 mL of ligand **1** as an example dissolved in methanol solution(10 mg/mL) and 30 μL of tetramethylammonium hydroxide(TMAH) were added to a 50 mL vial, the mixture was shaken at 40 °C in oil bath. For ligand exchange kinetics study, 2 mL of the above mixture was taken out at different reaction time and transferred into another vial containing 50 mL of ethyl acetate, after stirred for 30 min, 4 mL of purified water was added to the mixture, stirring was continued for 30 min. After the mixture was kept at room temperature for 1 h, orange-red QDs in the aqueous layer were found, at the same time, the organic layer became colorless.

Similarly, the kinetics of ligand exchange with ligands **2**, **3** and **4** were also investigated. The optical density of all the PAMAM-coated quantum dots named QDs1, QDs2, QDs3 and QDs4 were adjusted to 0.1 to ensure all the water-soluble QDs have the same concentration.

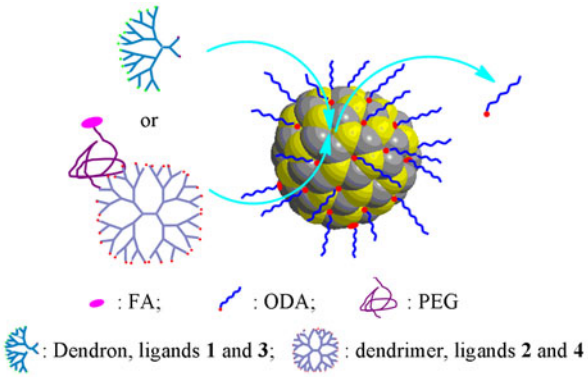
For efficiency measurement, 1 mL of 6×10^{-6} mol/L pyrene solution in acetone was added to the bottom of volumetric flask(10 mL), four different QDs were added to the flask until acetone was evaporated and the mixture was kept for 24 h. So that pyrene can reach a solubility balance in all the QDs solution. Finally, the fluorescence excitation and emission spectra were measured by a Shimadzu RF-5301PC fluorescence spectrometer.

2.3 Cellular Experiments

HeLa cells and SKOV3 cells were cultured in Dulbecco's minimum essential medium(DMEM) with 10%(volume ratio) fetal bovine serum(FBS) at 37 °C in CO_2 incubator. The cells were cultured until 80% confluence, then the cells were digested with 2.5 g/L trypsin and seeded in 6-well plates(Costar, USA) with a cell density of 5×10^4 cell/well. After 24 h, the medium was removed and replaced with fresh medium. QDs conjugates(non-FA:QDs1 and FA:QDs4) were added to each well and incubated with the cells for 2 h. Fluorescence imaging was performed with a spinning disk confocal microscope(Ultraview, Perkin-Elmer) with 380 nm laser excitation and CCD camera(ORCA-ER, Hamamatsu).

3 Results and Discussion

Four ligands used in this study have been synthesized. Functional groups of the ligands, such as amino groups(for ligands **1**, **2** and **4**) and carboxyl groups(for ligand **3**), have stronger binding affinity to cation on the QD surface than the original octadecylamine(ODA) ligand. Thus, stable and water-soluble QDs can be obtained *via* ligand exchange between hydrophilic dendron ligands or dendrimer ligands and hydrophobic ODA of ODA-QDs. After the ligand exchange, the QDs were transferred from the oil phase into the aqueous phase and all the as-prepared QDs were stable and no precipitate appeared over 90 d in water under ambient conditions. Scheme 1 depicts the ligand exchange process between ODA-QDs and fan-shaped PAMAM dendron ligands or star-shaped PAMAM dendrimer ligands.



Scheme 1 Schematic illustration of the ligand exchange process between ODA-QDs and fan-shaped PAMAM dendron ligands or star-shaped PAMAM dendrimer ligands modified with PEG and FA

The as-prepared new QDs named as QDs1, QDs2, QDs3 and QDs4 were found to be soluble in various polar media (Table 1). The UV-Vis absorption and fluorescent emission spectra of QDs1, QDs2, QDs3 and QDs4 in water and those of ODA-QDs in $CHCl_3$ were shown in Fig.1. The QDs in water present the same spectral characteristics as the original

Table 1 Solubility of the QDs in different solvents*

QDs	Solvent							
	Water	Methanol	THF	PBS buffer solution	Chloroform	DMF	Toluene	DMEM
ODA-QDs	–	–	+	–	+	–	+	–
QDs1	+	+	–	+	–	+	–	+
QDs2	+	+	–	+	–	+	–	+
QDs3	+	+	–	+	–	+	–	+
QDs4	+	+	–	+	–	+	–	+

* + Soluble; – insoluble.

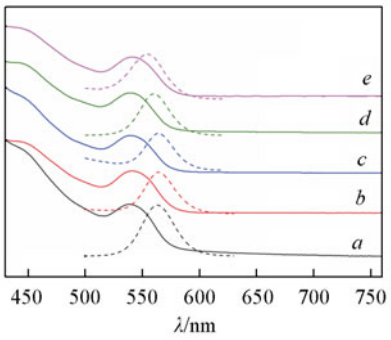


Fig.1 UV-Vis spectra(solid line) and PL spectra(dotted line) of ODA-QDs in $CHCl_3$(ODA-QDs)(*a*) and the corresponding QDs1(*b*), QDs2(*c*), QDs3(*d*), QDs4(*e*) in water

ODA-QDs dissolved in $CHCl_3$, indicating that upon ligand exchange, the QDs were protected very well by PAMAM ligands and no major surface defects generate. And the slight blue-shift phenomenon may be caused by the amount of amine, which could etch CdSe/ZnS QDs when ligand exchange took place[19].

Fan-shaped PAMAM dendron-coated QDs are more compact in size than those QDs capped with star-shaped PAMAM dendrimer ligands. For the same core-shell QDs(6.3 nm in diameter), dynamic light scattering(DLS) measurements showed that fan-shaped PAMAM dendron-coated QDs have hydrodynamic sizes(D_h) of 15.7 nm(QDs1) and 16.5 nm(QDs3), considerably smaller than those of star-shaped PAMAM dendrimer-coated QDs(25.2 nm for QDs2 and 24.5 nm for QDs4) (Table 2). The reason is that the PEG layer further increases the radius of nanoparticles. Furthermore, dendrimers have larger size than dendrons, it can identically enlarge the size of coated QDs. It is worth noting that this ligand exchange yielded single water- soluble QDs that remained well-dispersed in solution as confirmed by transmission electron microscopy(TEM, Fig.2), indicating that the ligand-exchange reaction did not cause QD aggregation. The hydrodynamic diameters are obviously smaller than the real ones in water, because dendron ligand or dendrimer ligand coatings shrink substantially upon drying.

Table 2 Hydrodynamic diameters and I_1/I_3 ratios of the QDs

Sample	D_h^*/nm	I_1/I_3
ODA-QDs	6.3	—
QDs1	15.7	1.12
QDs2	25.2	1.30
QDs3	16.5	1.37
QDs4	24.5	1.54
H_2O	—	1.81

* Measured by DLS.

Subsequently, we studied the ligand exchange efficiency utilizing pyrene probe. The intensity ratio of the first peak (I_1, λ_{max}=374 nm) to the third peak at fluorescence emission spectrum of pyrene(I_3, λ_{max}=385 nm), namely I_1/I_3 ratio has been utilized to study the micro-heterogeneity of the systems. For example, the I_1/I_3 value of water is 1.81, 0.95 for a polystyrene film, and about 0.5 for nonpolar solvents(*n*-hexane). Much higher I_1/I_3 ratio means stronger polarity of surroundings

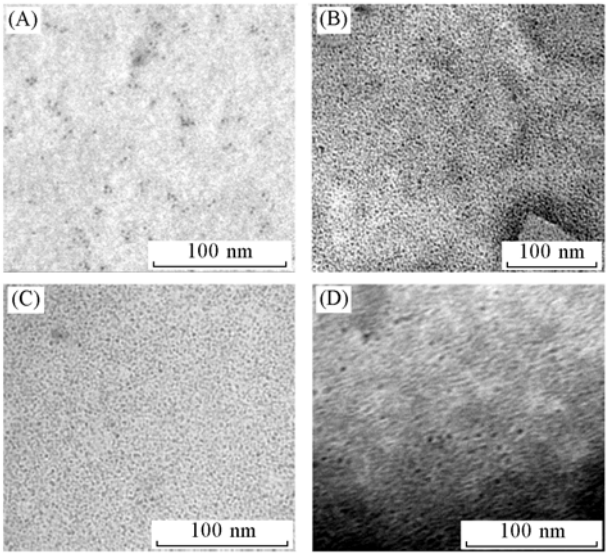


Fig.2 TEM images of the as-prepared water-soluble QDs
(A) QDs1; (B) QDs2; (C) QDs3; (D) QDs4.

around pyrene. Therefore, it's a very helpful probe for determining the dye preferentially partition into hydrophobic domains after mixing with QDs capped with dendrimer or dendron coatings. In this study, the I_1/I_3 ratios are 1.12, 1.30, 1.37 and 1.54 for QDs1, QDs2, QDs3 and QDs4, respectively (Table 2), Suggesting that there are residual hydrophobic original ODA ligands on the surface of QDs, and pyrene is trapped in the hydrophobic domain.

As is known to all, there are two processes in the ligand-exchange reaction, one is the desorption between cation on the QD surface and the original ODA ligand, the other is the adsorption between cation and hydrophilic dendron ligands or dendrimer ligands. These two processes are both reversible, therefore the ligand exchange reaction was reversible. For the dendron-coated QDs, there are two binding sites of a dendron ligand, amino groups for QDs1 and carboxyl groups for QDs3, respectively. And the carboxyl groups are stronger bonding groups to cations on the surface of QDs than amino groups. Thus, the reversibility of a ligand exchange reaction accompanied by a change of ODA and ligand showed QDs1 has more hydrophobic layer than QDs3(Table 2, I_1/I_3: QDs1=1.12, QDs3=1.37). There are more than two amino groups in ligands **2** and **4**, illustrating that ligands **2** and **4** have stronger binding affinity to cation on the QD surface than ligand **1**. Consequently, the I_1/I_3 ratios for QDs2 and QDs4 are higher than that for QDs1(Table 2). However, for dendrimer-coated QDs(QDs2 and QDs4), the original ODA coating are not completely replaced after ligand exchange reaction and kept a partial hydrophobic ligands on the QDs surface owning to steric hindrance. At the same time, the hydrophobic domains in dendrimer coatings have been examined by grafting different poly ethrylene glycol(PEG) chains on the dendrimer ligands(five chains for ligand **2** and one chain for ligand **4**).

Fluorescence experiments with pyrene probes were conducted in order to confirm the presence of small portion of the original hydrophobic ligands in dendrimer-QDs or dendron-QDs and to detect changes of the hydrophobic

microdomains in the ligand-exchange reaction. The study revealed that there always existed a small portion of the original ligands that could not be removed by any type of ligands under the present conditions, owing to the reversibility of ligand exchange reaction.

The fluorescence emission intensities of the samples at different reaction time are shown in Fig.3(A) and the change of I_1/I_3 ratios with reaction time is illustrated in Fig.3(B). The results showed that the hydrophobic layer decreased dramatically in the process of ligand-exchange reaction during the initial 4 h, and then maintained stable, suggesting that the optimal reaction time between ODA-QDs and ligand **3** was 4 h under the present conditions. More information on changes of hydrophobic microdomains can also be obtained from the analysis of pyrene excitation spectra[20]. As shown in Fig.4, the I_1/I_3 intensity ratio from emission spectra increased dramatically[Fig.4(A)], while the I_{335}/I_{332} intensity ratio from excitation spectra of pyrene decreased dramatically[Fig.4(B)] during the initial 3 h, which suggested that the optimal reaction time of QDs2 was 3 h. Dynamic light scattering(DLS) was used to confirm the hydrodynamic diameters of these QDs. For the core-shell QDs with ODA ligand in chloroform, DLS measurements showed that the ODA-QDs have a hydrodynamic size of 6.3 nm(Table 2). When the QDs were coated with ligand **2**, their hydrodynamic sizes increase from 6.3 nm to 24.2 nm in the initial 3 h(Fig.5), and then keep stable. The results showed that the ligand-exchange reaction between ODA-QDs and ligand **2** reaches equilibrium within 3 h, which was consistent with the results obtained from fluorescence experiments with pyrene probes.

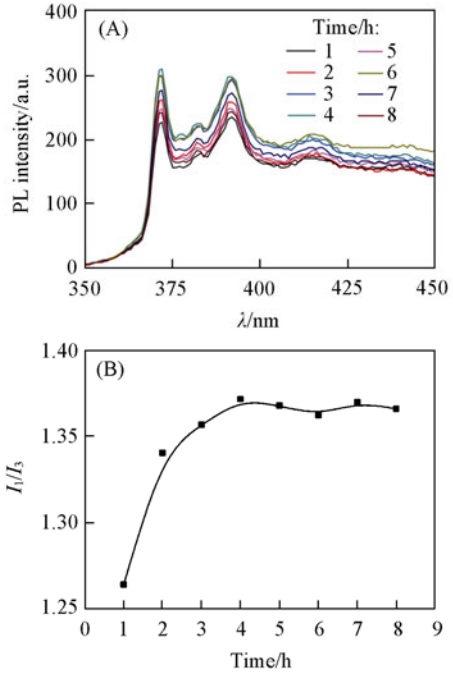


Fig.3 Influence of reaction time on reaction efficiency between ODA-QDs and the PAMAM ligand 3

(A) Fluorescence emission spectra of pyrene measured at different time; (B) ratios of fluorescence intensities at 374 and 385 nm(I_1/I_3) plotted against the time.

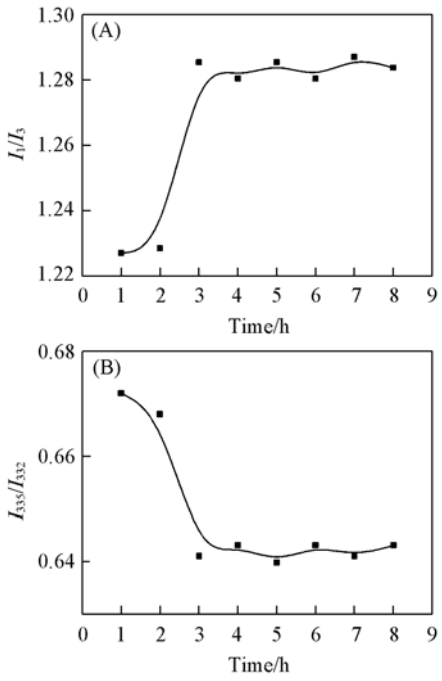


Fig.4 Influence of reaction time on reaction efficiency between ODA-QDs and the PAMAM ligand 2

(A) Ratios of fluorescence intensities at 374 and 385 nm(I_1/I_3) plotted against time; (B) ratios of excitation spectra of pyrene at 335 and 332 nm plotted against time.

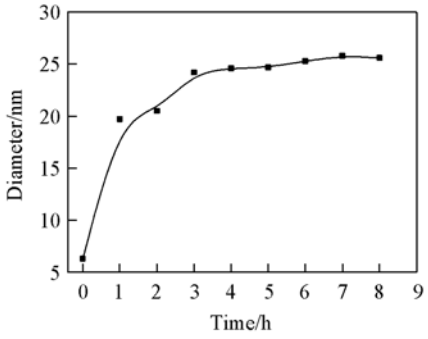


Fig.5 Influence of reaction time on the hydrodynamic diameter of QDs2 obtained by DLS

To evaluate the biological activity of the QDs, tumor cells (HeLa and SKOV3 cells) were treated with QDs(green-emitting) for 2 h(Fig.6). As shown in Fig.6(E) and (F), green fluorescence was observed in the cytoplasm of the cells, indicating that QDs4 have been successfully taken up by these tumor cells. Due to the specific binding of FA conjugates to the membrane, green signals could be detected on the surface of HeLa cells, which was associated with the expression of FA-receptor in the cell membrane[Fig.6(E)]. However, when HeLa cells were treated with FA-free conjugates(QDs1), no green fluorescence was observed, as shown in Fig.6(D). It has been shown that QDs can be specifically bound to SKOV3 cells *via* the membrane expression of FA receptors[21]. Similarly, the green signal was also observed from FA receptor over-expressing SKOV3 cells incubated with QDs4. The green signal was also observed from SKOV3 cells incubated with QDs4. These preliminary data demonstrated that FA-conjugated

molecules can specifically bind to FA receptor on the surface of the cells, which was responsible for the targeting ability of QDs4, and in turn beneficial for their entry into the cells through endocytosis or macropinocytosis. It apparently shown that FA-conjugated QDs exhibited efficient FA-mediated endocytosis in FA receptor-over expressing cancer cells, as compared to nontargeted QDs.

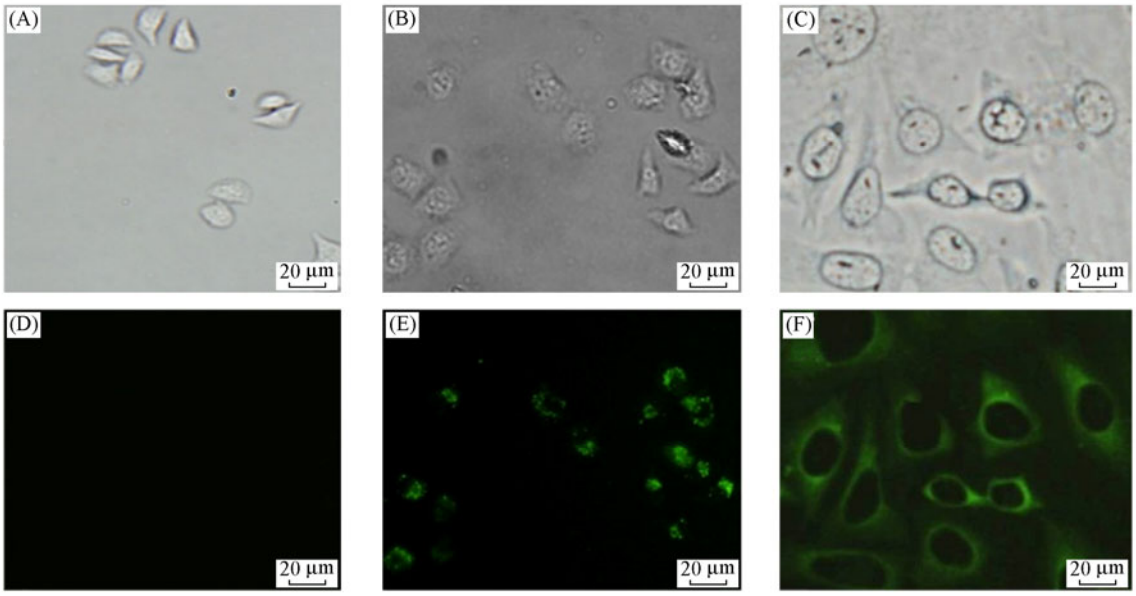


Fig.6 Cellular uptake of QDs to FA-receptor-expressing HeLa cells and SKOV3

(A—C) Phase contrast images; (D—F) fluorescence images. (A,D) HeLa cells were incubated with QDs1; (B,E) HeLa cells were incubated with QDs4; (C,F) SKOV3 cells were incubated with QDs4. All the cells were incubated for 2 h with QDs.

4 Conclusions

We have presented a new method, which used pyrene as a fluorescent probe, to detect the efficiency of the reaction between ODA-QDs and different ligands. The results are quite encouraging to other types of ligand-exchange reaction. The explorations are helpful for establishing reliable and rational strategies to study the ligand exchange kinetics or the efficiency of exchange reaction between hydrophilic dendron ligands or dendrimer ligands and hydrophobic capping on the surface of QDs.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s40242-015-5110-6.

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