Journal of Nanoparticle Research (2006) 8:1033–1038
DOI 10.1007/s11051-006-9142-1
© Springer 2006

Brief Communication

Liposome encapsulation of fluorescent nanoparticles: Quantum dots and silica nanoparticles

Chien-Sheng Chen[1], Jie Yao[2] and Richard A. Durst[1,*]
[1]*Department of Food Science & Technology, Cornell University, Geneva, NY, 14456, USA;* [2]*Department of Applied & Engineering Physics, Cornell University, Ithaca, NY, 14853, USA; *Author for correspondence (Tel.: +1-315-787-2297; Fax: +1-315-787-2397; E-mail: rad2@cornell.edu)*

Received 7 June 2005; accepted in revised form 10 July 2006

Key words: quantum dots, silica nanoparticles, fluorescence correlation spectroscopy, liposomes, encapsulation, biological imaging, nanomedicine

Abstract

Quantum dots (QDs) and silica nanoparticles (SNs) are relatively new classes of fluorescent probes that overcome the limitations encountered by organic fluorophores in bioassay and biological imaging applications. We encapsulated QDs and SNs in liposomes and separated nanoparticle-loaded liposomes from unencapsulated nanoparticles by size exclusion chromatography. Fluorescence correlation spectroscopy was used to measure the average number of nanoparticles inside each liposome. Results indicated that nanoparticle-loaded liposomes were formed and separated from unencapsulated nanoparticles by using a Sepharose gel. As expected, fluorescence self-quenching of nanoparticles inside liposomes was not observed. Each liposome encapsulated an average of three QDs. These studies demonstrated that nanoparticles could be successfully encapsulated into liposomes and provided a methodology to quantify the number of nanoparticles inside each liposome by fluorescence correlation spectroscopy.

Introduction

Fluorescent probes for biomolecular recognition have been widely used in bioassays and biological imaging (Schrock et al., 1996; Trau et al., 2002). However, organic fluorophores have characteristics that limit their effectiveness for these applications, including poor photostability, low brightness, and limited capability for multiplexed analysis (Goldman et al., 2002; Yang et al., 2004). Quantum dots (QDs) and silica nanoparticles (SNs) are relatively new classes of fluorescent probes that have the potential to overcome these limitations. QDs (fluorescent semiconductor nanocrystals) have broad excitation and size-dependent, tunable, narrow-emission spectra that allow the simultaneous excitation of several different-colored QDs at a single wavelength with little spectral emission overlap for multianalyte analysis (Alivisatos, 1996; Mattoussi et al., 1998; Rodriguez-Viejo et al., 2000). Also, QDs have been reported to be about 20 times brighter and 100 times more photostable in comparison with organic dyes such as rhodamine (Chan & Nie, 1998). Fluorescent SNs are synthesized by a sol–gel technique in which organic dyes are covalently attached to the silica precursor (Stober et al., 1968; Larson et al., 2003a). They are brighter and more photostable than free organic dyes (Larson et al., 2003a).

Liposomes are spherical vesicles consisting of phospholipid bilayers surrounding an aqueous

cavity. Because each liposome can contain up to several million fluorescent dye molecules, thereby providing greatly enhanced signals, liposomes have been successfully used as reporter particles in bioassays (Park & Durst, 2000; Esch et al., 2001; Ahn-Yoon et al., 2003; Baeumner et al., 2004; Ho et al., 2004). Therefore, liposomes have the potential of entrapping large amount of fluorescent nanoparticles to amplify signal. In addition, the biomimetic lipid bilayers of liposomes provide high biocompatibility (Struck & Pagano, 1980; Muller et al., 1995; Barber et al., 1996), thereby enhancing the effectiveness of fluorescent nanoparticles for biological detection *in vitro* and *in vivo*.

In this study, we encapsulated QDs and SNs into liposomes by the reverse-phase evaporation method. Nanoparticle-loaded liposomes were separated from unencapsulated nanoparticles by size-exclusion chromatography (SEC) and their characteristics were investigated. Dual-color, two-photon fluorescence correlation spectroscopy was used to determine the number of nanoparticles inside each liposome.

Experimental

Reagents

Common laboratory reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Sepharose CL-2B and Sepharose CL-4B were purchased from Sigma-Aldrich Co. Dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), dipalmitoylphosphatidylethanolamine-N-(biotin) (N-biotinyl-DPPE), lissamine rhodamine B-DPPE, nitrobenzoxadiazol-DPPE and polycarbonate syringe filters of 0.4 and 0.2 μm pore sizes were purchased from Avanti Polar Lipids (Alabaster, AL). Quantum dots (EviTags) were purchased from Evident Technologies (Troy, NY). Silica nanoparticles were kindly provided by Hooisweng Ow and Ulrich Wiesner, Cornell University, Ithaca, NY.

Encapsulant preparation

A 0.8 μM QD solution and a 122 nM SN solution were each prepared in HEPES buffer (0.01 M at pH 7.5, containing 0.2 M NaCl and 0.01% sodium azide). Sucrose was used to adjust the osmolality to 445 mOsmol/kg.

Preparation of nanoparticle-loaded liposomes

Liposomes were prepared using a modified version of the reverse-phase evaporation method described by Siebert et al. (1993). About 45 μmol DPPC, 5 μmol DPPG, 5 μmol N-biotinyl-DPPE, and 45 μmol cholesterol were dissolved in 3 ml of a chloroform/methanol solution (volume ratio, 5:1). While sonicating the suspension at 45°C, 0.6 ml of encapsulant was added. Using a vacuum rotary evaporator, the organic solvent was removed. Then, an additional 0.4 ml of encapsulant was added and the vacuum rotary evaporator was applied again. After the liposomes were formed, they were incubated for 30 min at 45°C and, finally, extruded 30 times through polycarbonate syringe filters with 0.4 μm pore size. Samples for fluorescence correlation spectroscopy were extruded with 0.4 and 0.2 μm pore-size filters in series.

Size exclusion chromatography (SEC) of liposomes

Unencapsulated QDs were separated from the liposomes by size exclusion chromatography using Sepharose CL-2B column (25×1.5 cm). HEPES buffer (0.01 M, pH 7.5) was used as the eluent containing 0.2 M NaCl and 0.01% sodium azide. Sucrose was used to adjust the osmolality to 515 mOsmol/kg. The flow rate was controlled at 25.1 ml/h. The eluted liposomes were collected at 1 ml/tube by a Retriever 500 fraction collector (ISCO, Lincoln, NE), followed by fluorescence measurement using a RF-551 spectrofluorometric detector (Shimadzu, Kyoto, Japan). Unencapsulated SNs were separated from the liposomes by size exclusion chromatography using Sepharose CL-4B column (25×1.5 cm). HEPES buffer (0.01 M, pH 7.5) was used as the eluent containing 0.2 M NaCl and 0.01% sodium azide. Sucrose was used to adjust the osmolality to 515 mOsmol/kg. The flow rate was controlled at 45.2 ml/h. The eluted liposomes were collected at 1.5 ml/tube by the Retriever 500 fraction collector, followed by fluorescence measurement using the RF-551 spectrofluorometric detector.

Measurement of the average number of nanoparticles inside each liposome

We used two-photon fluorescence correlation spectroscopy (FCS, Developmental Resource for Biophysical Imaging Opto-Electronics, Cornell University, Ithaca) to measure the number of fluorescent particles in the focal volume, thereby calculating the sample concentration (Larson et al., 2003b). Dual-color, cross-correlation experiments were performed to measure the intact liposome concentration and nanoparticle concentration released after liposome lysis, thus permitting measurements of the nanoparticle quantity inside each liposome. We incorporated 0.4 mol% red lissamine rhodamine B (LRB)-DPPE into the liposome bilayers for green QD encapsulation and 0.4 mol% green nitrobenzoxadiazol-DPPE for red SN encapsulation in the preparation of nanoparticle-loaded liposomes described above. We lysed liposomes by adding 0.5 ml of 30 mM n-OG to 5 μl liposome solution.

Results and discussion

Size exclusion chromatography of QD-loaded liposomes

The sizes of QDs and liposomes are approximately 50 nm and 300 nm, respectively. We separated unencapsulated QDs from the liposomes by SEC using Sepharose CL-2B. Figure 1 shows that QD-loaded liposomes were formed and separated by Sepharose CL-2B. Because QD-loaded liposomes contained fluorescent QDs, they produced higher fluorescence intensity than the empty (buffer-loaded) liposomes. Empty liposomes were detectable by fluorescence based on the property of large liposomes to scatter light, the degree of which can be detected and measured. The recovery of QDs from the column was poor due to the adsorption of some QDs to the top of the column matrix. Reynolds et al. also reported this problem while separating polystyrene beads using a size-exclusion column (Reynolds et al., 1983).

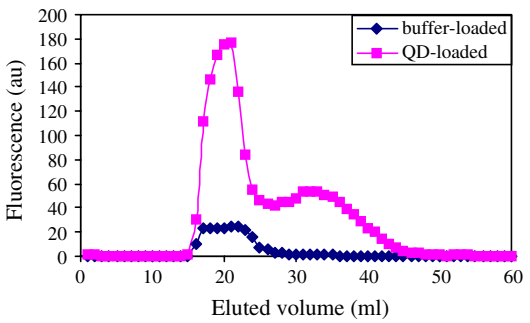

Figure 1. Elution profiles on Sepharose CL-2B of buffer-loaded liposomes and QD-loaded liposomes detected by fluorescence. The first peak at 20 ml corresponds to the eluted liposomes and the second broad peak is the unencapsulated QDs

Size exclusion chromatography of SN-loaded liposomes

Because of the size difference between SNs (~20 nm) and liposomes (~575 nm), we also used SEC to separate unencapsulated SNs from SN-loaded liposomes. Instead of Sepharose CL-2B, we used Sepharose CL-4B, which has smaller pore sizes because SNs are smaller than QDs. The elution profile of SN-loaded liposomes plotted in Figure 2 exhibits two distinct peaks at respective elution volumes of 16 and 32 ml, which correspond to liposome-entrapped and free SNs. This also demonstrated that SN-loaded liposomes were formed and separated well by Sepharose CL-4B. Like QDs, the recovery of unentrapped SNs from the column was also poor, thereby producing little fluorescence in Figure 2.

Characterization of nanoparticle-loaded liposomes

The absorption and emission spectra of both types of nanoparticle-loaded liposomes were measured

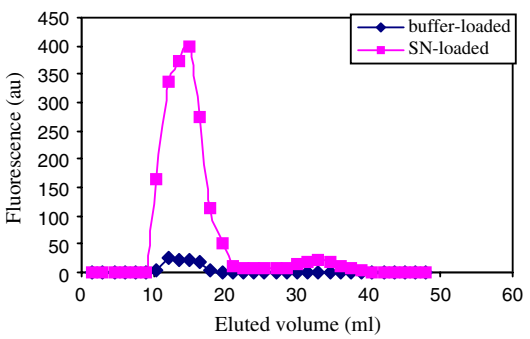

Figure 2. Elution profiles on Sepharose CL-4B of buffer-loaded and SN-loaded liposomes detected by fluorescence

by a spectrophotometer and spectrofluorometer, respectively. Since liposomes scatter light, their absorption spectrum is just a measure of scattered light, and the shorter the wavelength, the higher the intensity. The absorption intensity of liposomes, therefore, increased with decreasing wavelength. Diederichs (1996) also reported similar properties of the absorption spectrum of liposomes. On the other hand, liposome-entrapped nanoparticles exhibited fluorescent emission spectra similar to the free nanoparticles.

While the fluorescence of organic dyes in the liposomes is self-quenched, liposome-encapsulated nanoparticles produced a relatively strong signal. We also compared the fluorescence of intact *versus* lysed nanoparticle-loaded liposomes and our results showed no significant fluorescence difference between them (data not shown). This is a great benefit for bioassays since liposome lysis will not be required for fluorescence measurements. Also, fluorescent signals can be detected when liposome lysis is not feasible, such as in a lateral flow assay. Conventionally, the signal of organic dye-loaded liposomes on the test strip can only be read by color intensity (Park & Durst, 2000; Ahn-Yoon et al., 2003). Due to the absence of self-quenching in nanoparticle-loaded liposomes, the fluorescence intensity on a test strip can be detected, thereby providing increased sensitivity.

Number of nanoparticles inside each liposome based on FCS

Fluorescence correlation spectroscopy (FCS) is a technique that analyzes fluorescence intensity fluctuations arising from molecules diffusing in and out of a microscopic detection volume of about one femtoliter defined by a tightly focused laser beam (Rigler, 1995; Maiti et al., 1997). In dual-color, cross-correlation FCS, the fluorescence signals from the two fluorophores are recorded simultaneously, and the fluctuations in the fluorescence signal of one fluorophore are correlated with those of the other fluorophore (Kim & Schwille, 2003). If fluorescent nanoparticles are encapsulated inside a dye-labeled liposome, they will pass through the FCS detection volume together, resulting in coincident fluctuations in both detector channels, which can be detected by cross-correlation analysis of fluctuations in the two channels.

In Figure 3, the cross-correlation between QDs and liposomes is shown for the intact liposome sample, which indicates that QDs were encapsulated inside the liposomes. After liposome lysis, the cross-correlation amplitude was observed to be zero due to the dissociation of the QDs and lysed liposomes.

For measuring the quantity of QD-loaded liposomes and released QDs after liposome lysis, autocorrelation analysis was used. The amplitude of the autocorrelation curve at $\tau = 0$ is inversely proportional to the average number of fluorescent molecules in the detection volume. This provides a direct measure of the concentration (Levin & Carson, 2004). Figure 4 shows the autocorrelation curves of QD-loaded liposomes and released QDs for the same concentration of liposomes. The concentrations of QD-loaded liposomes and released QDs were 0.32 and 0.94 nM, respectively. Hence, we calculated that each liposome contained an average of three QDs. Unfortunately, we could not measure the SN quantity inside each liposome due to the spectral overlap between SNs and nitrobenzoxadiazol-liposomes.

Although liposomes did not provide much signal amplification due to the small number of QDs encapsulated, they still provided protection and biocompatibility for QDs during biological detection *in vitro* and *in vivo*. In addition, the small number of encapsulated QDs can be explained by the large size (~50 nm diameter) and low

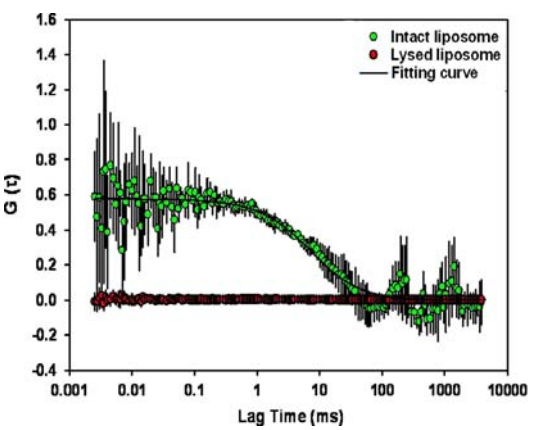

Figure 3. Cross-correlation curves of intact and lysed QD-loaded liposomes

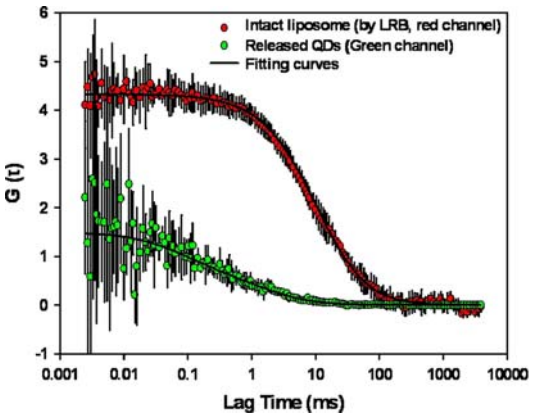

Figure 4. Autocorrelation curves of intact QD-loaded liposomes and released QDs after liposome lysis

concentration (0.8 μM) of the QD solution (encapsulant) for liposome preparation.

Conclusions

Demonstration of the successful encapsulation of QDs and SNs into liposomes was achieved. As expected, self-quenching of the fluorescence was not observed for either QD-loaded or SN-loaded liposomes. When a 0.8 μM QD solution was used for liposome encapsulation, each liposome contained an average of three QDs based on dual-color fluorescence correlation spectroscopy. In the future, smaller and more highly concentrated QDs will be needed for liposome encapsulation in an attempt to increase the number of quantum dots inside each liposome.

References

Ahn-Yoon S., T.R. DeCory, A.J. Baeumner & R.A. Durst, 2003. Ganglioside-liposome immunoassay for the ultrasensitive detection of cholera toxin. Anal. Chem. 75, 2256–2261.

Alivisatos A.P., 1996. Semiconductor clusters, nanocrystals, and quantum dots. Science 271, 933–937.

Baeumner A.J., J. Pretz & S. Fang, 2004. A universal nucleic acid sequence biosensor with nanomolar detection limits. Anal. Chem. 76, 888–894.

Barber K., R.R. Mala, M.P. Lambert, R.Z. Qiu, R.C. MacDonald & W.L. Klein, 1996. Delivery of membrane-impermeant fluorescent probes into living neural cell populations by lipotransfer. Neurosci. Lett. 207, 17–20.

Chan W.C. & S. Nie, 1998. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281, 2016–2018.

Diederichs J.E., 1996. Plasma protein adsorption patterns on liposomes: establishment of analytical procedure. Electrophoresis 17, 607–611.

Esch M.B., A.J. Baeumner & R.A. Durst, 2001. Detection of Cryptosporidium parvum using oligonucleotide-tagged liposomes in a competitive assay format. Anal. Chem. 73, 3162–3167.

Goldman E.R., G.P. Anderson, P.T. Tran, H. Mattoussi, P.T. Charles & J.M. Mauro, 2002. Conjugation of luminescent quantum dots with antibodies using an engineered adaptor protein to provide new reagents for fluoroimmunoassays. Anal. Chem. 74, 841–847.

Ho J.A., H.W. Hsu & M.R. Huang, 2004. Liposome-based microcapillary immunosensor for detection of Escherichia coli O157:H7. Anal. Biochem. 330, 342–349.

Kim S.A. & P. Schwille, 2003. Intracellular applications of fluorescence correlation spectroscopy: Prospects for neuroscience. Curr. Opin. Neurobiol. 13, 583–590.

Larson D.R., A. Heikal, H. Ow, M. Srivastava, U. Wiesner, B. Baird & W.W. Webb, 2003a. Development of fluorescent silica nanoparticles for biological imaging. Biophys. J. 84, 586A–586A.

Larson D.R., W.R. Zipfel, R.M. Williams, S.W. Clark, M.P. Bruchez, F.W. Wise & W.W. Webb, 2003b. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science 300, 1434–1436.

Levin M.K. & J.H. Carson, 2004. Fluorescence correlation spectroscopy and quantitative cell biology. Differentiation 72, 1–10.

Maiti S., U. Haupts & W.W. Webb, 1997. Fluorescence correlation spectroscopy: diagnostics for sparse molecules. Proc. Natl. Acad. Sci. USA 94, 11753–11757.

Mattoussi H., L.H. Radzilowski, B.O. Dabbousi, E.L. Thomas, M.G. Bawendi & M.F. Rubner, 1998. Electroluminescence from heterostructures of poly(phenylene vinylene) and inorganic CdSe nanocrystals. J. Appl. Phys. 83, 7965–7974.

Muller W.J., K. Zen, A.B. Fisher & H. Shuman, 1995. Pathways for Uptake of Fluorescently Labeled Liposomes by Alveolar Type-Ii Cells in Culture. Am. J. Physiol. Lung Cell Mol. Physiol. 13, L11–L19.

Park S. & R.A. Durst, 2000. Immunoliposome sandwich assay for the detection of Escherichia coli O157:H7. Anal. Biochem. 280, 151–158.

Reynolds J.A., Y. Nozaki & C. Tanford, 1983. Gel-exclusion chromatography on S1000 Sephacryl: application to phospholipid vesicles. Anal. Biochem. 130, 471–474.

Rigler R., 1995. Fluorescence correlations, single molecule detection and large number screening. Applications in biotechnology. J Biotechnol 41, 177–186.

Rodriguez-Viejo J., H. Mattoussi, J.R. Heine, M.K. Kuno, J. Michel, M.G. Bawendi & K.F. Jensen, 2000. Evidence of photo- and electrodarkening of (CdSe)ZnS quantum dot composites. J. Appl. Phys. 87, 8526–8534.

Schrock E., S. du Manoir, T. Veldman, B. Schoell, J. Wienberg, M.A. Ferguson-Smith, Y. Ning, D.H. Ledbetter, I. Bar-Am, D. Soenksen, Y. Garini & T. Ried, 1996. Multicolor spectral karyotyping of human chromosomes. Science 273, 494–497.

Siebert S.T.A., S.G. Reeves & R.A. Durst, 1993. Liposome immunomigration field assay device for Alachlor determination. Anal. Chim. Acta 282, 297–305.

Stober, W., A. Fink & E. Bohn, 1968. Controlled growth of monodisperse silica spheres in micron size range. J. Colloid Interf. Sci. 26, 62.

Struck D.K. & R.E. Pagano, 1980. Insertion of fluorescent phospholipids into the plasma-membrane of a mammalian-cell. J. Biol. Chem. 255, 5404–5410.

Trau D., W. Yang, M. Seydack, F. Caruso, N.T. Yu & R. Renneberg, 2002. Nanoencapsulated microcrystalline particles for superamplified biochemical assays. Anal. Chem. 74, 5480–5486.

Yang W., C.G. Zhang, H.Y. Qu, H.H. Yang & J.G. Xu, 2004. Novel fluorescent silica nanoparticle probe for ultrasensitive immunoassays. Anal. Chim. Acta 503, 163–169.