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Gatekeeping Layer Effect: A Poly(lactic acid)-coated Mesoporous Silica Nanosphere-Based Fluorescence Probe for Detection of Amino-Containing Neurotransmitters

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Synthesizing molecular receptors that can differentiate between various extracellular amino acid-based neurotransmitters has long been a research challenge. For example, dopamine and glutamic acid are two essential neurotransmitters that are often simultaneously exchanged between various neural cells.[1] Despite their importance in understanding interneuronal chemical communication, to the best of our knowledge, no synthetic molecular receptor has been reported in the literature that can distinguish dopamine from glutamic acid.

Selective functionalization of the exterior and interior surface of structurally uniform mesoporous materials, such as MCM- or SBA-type of silica, with different organic moieties[2] allows for precise regulation of the penetration of selective molecules with certain sizes and chemical properties into the nanoscale pores. Herein, we report on the synthesis and characterization of a poly(lactic acid)-coated, MCM-41-type mesoporous silica nanosphere (PLA-MSN) material that can serve as a fluorescence probe for selective detection of amino-containing neurotransmitters under physiological conditions. By utilizing the PLA layer as a *gatekeeper* to regulate the penetration of molecules in and out of the nanoscale pores, we investigated the molecular recognition events between several structurally simple neurotransmitters, i.e., dopamine, tyrosine, and glutamic acid and a pore surface-anchored *o*-phthalic hemithioacetal (OPTA) group.[3] As depicted in the bottom of Figure 1, OPTA functions as a fluorescence-sensing group that reacts with these neurotransmitters that contain primary amines to form the fluorescent isoindole products.[3]

First, we synthesized a mercaptopropyl-functionalized mesoporous silica nanosphere (thiol-MSN) material with average pore diameter of 2.5 nm via our previously reported method.[3a,4] As outlined in Figure 1, 5,6-epoxyhexyltriethoxysilane (EHTES) was grafted onto the exterior surface of the thiol-MSN-containing cetyltrimethylammonium bromide (CTAB) surfactants inside the mesopores. The resulting material (1.50 g) was refluxed in a 162-mL methanol solution of hydrochloric acid (1.57 M) for 12 h to remove the CTAB template and to convert the thiol-MSN with epoxyhexyl groups to a 5,6-dihydroxyhexyl-coated thiol-MSN material (DH-MSN).[5] Incorporation of the 5,6-dihydroxyhexyl group was confirmed by ^{29}Si and ^{13}C CP- and DP-MAS NMR spectroscopy, and the surface coverage was measured to be 43% (2.1 mmol/g).[6] The vacuum-dried DH-MSN material (0.68 g) was sonicated for 30 min in 10 mL of anhydrous THF to disperse the particles uniformly. L-Lactide (0.36 g, 2.50 mmol) was mixed with a catalyst, tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, 0.16 mL, 0.50 mmol), in 15 mL of anhydrous THF. The lactide/catalyst solution was added to the DH-MSN THF suspension via injection and stirred at 80 °C for 72 h to yield the PLA-coated thiol-MSN material. The crude solid product was further purified by a method previously published by Langer's group.[7] As shown in Figure 2, the average thickness (ca. 11 nm) of the PLA layer was determined by transmission electron microscopy (TEM). The chemically accessible thiol density

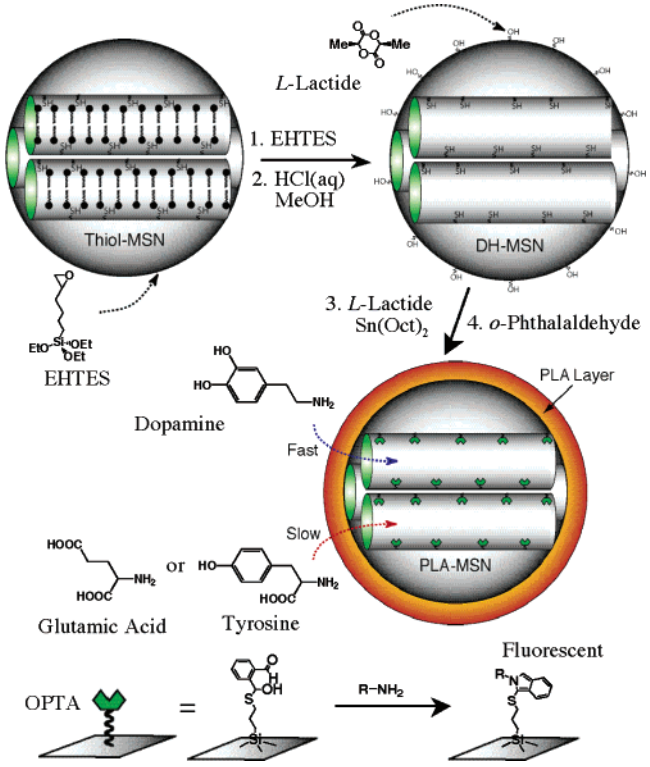

Figure 1. Schematic representation of the synthesis of PLA-coated MSN-based fluorescence sensor system for detection of amine-containing neurotransmitters, i.e., dopamine, glutamic acid, and tyrosine (R-NH_2). (5,6-epoxyhexyltriethoxysilane = EHTES, cetyltrimethylammonium bromide (CTAB) surfactant = ●∼∼∼∼).

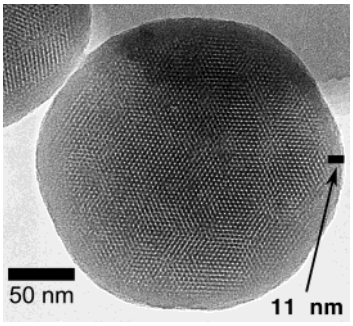

Figure 2. Transmission electron micrograph (TEM) of an ultramicrotomed PLA-MSN material. The layer of PLA can be visualized by the rim of amphorous structure surrounding the MCM-41-type of MSN core with mesopores packed in a hexgonal symmetry.

(0.22 mmol/g) of the purified PLA-MSN was measured by our previously published method.[3a] The mercaptopropyl functionality was then converted to the amine-sensitive OPTA group by reacting

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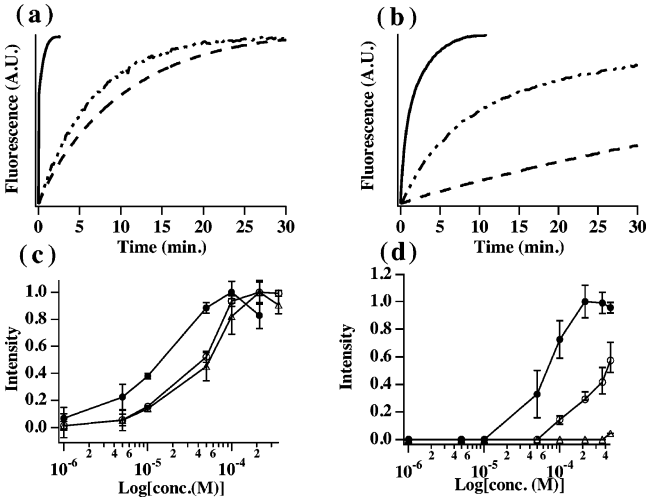

Figure 3. Kinetic measurements of the fluorescence detection of dopamine (—), tyrosine (— - — - — - —), and glutamic acid (- - -) with OPTA-SS **(a)** and PLA-MSN **(b)**. Fluorescence increase of OPTA-SS **(c)** and PLA-MSN **(d)** as a function of dopamine (●), tyrosine (○), and glutamic acid (△) concentrations. The fluorescence intensities were measured 5 min after the introduction of every concentration of each neurotransmitter.

85.0 mg of PLA-coated thiol-MSN with 170.0 mg (1.26 mmol) of phthalic dicarboxaldehyde (*o*-phthalaldehyde, OPA) in 10 mL of methanol solution for 5 h. After filtration, the resulting material (PLA-MSN) was thoroughly washed with methanol and dried under vacuum. The morphology, particle size distribution, and the structure of organic functionalities of PLA-MSN were scrutinized by XRD, SEM, TEM, N_2 sorption isotherms, and ^{13}C CP-MAS NMR spectroscopy.[6]

To examine the gatekeeping effect of the PLA layer in our PLA-MSN system, we prepared and characterized an amorphous silica material grafted with the same OPTA functionality (OPTA-SS) as a control system.[6] The surface coverage of the OPTA group was determined to be 0.08 mmol/g.[2a,4] Both the OPTA-SS and our PLA-MSN materials were dispersed in pH 7.4 PBS buffer (10 mM) for the fluorescence-sensing experiments of neurotransmitters. In the case of OPTA-SS, dopamine, tyrosine, and glutamic acid (230 μM each) reacted with the surface-bound OPTA groups rapidly, as evidence by the fluorescence emission data shown in Figure 3a. It is noteworthy that both tyrosine and glutamic acid reacted to the OPTA-SS with very similar rates and therefore could not be distinguished. In contrast, the reactions of these analytes (230 μM) with our OPTA-derivatized PLA-MSN exhibited significantly different and lower reaction rates (Figure 3b), by a factor of 4, 10, and 57, respectively. In the case of dopamine, the lower reaction rate could be attributed to the additional diffusional penetration through the PLA layer into the OPTA-functionalized mesopores. Clearly, the reaction rates of tyrosine and glutamic acid were further slowed by the gatekeeping effect of the PLA layer on these two analytes. In addition, our study (Figure 3c) showed that the fluorescence intensity of OPTA-SS increased similarly with the increasing concentrations of all three neurotransmitters. However, in the case of the PLA-MSN (Figure 3d), the dopamine binding gave the most significant increase of fluorescence intensities at all concentrations. We also note that a similar set of kinetic and titration experiments performed on the DH-MSN material (without PLA) showed no evidence of the gatekeeping effect.[6]

To examine the substrate selectivity of our PLA-MSN system in the presence of a mixture of neurotransmitters, PLA-MSN nanoparticles (2 mg) were introduced to a pH 7.4 PBS buffer (10 mM) solution of dopamine (0.5 mM) and glutamic acid (10 mM) at 25 °C. After 10 min of mixing, the suspension was centrifuged, and the individual concentrations of dopamine and glutamic acid in the supernatant were analyzed by HPLC.[6] Given that the signal transduction mechanism of the PLA-MSN system is based on the covalent capture of substrates by the surface-bound OPTA groups, the different degrees of concentration decrease of these two analytes in solution would represent the selectivity of the PLA-MSN system. Despite the initial 20:1 concentration ratio between glutamic acid and dopamine, the results showed a 96% decrease of dopamine concentration, whereas only a 2% decrease of the concentration of glutamic acid was observed.[6]

The observed large difference in the rates of diffusion is most likely due to the different electrostatic, hydrogen bonding, and dipolar interactions between these neurotransmitters and the PLA layer in pH 7.4 buffer. The isoelectric points (pIs) of dopamine, tyrosine, and glutamic acid are 9.7, 5.7, and 3.2, respectively, whereas the pI of PLA is typically below 2.0,[8] which means the dopamine will be positively charged and the others will be negatively charged under our experimental conditions. Similar effects of pI have also been reported previously.[9] For example, Blanco et. al reported that proteins with low pI values, such as bovine serum albumin (pI = 4.6) were released faster from a PLGA-based polymer than those with high pIs, such as lysozyme (pI = 11.2).[9a]

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Supporting Information Available: Syntheses and spectroscopic characterizations of the OPTA-SS and PLA-MSN materials (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) See the Supporting Information for experimental details on materials preparation, N_2 adsorption isotherms, particle size distributions, SEM micrograph, XRD spectra, HPLC analysis of the competitive neurotransmitter detection of PLA-MSN, kinetic and titration measurements of DH-MSN, as well as detailed analysis of solid state ^{13}C and ^{29}Si NMR experiments.

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