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Accelerated Oxidation of Epinephrine by Silica Nanoparticles

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We have measured the influence of mesoporous silica (MCM-41 and SBA-15) nanoparticles and dense silica nanoparticles on epinephrine oxidation, a pH-dependent reaction, whose rate is small in acidic or neutral solutions but much greater at higher pH. The reaction was measured by monitoring adrenochrome at 480 nm, the product of epinephrine oxidation. In distilled water (dH₂O) with no particles present, the oxidation of epinephrine occurs slowly but more rapidly at higher pH. The presence of MCM-41 or silica spheres does not accelerate the oxidation, but SBA-15 does, showing that the difference in the structures of nanomaterials leads to differing effects on the epinephrine oxidative process. In phosphate buffered saline (PBS, pH = 7.4), epinephrine undergoes a much quicker oxidation, and, in this case, the presence of SBA-15 and MCM-41 makes it even more rapid. Silica spheres have no noticeable influence on the oxidation in PBS or in dH₂O. The possibility that the catalytic effect of mesoporous silica nanoparticles (MSN) could result from the residue of templating chemicals, however, can be excluded due to the postsynthesis calcinations. Experiments with dithionite, added either earlier than or at the same time as the epinephrine addition, show that fast oxidation takes place only when dithionite and epinephrine are simultaneously added into PBS solution. This confirms a vital role of oxygen radicals (probably $\cdot O_2^-$) in the oxidation of epinephrine. These oxygen radicals are likely to form and accumulate within the phosphate buffer or in the presence of MSN. Comparing the three kinds of silica nanoparticles applied, we note that mesoporous SBA-15 and MCM-41 materials own much larger surface area than solid silica particles do, whereas MCM-41 possesses a much narrower pore size (0.4-fold) than SBA-15. It seems, therefore, that large surface area, characteristic mesoporosity, and surface structures aid in the deposit of oxygen radicals inside MSN particles, which catalyze the epinephrine oxidation in a favorable phosphate environment.

Introduction

A family of biogenic amines, catecholamines take major responsibilities for the acute stress response perceived by mammals when sensing unexpected stimuli from either external or internal environment.^{1,2} Chemically, catecholamines are a group of hormones, biosynthesized from tyrosine and phenylalanine via hydroxylation to produce, among others, dopamine, norepinephrine, and epinephrine.³ All these hormones contain catechol and amine moieties as implied in the name. Among them, epinephrine (also known as adrenalin) is the most potent agonist to adrenergic receptors in a variety of cells.⁴⁻⁷ Once bound to a cell, epinephrine transmits neuronal signals to G-protein coupled receptors on the cell membrane, and triggers a cascade of intracellular enzymatic reactions. As a consequence, a large number of cyclic adenosine monophosphates are produced to activate glycogen phosphorylases, which further accelerate glycogenolysis and liberate glucoses into the bloodstream.⁵⁻⁷ This sudden burst of energy finally stimulates the body to make a spontaneous decision-to fight or flight.

A continual alternation of stress and depression, which accumulates a high level of epinephrine, puts our lives at high risks of tumorigenesis and cardiac dysfunction.^{8,9} Besides, a recent finding suggested that this hormone could sabotage cancer treatment by inactivating the pro-apoptotic proteins.¹⁰ Thus, the regulation of epinephrine production in order to either sustain a normal physical condition or reinforce cancer therapy becomes demanding as well as challenging.

The mission of epinephrine as a neurotransmitter can be aborted due to its oxidation. However, under physiological conditions (pH = 7.4), the autoxidation of epinephrine proceeds very slowly, although its rate can be escalated by an increase in pH. The oxidation produces O_2^- and H_2O_2 as intermediates and adrenochrome at the end.^{11,12} With superoxides present, epinephrine oxidation involves a cyclization of the quinone moiety.¹³ This step promotes the formation of more reactive oxygen species and appears more dominant at higher pH.¹³ It is well-known that $\cdot O_2^-$ reacts with H_2O_2 at neutral or basic pH to give $\cdot OH (+ O_2 + OH^-)$, which may be the active species in epinephrine oxidation.¹⁴ (In acid solutions, $\cdot O_2^-$ becomes HO₂·, which reacts with H_2O_2 to give $\cdot OH + O_2 + H_2O$.)¹⁴ Many enzymes, notably catechol oxidase and amine oxidase, are known

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⁽¹⁾ Lodish, H.; Berk, A.; Zipursky, L; Matsudaira, P.; Baltimore, D.; Darnell, J. Molecular Cell Biology, 4th ed.; W. H. Freeman Press: New York, 1999.

⁽²⁾ Eichelman, B. S. Annu. Rev. Med. 1990, 41, 149–158.

⁽³⁾ Blaschko, H. Br. Med. Bull. 1973, 29, 105-109.

⁽⁴⁾ Furchgott, R. F. Pharmacol, Rev. 1959, 11, 429-441.

⁽⁵⁾ Rizza, R. A.; Cryer, P. E.; Haymond, M. W.; Gerich, J. E. J. Clin. Invest 1980, 65, 682–689.
(6) Minneman, K. P.; Pittman, R. N.; Molinoff, P. B. Annu. Rev. Neurosci. 1981,

⁽a) Winnennan, K. F., Freinan, K. W., Monnori, F. B. Anna, K. W. Wearoser. 4, 419–461.

⁽⁸⁾ Antoni, M. H.; Lutgendorf, S. K.; Cole, S. W.; Dhabhar, F. S.; Sephton, S.

<sup>E.; McDonald, P. G.; Stefanek, M.; Sood, A. K. Nat. Rev. Cancer 2006, 6, 240–248.
(9) Tang, W.; Weil, M. H.; Sun, S.; Noc, M.; Yang, L.; Gazmuri, R. J. Circulation 1995, 92, 3089–3093.</sup>

⁽¹⁰⁾ Sastry, K. S.; Karpova, Y.; Prokopovich, S.; Smith, A. J.; Essau, B.; Gersappe, A.; Carson, J. P.; Weber, M. J.; Register, T. C.; Chen, Y. Q.; Penn, R. B.; Kulik, G. J. Biol. Chem. 2007, 282, 14094–14100.

⁽¹¹⁾ Misra, H. P.; Fridovich, I. J. Biol. Chem. 1972, 247, 3170-3175.

⁽¹²⁾ Babior, B. M.; Kipnes, R. S. Blood 1976, 47, 461-471.

⁽¹³⁾ Alhasan, R.; Njus, D. Anal. Biochem. 2008, 381, 142-147.

⁽¹⁴⁾ Ross, A. B.; Mallard, W. G.; Heiman, W. P.; Buxton, G. V.; Hule, R. E.; Neta, P. *NDRL-NIST Solution Kinetics Database, version 3.0*, 1998, Notre Dame Radiation Laboratory and National Institute of Standards and Technology, Gaithersburg, MD.

to catalyze the oxidation of catecholamines, as well as a number of organic and inorganic compounds and materials.^{15,16}

Mesoporous silica nanoparticles (MSN) are a group of nanosized spherical or rod-like silica particles with different porous structures, varying in their pore volume, wall thickness, and surface area. Their unique mesopores with large internal space make these nanomaterials widely applicable to catalysis, biosensing, and, in particular, drug delivery.^{17–20} We report here the accelerated oxidation of epinephrine in the presence of two MSN, SBA-15 and MCM-41. In order to emphasize the effect of mesoporous structure on the oxidative reaction, silica microspheres (SMS) were also tested in this study as a control.

Experimental Section

Materials and Methods. Epinephrine was purchased from Sigma-Aldrich (St. Louis, MO). Cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), and poly(ethylene oxide)-block-poly(butylene oxide)-block-poly(ethylene oxide) (P123, EO₂₀PO₇₀EO₂₀) were obtained from Sigma-Aldrich. Sodium dithionite (MW 174.11) was purchased from Fluka, UK. Working solutions (1.0 M) were freshly made in distilled water (dH_2O) under argon shield. Phosphate-buffered salt solution (1× PBS, without Mg^{2+} and Ca^{2+} , pH = 7.4) was purchased from Mediatech (Herndon, VA). When studying the pH effect on epinephrine oxidation, all water solutions with different pH values were first prepared $2 \times$ higher in [H⁺] (by making different NaOH or HCl solutions) and then immediately mixed with the equal amount of 250 μ M epinephrine at time zero. The same mixture with no addition of epinephrine was tested to confirm the desired starting pH values.

Synthesis of Mesoporous Silica Nanomaterials. MCM-41 and SBA-15 type mesoporous silica nanospheres (MSN) were synthesized by supramolecular self-assembly.²¹⁻²⁴ SBA-15 was synthesized as reported by using P123 in acidic solution as a template. 21,22 Typically, a solution of EO₂₀PO₇₀EO₂₀/2 M HCl/ tetraethoxysilane (TEOS)/ $H_2O = 2:60:4.25:15$ (mass ratio) was stirred at 40 °C for 20 h and then aged at 80 °C for another 24 h. The solution was then filtered, and the solid was washed with a large amount of water resulting in "as-synthesized" SBA-15. The synthesis of MCM-41 was done by following a reported procedure with minor modification.^{23,24} 4.0 g (1.1 mmol) CTAB was dissolved in 960 mL of Millipore water and then mixed with 14 mL of 2.0 M NaOH solution. The solution was moderately stirred at 80 °C for 30 min; stirring was followed by the addition of 22.6 mL (101.2 mmol) TEOS. After stirring for another 2 h at 80 °C, the solution was filtered and the precipitate was rinsed with Millipore water ($4 \times 80 \text{ mL}$), followed by rinsing with ethanol (4 \times 80 mL) and drying in the oven at 80 °C. For both SBA and MCM materials, the as-synthesized samples were calcined, to remove the template, at 600 °C for 6 h with a heating ramp of 1 °C/min, followed by a cooling ramp of 2 °C/min.



Figure 1. Transmission electron micrographs of (A) calcined MCM-41, (B) calcined SBA-15, and (C) silica nanospheres.

Synthesis of Dense Silica Nanomaterials. Dense silica nanospheres were synthesized by following the well-known Stöber method.^{25–27} Typically, 5.84 g of TEOS was added to 10 mL of 5 M ammonia solution (30 wt %) in a mixture of 50 mL ethanol and 3.6 g Millipore water under stirring to allow the hydrolysis of TEOS. After stirring for 12 h, a colloidal solution of silica spheres was obtained. The solution was centrifuged at 6500 rpm for 5 min and the supernatant was dumped. The precipitate was then redispersed in a mixture of 20 mL Millipore water and 20 mL ethanol. The centrifugation and redispersing process was repeated several times to remove any unreacted chemicals. The resulting silica microspheres were dried before further modification.

Results and Discussion

Synthesis and Characterizations of Nanoparticles. The synthesis of MCM-41 or SBA-15 was achieved by following the procedures reported in refs 21-24. The synthesis was followed by calcinations to remove the template, leading to the formation of ordered mesoporous silica nanoparticles (MSN). The transmission electron microscopy (TEM) images (Figure 1A,B) show that the MCM-41 nanomaterials are rather regular spherical nanoparticles of ~600 nm diameter, while the SBA-15 materials are irregularly shaped particles of various sizes. These two kinds of MSN were further characterized by nitrogen physisorption measurements, and both showed type IV isotherms with steep capillary condensation steps (Supporting Information Figure S1), confirming the presence of particles with highly uniform mesopores. For MCM-41, measured BET surface area was $1143 \text{ m}^2/\text{g}$. Porosity by the Barrett-Joyner-Halenda (BJH) method exhibits average pore diameter of 28.4 Å and cumulative pore volume of $0.91 \,\mathrm{cm}^3/\mathrm{g}$. For SBA-15 nanomaterials, the measured surface area is 883 m²/g, the average pore width is 63.8 Å, and the cumulative pore volume is 1.10 cm³/g. These results are summarized in Supporting Information Table S1. Thus, compared to SBA-15, MCM-41 has a bigger surface area but smaller pore volume and width. Spherical silica nanoparticles were synthesized by following the procedure stated in the Experimental Section. After adjustment of ammonia concentrations, it produced quite symmetrical silica spheres with a diameter of \sim 300 nm (Figure 1C). The nitrogen physisorption measurement detected a surface area

⁽¹⁵⁾ Kitamura, Y.; Mifune, M.; Takatsuki, T.; Iwasaki, T.; Kawamoto, M.; Iwado, A.; Chikuma, M.; Saito, Y. Catal. Commun. 2007, 9, 224–228.

 ⁽¹⁶⁾ Ryan, T. P.; Miller, D. M.; Aust, S. D. J. Biochem. Toxicol. 2006, 8, 33–39.
 (17) De, M.; Ghosh, P. S.; Rotello, V. M. Adv. Mater. 2008, 20, 1–17.

⁽¹⁸⁾ Slowing, I. I.; Trewyn, B. G.; Giri, S.; Lin, V. S.-Y. Adv. Funct. Mater. 2007, 17, 1225–1236.

⁽¹⁹⁾ Trewyn, B. G.; Giri, S.; Slowing, I.; Lin, V. S.-Y. Chem. Commun. 2007, 3236–3245.

⁽²⁰⁾ Vallet-Regi, M.; Balas, F.; Arcos, D. Angew. Chem., Int. Ed. 2007, 46, 7548-7558.

⁽²¹⁾ Zhao, D.; Feng, J.; Huo, Q.; Melosh, N.; Fredrickson, G. H.; Chmelka, B. F.; Stucky, G. D. *Science* **1998**, *279*, 548–552.

⁽²²⁾ Zhao, D. Y.; Huo, Q. S.; Feng, J. L.; Chmelka, B. F.; Stucky, G. D. J. Am. Chem. Soc. **1998**, *120*, 6024–6036.

⁽²³⁾ Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. *Nature* **1992**, *359*, 710–712.

⁽²⁴⁾ Huh, S.; Wiench, J. W.; Yoo, J. C.; Pruski, M.; Lin, V. S.-Y *Chem. Mater.* **2003**, *15*, 4247–4256.

⁽²⁵⁾ Stöber, W; Fink, A.; Bohn, E. J. Colloid Interface Sci. 1968, 26, 62-69.

⁽²⁶⁾ Shi, Y.-L.; Asefa, T. *Langmuir* **2007**, *23*, 9455–9462.

⁽²⁷⁾ Asefa, T.; Shi, Y.-L. J. Mater. Chem. 2008, 18, 5604–5614.



Figure 2. (A) UV absorption spectrum for epinephrine for concentrations of $20-240 \ \mu$ M. (B) Band intensities for epinephrine as functions of epinephrine concentration with fits. $\blacklozenge = 195-205 \text{ nm}$, $\blacksquare = 210-290 \text{ nm}$, $\blacktriangle = 270-290 \text{ nm}$. Linear fits are given for the first two, whereas the third is fit to two intersecting lines.

of 11 m²/g. Such a small value reflects the nonporosity of SMS nanoparticles, corroborating their solid-cored structure.

UV-vis Spectra of Epinephrine. We first investigate the spectra of epinephrine in solutions, using UV-vis spectrophotometry. Different concentrations of epinephrine (20–240 μ M) in dH₂O were freshly prepared and immediately scanned at wavelengths from 800 to 200 nm. Results are shown in Figure 2A. In each spectrum, two absorbance peaks can be observed, one at \sim 200 nm and the other at \sim 280 nm. In addition, one absorbance shoulder was evident, in the wavelength region 210-230 nm. All the spectra are concentration-dependent, showing stronger intensity resulting from higher concentration of epinephrine. However, changing concentration also produces a small shift in the wavelength of the absorbance peak. Because of the shift of the peak, we report here integrated absorbance over a band, i.e., the sum of intensities for a number of wavelengths, in lieu of the absorbance at a single wavelength. In Figure 2B, the summed intensities for 195-205 nm (11 intensities), 210-230 nm (21 intensities), and 270–290 nm (21 intensities) are plotted as functions of epinephrine concentration.

For the 195–205 nm band, the sum of intensities (diamonds) first increases with addition of epinephrine, but then apparently reaches a plateau. The plateau is associated with an instrumental limitation. We therefore fit the sum of intensities covering 195–205 nm into a two-line function of [epinephrine] (i.e., [EP]): $S_{195-205}$ (the sum of intensities) = 0.250 [EP] (for [EP] < 129.6 μ M) and $S_{195-205}$ = 32.4 (for [EP] \geq 129.6 μ M). Only the slope of the first line is used in later measurements. For the 210–230 nm and 270–290 nm bands (squares and triangles), the integrated intensities increased with [EP] over the entire concentration range. The fitting of the sum of intensities for each individual band vs [EP] into a linear function passing through zero gives: for the 210–230 nm band, $S_{210-230}$ = 0.121 [EP] (r^2 = 0.979); and for the 270–290 nm band, $S_{270-290}$ = 0.044 [EP] (r^2 = 0.983). Therefore,

Scheme 1. Mechanism of Epinephrine Oxidation, Undergoing the Sequential Loss of One Electron to Generate Adrenchrome as the Final Product^{13,31}



except for the instrumental limitation, the integrated intensities for each band demonstrated proportionality to [EP]. Since the ratio of any two of the three variables ($S_{195-205}$, $S_{210-230}$, and $S_{270-290}$) remains unchanged, these three absorbances very likely result from the same epinephrine species, which prevails in the freshly made epinephrine solutions.

pH-Dependent Oxidation Rate of Epinephrine. Extensive studies on transient free radical forms of catecholamines have enabled a good understanding of in situ oxidation of epinephrine to adrenochrome through a sequential one-electron loss, although it is possible that other intermediate steps also exist.^{11-13,28-30} Scheme 1 illustrates the oxidative process of epinephrine to produce adrenchrome. Instead of reacting directly with O₂ dissolved in solutions, epinephrine is much more liable to be oxidized by the hydroxyl radical (•OH, a product of the reaction of O_2^- and H_2O_2) to form *o*-semiquinone radical.¹¹⁻¹⁶ Under neutral or acidic conditions, the production of O_2^- and sogenerated •OH is very low, which results in a very slow oxidation of epinephrine. Conversely, in alkaline solutions, active O_2^- can be formed quickly, followed by its quick reduction to H₂O₂ that further reacts with O_2^- to produce $\cdot OH$. Moreover, as the oxidative product, adrenochromes are unstable in alkali medium due to their easy reaction with OH⁻. These processes add up to a rapid oxidation of epinephrine at elevated pH. It has been reported that the rate of epinephrine oxidation at pH = 8 is almost four times higher than that at pH = 4.³¹

We thus examine the pH effect on the oxidation rates of epinephrine, by monitoring the absorbance of the generated adrenochrome at 480 nm over time.^{13,32} A series of 125 μ M solutions of epinephrine in dH₂O with different pH (pH = 1–12) was prepared. The pH was adjusted by additions of either HCl or NaOH. Absorbance readings were taken every 5 s for 30 min, and the results are shown in Figure 3. At acidic conditions, the production of adrenochrome is minimal (Figure 3A,B). The gradual decrease in intensity, which appears similar in most of these conditions, may be due to the drift in the instrument. However, the decrease over the first few minutes likely represents an induction time, required for the formation of \cdot OH to oxidize the epinephrine. For a quantitative measure of the epinephrine oxidation, we obtain linear fits to only the data for $t \ge 200$ s. This gives the slopes (×10⁻⁷ s⁻¹): -3.12 ± 0.07 ($r^2 = 0.85$), -4.85 ± 0.11 ($r^2 = 0.87$), 0.37 ± 0.08 ($r^2 = 0.07$), -0.88 ± 0.07

⁽²⁸⁾ Borg, D. Proc. Natl. Acad. Sci. U.S.A. 1965, 53, 829-836.

⁽²⁹⁾ Ball, E. G.; Chen, T.-T. J. Biol. Chem. 1933, 102, 691-719.

⁽³⁰⁾ Adak, S.; Bandyopadhyay, U.; Bandyopadhyay, D.; Banerjee, R. K.

Biochemistry 1998, 37, 16922–1693.
 (31) Parnass, S. M.; Baughman, V. L.; Miletich, D. J.; Albrecht, R. F. Anesthesiology 1987, 67, A280.

⁽³²⁾ Heacock, R. A. Chem. Rev. 1959, 59, 181-237.



Figure 3. Absorbance of adrenochrome (product of epinephrine oxidation) at 480 nm as a function of time. The different plots correspond to different pH of the original epinephrine solutions. The slopes of the plots give the rate of epinephrine oxidation. (A) \bigcirc = pH 1, \blacktriangle = pH 2, \bigtriangledown = pH 3, \blacklozenge = pH 4, \blacklozenge = pH 5, \square = pH 6; (B) \square = pH 6, \blacktriangle = pH 7, \bigtriangledown = pH 8, \bigcirc = pH 9; (C) \bigcirc = pH 9, \blacksquare = pH 10, \triangle = pH 11, \blacklozenge = pH 12.

 $(r^2 = 0.36), -0.13 \pm 0.09 (r^2 = 0.01), \text{ and } 3.65 \pm 0.13 (r^2 = 0.72)$ for solutions at pH = 1, 2, 3, 4, 5, and 6, respectively. At pH = 7-10 (Figure 3B,C), the absorbance at 480 nm first declines a little bit, but then increases linearly with time. (Note the difference in scale between Figures 3A,B,C). As before, the initial drop could be due to an induction time required to generate active oxygen radicals. These plots were fit to V-shaped functions (the overall r^2 values are all greater than 0.98); in all cases, the bottom of the V was at ~220 s, suggesting that a certain fixed time is required for the fast oxidization of epinephrine to begin under these neutral or weakly alkaline conditions. The slopes after the minimum, when [adrenochrome] increases linearly with time, were (×10⁻⁶ s⁻¹) 1.20, 3.68, 3.51, and 2.53 for the solutions of pH = 7, 8, 9, and 10, representing an order-of-magnitude increase in rate over the slopes at non-acidic conditions.

In solution with pH = 11 (Figure 3C), the production of adrenochrome drops off initially and then quickly increases, showing the rapid oxidization of epinephrine. Later, it declines again, probably due to the instability of high concentrations of adrenochrome in this solution. Further evidence for the destruction of adrenochrome is the shape of the plot for pH 11. Excluding the first decreasing part before oxidation occurs, we fit the increasing part of the data to a cubic, i.e., [Intensity] = $(-9.6 \times$ $10^{-9}t^{2} + (2.2 \times 10^{-5})t - 3.4 \times 10^{-3}$ (75 < t < 1030 s, $r^{2} = 0.99$). The rate of adrenochrome production is then equal to (2.2 \times $10^{-5} - 1.9 \times 10^{-8} t$) s⁻¹, much higher than those at smaller pH. At pH = 12, the oxidation of epinephrine starts immediately. After a sharp increase, the production of adrenochrome slows down abruptly, so that the concentration almost levels off. By fitting these data to a two-line function, we get the slope for the rapid oxidation 3.3 \times 10 $^{-5}$ and the slope for the slow one 9.9 \times 10^{-7} (overall r^2 value = 0.95). The apparent "slow oxidation" is really a net rate of production of adrenochrome, i.e., its rate of production minus that of destruction. These results show that the oxidation of epinephrine is highly pH-dependent, and the rate of adrenochrome production is clearly higher at higher pH. It is worth noting that the mathematical fitting we have conducted helps to quantitatively interpret oxidation profiles of epinephrine under various conditions. In particular, the slope calculated from each fitting represents the oxidation rate of epinephrine and makes the comparisons between different catalysts easier.

Effect of Silica Nanoparticles on Epinephrine Oxidation. In the following experiments, we measure the rates of epinephrine oxidation in dH₂O with or without silica nanoparticles. 10 mg/mL SBA-15, MCM-41, or silica spheres were individually dispersed in dH₂O and sonicated until well-suspended. Each suspension was then centrifuged, and the resulting supernatant was mixed with 125 μ M freshly prepared epinephrine solution and instantly collected for the spectroscopic scan. Our previous studies on drug adsorption by silica nanoparticles showed that centrifugations were not sufficient to remove all nanoparticles from the supernatant.³³ Therefore, these experiments measure epinephrine oxidation in the presence of these silica nanoparticle residues.

The results are shown in Figure 4. The absorbance at 480 nm was fit to a linear function of t in each case, with the slope giving the rate of epinephrine oxidation. The different intercepts in the beginning of oxidation reveal the "pseudo-adsorption" due to the scattering of 480 nm radiation from the nanoparticle residues; only the slopes are significant. In the absence of nanoparticles, epinephrine (solid curve) oxidizes to adrenochrome at a rate of $(10.00 \pm 0.07) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.98)$. With particles present, the oxidation rate becomes $(9.76 \pm 0.06) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.99)$ for MCM-41 addition (long-dashed curve), $(15.74 \pm 0.08) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.99)$ for SBA-15 addition (hatched curve) and $(9.89 \pm 0.10) \times 10^{-7} \text{ s}^{-1} (r^2 = 0.96)$ for silica sphere addition (short-dashed curve). Obviously, the presence of MCM-41 or silica spheres does not accelerate the oxidation,

⁽³³⁾ Tao, Z.; Xie, Y.; Toms, B.; Goodisman, J.; Asefa, T. Manuscript Submitted.



Figure 4. Absorbance at 480 nm due to adrenochrome as a function of time for epinephrine solutions without (solid line) and with silica nanoparticles. Long-dashed line = addition of MCM-41, hatched line = addition of SBA-15, short-dashed line = addition of silica spheres.

whereas SBA-15 exhibits an exceptional ability to enhance the oxidation rate of epinephrine to adrenochrome (by 57.4%), although all the oxidations proceed very slowly in dH₂O. The same experiments done with no epinephrine show that the slopes of absorbance at 480 nm vs t (due to nanoparticles) are essentially zero for all nanoparticles (data not shown).

We next examine the oxidation of epinephrine in PBS, with or without silica nanoparticles. The experiments were performed the same as the above, except dH₂O was replaced with PBS solutions. Results are shown in Figure 5. With no nanoparticles added (hatched line), epinephrine oxidizes much more quickly in PBS than in dH₂O. The absorbance due to the produced adrenochrome increases at a rate of 5.04×10^{-5} s⁻¹, over 50 times higher than in dH₂O. This enhancement of oxidation rate could be due to the weak acidity of $dH_2O(pH = 6)$; as we have shown, oxidation is slower at lower pH. The higher ionic strength in PBS solutions (~0.15 M, as in physiological condition) may contribute to this increased oxidation velocity as well. The addition of silica spheres (short-dashed line) leads to a rate of oxidation of epinephrine to adrenochrome of $5.19 \times 10^{-5} \text{ s}^{-1}$, so there is no sign of silica spheres accelerating the oxidation in PBS. However, with either MCM-41 (solid line) or SBA-15 (long-dashed line) nanoparticles present, the oxidation is clearly enhanced. These curves cannot be fit by straight lines, since the apparent rates decrease with time. The later reductions in the rate of production of adrenochrome reflect the instability of this product in these solutions. By fitting each absorbance vs t into a quadratic form, we find [Intensi $ty]_{MCM} = (-1.42 \times 10^{-8})t^2 + (9.61 \times 10^{-5})t + 8.16 \times 10^{-3}(r^2 > 10^{-3})t^2 + 10^{-3}(r^2 > 10^{-3})$ 0.99) and [Intensity]_{SBA} = $(-1.39 \times 10^{-8})t^2 + (8.60 \times 10^{-5})t + 1.68 \times 10^{-2} (r^2 > 0.99)$. Thus, in the presence of MCM-41 and SBA-15, the oxidative reaction of epinephrine to adrenochrome occurs at a rate of $(9.61 \times 10^{-5} - 2.84 \times 10^{-8} t)$ s⁻¹ and $(8.60 \times 10^{-5} - 2.84 \times 10^{-8} t)$ s⁻¹ $10^{-5} - 2.78 \times 10^{-8} t$) s⁻¹, respectively. The initial rates are 9.61 × 10^{-5} s⁻¹ and 8.60 × 10^{-5} s⁻¹, both higher than that for epinephrine oxidation in PBS with no nanoparticles. The same experiments done without epinephrine show that the slopes of absorbance at 480 nm vs t in PBS (due to nanoparticles) are essentially zero (data not shown).

To further investigate the accelerated oxidation of epinephrine by MSN in PBS and/or dH_2O , we perform experiments in which dithionite is added into epinephrine solutions in different time



Figure 5. Absorbance of adrenochrome at 480 nm as a function of time, from epinephrine solutions in PBS, with and without silica nanoparticles. Hatched line = no particles, short-dashed line = silica spheres, solid line = MCM-41, and long-dashed line = SBA-15.



Figure 6. Absorbance of adrenochrome at 480 nm as a function of time, from solutions of dithionite + epinephrine. Dotted curve = dH_2O with dithionite added 5 min before epinephrine, long-dashed curve = dH_2O with dithionite and epinephrine added simultaneously, short-dashed curve = PBS with dithionite added 5 min before epinephrine, solid curve = PBS with dithionite and epinephrine added simultaneously.

sequences. Sodium dithionite (Na₂S₂O₄) is well-known to consume O₂ molecules rapidly in aqueous solutions, producing bisulfate and bisulfite, where the reaction involves free radical intermediates, including $\cdot O_2^{-.34,35}$ In the present experiments, dithionite was first dissolved in dH₂O at a high concentration (1 M) and then diluted into dH₂O or PBS solutions (final concentration [S₂O₄²⁻] = 500 μ M), either 5 min prior to, or simultaneously with, the addition of 125 μ M epinephrine. Results are shown in Figure 6. Time zero corresponds to the addition of epinephrine. In dH₂O, dithionite added 5 min before (dotted curve) or at the same time as (long-dashed curve) the epinephrine addition prevents production of adrenochrome (which was low in any case). However, in PBS solutions, rapid oxidation of

⁽³⁴⁾ Creutz, C.; Sutin, N. Inorg. Chem. 1974, 13, 2041-2043.

⁽³⁵⁾ Tao, Z.; Goodisman, J.; Souid, A.-K. J. Phys. Chem. A 2008, 112, 1511– 1518.

epinephrine occurs when epinephrine is added concurrently with dithionite (solid curve), whereas epinephrine is not oxidized if it is added 5 min after the dithionite addition. The addition of dithionite alone to either dH₂O or PBS gives no rise in the absorbance at 480 nm (data not shown). Since $500 \,\mu\text{M}$ dithionite can scavenge dissolved O_2 in seconds and simultaneously generate radical intermediates like $\cdot O_2^{-,35,36}$ this result confirms that, rather than O₂ molecules, oxygen radicals are responsible for the rapid oxidation of epinephrine in a phosphate buffer environment. Concomitantly, it suggests that the accelerated epinephrine oxidations with MSN present in PBS are due to the formation and accumulation of reactive oxygen species by the nanoparticles. Apparently, MCM-41 or SBA-15 silica nanoparticles, but not silica spheres, provide a hotbed for oxygen radicals. In dH₂O, the epinephrine oxidation is not accelerated by dithionite, even when it is added simultaneously with epinephrine. A possible explanation for this is that dithionite generates, in addition to oxygen radicals that should be able to execute the epinephrine oxidation, HSO_3^- and HSO_4^- ions. In an unbuffered solution, these ions create an acidic environment, which undermines the reaction. In fact, the measured pH of a 500 μ M dithionite solution in dH₂O is 3.7 ± 0.1 .

Conclusions

We have measured the influence of mesoporous silica (MCM-41 and SBA-15) nanoparticles and dense silica nanoparticles on epinephrine oxidation, a pH-dependent reaction whose rate is small in acidic or neutral solutions but much larger at higher pH. While MCM-41 or silica spheres do not accelerate the oxidation in dH₂O, SBA-15 does, showing that the difference in the structures of nanomaterials leads to differing effects on the epinephrine oxidation. In contrast to MCM-41, SBA-15 has a unique microporosity and interconnectivity in the mesopore walls, which contributes to a substantial part of total surface area.³⁶

(36) Ryoo, R.; Ko, C. H. J. Phys. Chem. B 2000, 104, 11465-11471.

This feature could lead to more trapping of oxygen radicals inside the mesoporous channel, significantly enhancing the oxidation of epinephrine even in weak acid. In PBS solutions, the presence of SBA-15 and MCM-41 makes the oxidation even more rapid. Silica spheres have no noticeable influence on the oxidation in either PBS or dH₂O. The possibility that the catalytic effect of MSN could result from the residue of templating chemicals, however, can be excluded, since residues are removed by the postsynthesis calcinations. Experiments with dithionite, added either earlier than or at the same time as the epinephrine addition, show that fast oxidation takes place only when dithionite and epinephrine are simultaneously added into PBS solution. This confirms a vital role of oxygen radicals (probably $\cdot O_2^{-}$) in the oxidation of epinephrine. These oxygen radicals are likely to form and accumulate within the phosphate buffer or in the presence of mesoporous silica nanoparticles. Comparing the three kinds of silica nanoparticles applied, we note that mesoporous SBA-15 and MCM-41 materials own much larger surface area than solid silica particles do, whereas MCM-41 possesses a much narrower pore size (0.4-fold) than SBA-15. It seems, therefore, that large surface area plus characteristic mesoporosity and surface structures aid in the generation and deposit of oxygen radicals inside MSN particles, which catalyze the epinephrine oxidation in a favorable phosphate environment.

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Supporting Information Available: Nitrogen gas adsorption isotherms and pore size distributions of the mesoporous and dense silica nanospheres. This material is available free of charge via the Internet at http://pubs.acs.org.