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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 -O2-) 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 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 -O2- reacts with H2O2 at neutral or basic pH to give -OH (+ O2 + OH-), which may be the active species in epinephrine oxidation.14 In acid solutions, -O2- becomes HO2-, which reacts with H2O2 to give -OH (+ O2 + H2O).14 Many enzymes, notably catechol oxidase and amine oxidase, are known to catalyze these reactions. Several studies have reported that catecholamines when oxidized can release oxygen radicals.1,2,15,16

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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 an effort 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)-b-poly(butylene oxide)-b-poly(ethylene oxide) (P123, EO$_2$PO$_{10}$EO$_2$) 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$_2$O) 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× higher in [H$^+$] (by making different NaOH or HCl solutions) and then immediately mixed with the equal amount of 250 μM 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 nanoparticles (MSN) were synthesized by supramolecular self-assembly.21–24 SBA-15 was synthesized as reported by using P123 in acidic solution as template.21,22 Typically, a solution of EO$_2$PO$_{10}$EO$_2$/2 M HCl/tetraethoxysilane (TEOS)/dH$_2$O = 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× 80 mL), followed by rinsing with ethanol (4× 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 nanoparticles.

Results and Discussion

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 re-dispersed 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.

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UV–vis Spectra of Epinephrine. We first investigate the spectra of epinephrine in solutions, using UV–vis spectrophotometry. Different concentrations of epinephrine (20–240 μM) 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 ~200 nm and the other at ~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 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 195–290 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 195–230 nm into a two-line function of [epinephrine] (i.e., [EP]):

\[ S_{195-205} = 0.250 \text{[EP]} \text{ for } [\text{EP}] < 129.6 \mu M \]

and

\[ S_{195-205} = 32.4 \text{[EP]} \text{ for } [\text{EP}] \geq 129.6 \mu M. \]

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, \( S_{210-230} = 0.121 \text{[EP]} (r^2 = 0.979); \) and for the 270–290 nm band, \( S_{270-290} = 0.044 \text{[EP]} (r^2 = 0.983). \) Therefore, 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 adrenochrome. 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 \( \alpha \)-semiquinone radical.\(^{11-16}\) Under neutral or acidic conditions, the production of \( O_2^- \) and \( \alpha \)-generated \(-OH \) 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 \( H_2O_2 \) that further reacts with \( O_2^- \) to produce \(-OH \). Moreover, as the oxidative product, adrenochromes are unstable in alkaline medium due to their easy reaction with \( \text{OH}^- \). 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.\(^{13,12}\) A series of 125 μM 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 \(-OH \) to oxidize the epinephrine. For a quantitative measure of the epinephrine oxidation, we obtain linear fits to only the data for \( t \geq 200s \). This gives the slopes (×10\(^{-7}\) s\(^{-1}\)):

\(-3.12 \pm 0.07 (r^2 = 0.85), -4.85 \pm 0.11 (r^2 = 0.87), 0.37 \pm 0.08 (r^2 = 0.07), -0.88 \pm 0.07\)

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The slopes of the plots give the rate of epinephrine oxidation. The different intercepts in each case, with the slope giving the rate of epinephrine oxidation in \( \text{dH}_2\text{O} \) with or without silica nanoparticles. 10 mg/mL SBA-15, MCM-41, or silica spheres were individually dispersed in \( \text{dH}_2\text{O} \) and sonicated until well-suspended. Each suspension was then centrifuged, and the resulting supernatant was mixed with 125 \( \mu\text{M} \) 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. 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 \( (33) \) Tao, Z.; Xie, Y.; Toms, B.; Goodisman, J.; Asefa, T. Manuscript Submitted.

Effect of Silica Nanoparticles on Epinephrine Oxidation.

The rate of adrenochrome production is then equal to \((2.2 \times 10^{-3} - 1.9 \times 10^{-2}) \text{ s}^{-1}\), much higher than those at smaller pH. At \( \text{pH} = 12 \), 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 \( 3.3 \times 10^{-3} \) 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.

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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 dH$_2$O. 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 dH$_2$O 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 dH$_2$O. The absorbance due to the produced adrenochrome increases at a rate of $5.04 \times 10^{-5}$ s$^{-1}$ over 50 times higher than in dH$_2$O. This enhancement of oxidation rate could be due to the weak acidity of dH$_2$O (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 $5.19 \times 10^{-5}$ 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 $[\text{Intensity}]_\text{MCM} = (-1.42 \times 10^{-8})t^2 + (9.61 \times 10^{-5})t + 8.16 \times 10^{-3}$ ($r^2 > 0.99$) and $[\text{Intensity}]_\text{SBA} = (-1.39 \times 10^{-8})t^2 + (8.60 \times 10^{-5})t + 1.68 \times 10^{-2}$ ($r^2 > 0.99$). 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 $(8.60 \times 10^{-5} - 2.78 \times 10^{-8} t)$ s$^{-1}$, respectively. The initial rates are $9.61 \times 10^{-5}$ s$^{-1}$ and $8.60 \times 10^{-5}$ 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$_2$O, we perform experiments in which dithionite is added into epinephrine solutions in different time sequences. Sodium dithionite (Na$_2$S$_2$O$_4$) is well-known to consume O$_2$ molecules rapidly in aqueous solutions, producing bisulfite and bisulfite, where the reaction involves free radical intermediates, including O$_2^-$ (34,35). In the present experiments, dithionite first dissolved in dH$_2$O at a high concentration (1 M) and then diluted into dH$_2$O or PBS solutions (final concentration [S$_2$O$_4^{2-}$] = 500 μM), either 5 min prior to, or simultaneously with, the addition of 125 μM epinephrine. Results are shown in Figure 6. Time zero corresponds to the addition of epinephrine. In dH$_2$O, 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

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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 μM dithionite can scavenge dissolved O2 in seconds and simultaneously generate radical intermediates like -O2-, 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, HSO3- 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 μM 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.

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 -O2-) 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.

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