Protonation of Epigallocatechin-3-gallate (EGCG) Results in Massive Aggregation and Reduced Oral Bioavailability of EGCG-Dispersed Selenium Nanoparticles

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ABSTRACT: The current results show that epigallocatechin-3-gallate (EGCG), in the form of phenolic anions at pH 8.0, can effectively disperse selenium nanoparticles. However, at gastric juice pH (1.0), the EGCG-dispersed selenium nanoparticles (referred to as E-Se) extensively aggregated, so that nano features largely disappeared. This demonstrates that deprotonated phenolic anions of EGCG play an important role in maintaining E-Se stability and suggests that E-Se would suffer from reduced oral bioavailability. To validate this conjecture, size-equivalent E-Se and bovine serum albumin (BSA)-dispersed selenium nanoparticles (B-Se), whose physicochemical properties were not altered at pH 1.0, were orally administered to selenium-deficient mice. In comparison to B-Se, the bioavailabilities of E-Se as indicated with hepatic and renal glutathione peroxidase activity and hepatic selenium levels were significantly ($p < 0.01$) reduced by 39, 32, and 31%, respectively. Therefore, the present study reveals that size-equivalent selenium nanoparticles prepared by different dispersers do not necessarily guarantee equivalent oral bioavailability.

KEYWORDS: Bioavailability, epigallocatechin-3-gallate, nanoparticles, selenium

■ INTRODUCTION

Selenium is an essential trace mineral for humans. Dietary selenium exerts biological activities, mainly through its incorporation into a class of selenocysteine-containing proteins referred to as selenoproteins. Among the identified 25 types of selenoproteins, the biological functions of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), also known as selenoenzymes with antioxidant attributes, have been well-elucidated.[1,2] Because selenium has a narrow safety margin and the form of selenium has a significant impact on its bioactivities and toxicity, the issue of optimal selenium forms for supplementation has received considerable attention.[3]

Elemental selenium in the redox state of zero was previously considered to be biologically inert in general.[4] Newborn elemental selenium atoms generated from the redox system of sodium selenite and glutathione are liable to aggregate into bulky particles in the micrometer range. In the presence of bovine serum albumin (BSA) in the redox system, we showed that elemental selenium nanoparticles could be obtained[5] and the sizes were BSA-concentration-dependent.[6,7] We have demonstrated that BSA-dispersed selenium nanoparticles, referred to as B-Se herein, have significantly lower toxicity but still possess an equivalent capacity for increasing selenoenzyme activities compared to selenomethionine and methylselenocysteine, two naturally occurring organic selenium compounds used nowadays for selenium supplementation. Therefore, B-Se can be regarded as a novel selenium source with a reduced risk of selenium toxicity.[8,9] Subsequently, other investigators have found that certain kinds of substances, such as various types of polysaccharides,[10−12] melatonin,[13] polyethylene glycol,[14] and adenosine triphosphate,[15] can be used for preparing selenium nanoparticles. However, in terms of elevating selenoenzyme activities, none of these selenium nanoparticles has been evaluated. Whether these selenium nanoparticles possess optimal bioavailability, as with B-Se, constitutes an issue of concern in light of the fact that payloads encapsulated in poly(lactic acid)/poly(lactic-co-glycolic acid) (PLA/PLGA) nanoparticles often suffer from reduced oral bioavailability because of the degradation of PLA/PLGA in gastric fluid.[16]

Epigallocatechin-3-gallate (EGCG), a major component of green tea, has numerous health-promoting effects. It has been reported that EGCG encapsulated in nanoparticles could maintain its chemical stability and enhance its biological activity.[17,18] On the other hand, recent studies have shown that EGCG can act as a disperser for single-walled carbon nanotubes or gold nanoparticles in aqueous solution.[19,20] The present study addressed the question as to whether selenium nanoparticles could be dispersed by EGCG. Once this specific purpose was fulfilled, we evaluated whether the EGCG-dispersed selenium nanoparticles (referred to as E-Se) could maintain the same bioavailability as B-Se. We found that, although E-Se could be prepared, it suffered from reduced oral bioavailability compared to size-equivalent B-Se. Therefore, the present study revealed that size-equivalent selenium nanoparticles prepared by different dispersers do not necessarily guarantee equivalent bioavailability.
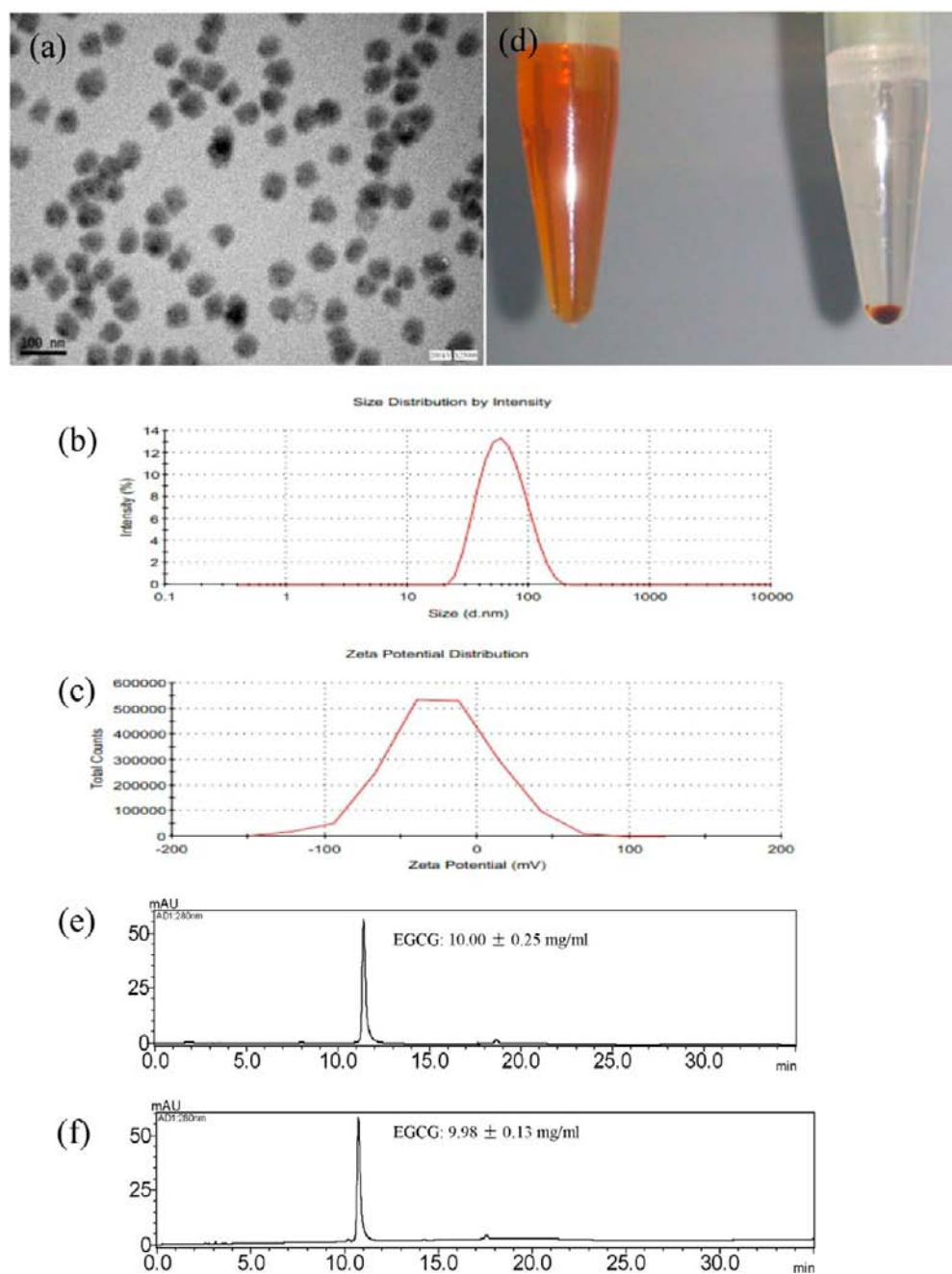
■ MATERIALS AND METHODS

Chemicals. NADPH, BSA, 5,5′-dithiobis(2-nitrobenzoic acid), reduced glutathione, glutathione reductase (from *Escherichia coli*),

Figure 1. Characterization of E-Se. (a) TEM image of E-Se. (b) Size distribution of E-Se. (c) ζ potential of E-Se. (d) E-Se solution before (left panel) and after (right panel) centrifugation. (e) HPLC analysis of EGCG before E-Se preparation. (f) HPLC analysis of EGCG after centrifugation of E-Se solution. Data are presented as the mean $\pm$ SEM (n = 3).

and 2,3-diaminonaphthalene were purchased from Sigma (St. Louis, MO). Auranofin was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). EGCG (purity over 98%) was a gift provided by Roche (Basel, Switzerland). Other chemicals were of the highest grade available.

Preparation and Characterization of E-Se. First, 156.0 mg of glutathione and 250.0 mg of EGCG were dissolved in 23.5 mL of deionized water. Then, 1.0 mL of sodium selenite solution (10.0 mg of Se/mL) was added to the reaction mixture with constant stirring for 3 min. The solution pH was adjusted to 8.0 with sodium hydroxide solution. The total volume was compensated to 25.0 mL with deionized water. The resultant red colloidal solution indicated successful formation of selenium nanoparticles. The as-prepared solution was stored in a −80 °C freezer instantly before usage. The particle size was assessed using transmission electron microscopy

(TEM, JEOL1400); the size distribution was evaluated using a laser particle analyzer (Malvern Mastersize 2000); and the ζ potential indicating colloid stability was measured using a particle size and ζ potential analyzer (Malvern Zetasizer Nano-ZS90).

Animals and Treatments. Male Kunming mice (18−22 g body weight) and their diet were purchased from the animal center of Anhui Medical University, People's Republic of China. The mice were housed in plastic cages in a room with controlled humidity (50 $\pm$ 10%) and temperature (22 $\pm$ 1 °C) on a 12 h light/dark cycle. The mice were allowed free access to food and water. All animal treatment procedures complied with the guidelines of Anhui Agricultural University for the care and use of laboratory animals.

Validation of Selenium Deficiency in the Experimental Mice. To confirm that the employed mice were selenium-deficient, 10 mice were randomly divided into 2 groups with 5 mice per group. The mice
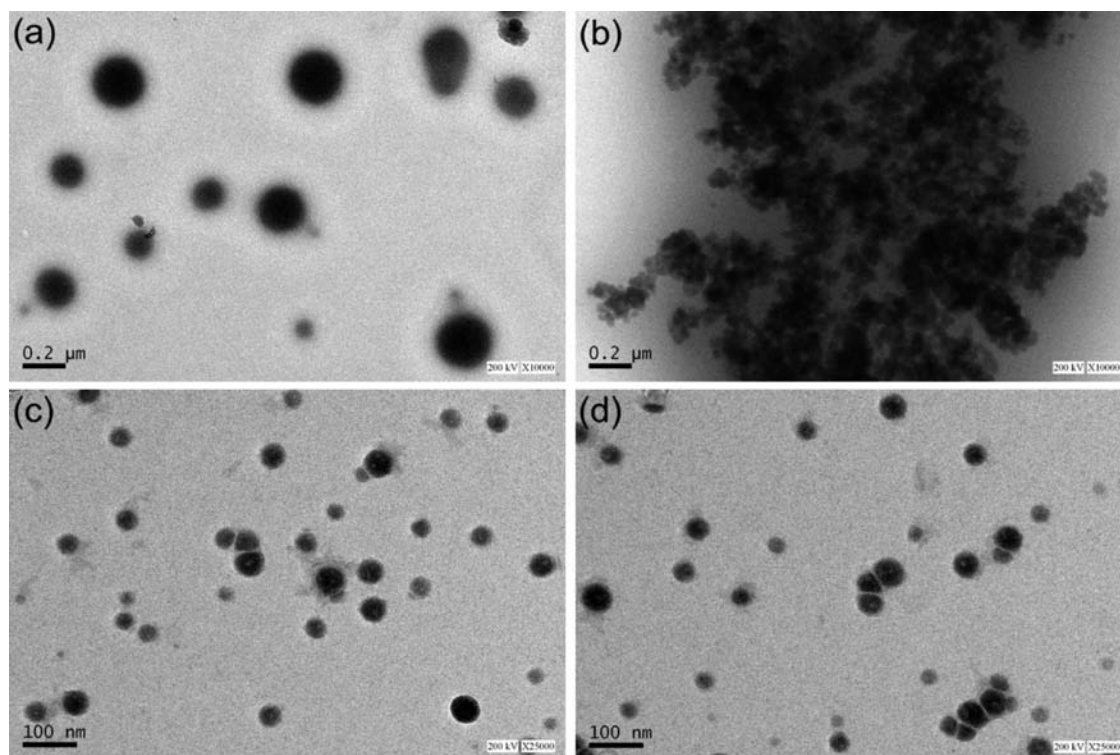
Figure 2. Sizes of E-Se and B-Se at different pH values. (a) Size of E-Se subjected to pH 1.0 for 3 min. (b) Size of E-Se subjected to pH 1.0 for 1 h. (c) Size of B-Se at pH 8.0. (d) Size of B-Se subjected to pH 1.0 for 1 h.

were orally administered with saline as a control or sodium selenite at a dose of 300 μg of Se/kg once daily for 7 consecutive days and then sacrificed 24 h after the last dose.

Bioavailability Comparison between E-Se and B-Se. B-Se with a size equivalent to that of E-Se was prepared using BSA according to the approach reported in our previous study.[6,7] A total of 30 mice were randomly divided into 5 groups with 6 mice per group. The mice were orally administered with saline as a control, E-Se, or B-Se at a dose of 40 or 80 μg of Se/kg once daily for 7 consecutive days and then sacrificed 24 h after the last dose.

Moreover, the bioavailability of E-Se and B-Se administered intraperitoneally was evaluated using 18 mice that were randomly divided into 3 groups with 6 mice per group. The mice were intraperitoneally injected with saline as a control, E-Se, or B-Se at a dose of 20 μg of Se/kg once daily for 7 consecutive days and then sacrificed 24 h after the last dose.

To investigate whether EGCG affects selenium bioavailability, 24 mice were randomly divided into 4 groups with 6 mice per group. The mice were orally administered with saline as a control or B-Se at a dose of 50 μg of Se/kg either alone or together with EGCG, which was orally administered 30 min ahead of selenium administration at a dose of 1.25 or 125 mg/kg, once daily for 7 consecutive days. Mice were sacrificed 24 h after the last dose.

Tissue Preparation and Selenium-Related Biomarker Assessments. At the end of each set of experiments, the mice were sacrificed by cervical dislocation. Livers and kidneys were excised and rinsed in ice-cold saline and then stored at −24 °C for less than 1 week. The tissue samples were homogenized with ice-cold phosphate buffer solution (150 mM, pH 7.2) containing 1 mM EDTA·Na$_2$ (1:9, w/v). The tissue homogenate samples were centrifuged at 15000g and 4 °C for 20 min, prior to biomarker assessments; the resultant supernatants were stored at −24 °C for less than 1 week. Protein levels were determined by the Bradford dye-binding assay with BSA as the standard. GPx and TrxR activities were measured according to the method by Smith and Levander.[21] Both GPx and TrxR activities were calculated in terms of micromoles of NADPH oxidized per minute per milligram of protein. The selenium content was assessed using a 2,3-

diaminonaphthalene-based fluorescent method.[22] Fluorescence was excited at 365 nm, and the intensity was recorded at 520 nm, with sodium selenite as a standard.

EGCG Assessment. For measuring the amount of EGCG in E-Se solution, after E-Se preparation, the pH of the E-Se solution was immediately adjusted to 5.5 to reduce the auto-oxidation of EGCG, then the red E-Se solution was centrifuged at 20000g and 4 °C for 30 min to fully precipitate selenium nanoparticles. The resultant colorless supernatant was instantly analyzed on a high-performance liquid chromatograph (HPLC, Shimadzu-20AD) equipped with a C18 reversed-phase column (250 × 4.60 mm, 5 μm particle size) that was maintained at 25 °C. The gradient elution was performed at a flow rate of 1.0 mL/min over 35 min using mobile phases containing 1% acetic acid and acetonitrile according to the following procedures: 0% acetonitrile, 0−2 min; 13% acetonitrile, 2−30 min; 30% acetonitrile, 30−32 min; and 13% acetonitrile, 32−35 min. EGCG was monitored at 280 nm.

Statistical Analysis. Data are presented as the mean ± standard error of the mean (SEM). The differences between groups were examined by Student's t test or one-way analysis of variance with the Newman−Keuls multiple comparison post-hoc test as appropriate using GraphPad software (Prism, version 5, San Diego, CA). A p value of less than 0.05 was considered statistically significant.

■ RESULTS

EGCG at Neutral to Alkaline pH Conditions Is an Effective Stabilizer for Selenium Nanoparticles. TEM showed that the selenium nanoparticles prepared using EGCG at pH 8.0 displayed spherical structures (Figure 1a) with a narrow size distribution and an average size of 60 nm, which was identical to the peak size, a surrogate of the mean size, evaluated using a laser particle analyzer (Figure 1b). The ζ potential of the E-Se solution was −23.7 mV (Figure 1c), suggesting that selenium nanoparticles in the EGCG solution had a tendency to repel each other against aggregation. The selenium nanoparticles could be effectively separated from the
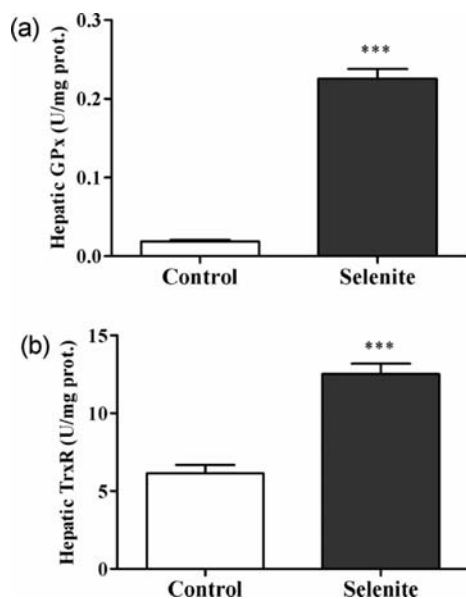
Figure 3. Validation of selenium-deficient mice. Mice were orally administered with saline as a control or sodium selenite at a dose of 300 μg of Se/kg once daily for 7 consecutive days. (a) Hepatic GPx activity. (b) Hepatic TrxR activity. Data are presented as the mean ± SEM ($n = 5$). (∗∗∗) $p < 0.001$ compared to the control.

EGCG solution after centrifugation at 20000g for 30 min (Figure 1d), and EGCG amounts remaining in the supernatant were equal to the level of EGCG added during E-Se preparation, as evidenced by HPLC analysis (panels e and f of Figure 1). Altogether, these results suggest that EGCG in the redox system of glutathione and selenite neither participates in selenite reduction nor covalently conjugates with the resultant selenium nanoparticles; rather, it actually acts as a stabilizer in neutral to alkaline pH conditions.

E-Se Suffers Rapid Destruction in Strong Acidic Conditions. When the E-Se pH was adjusted from the original 8.0 to a final pH of 1.0, selenium nanoparticles with an average size of 60 nm, as shown in Figure 1a, instantly increased their dimensions, reaching 300 nm within the first 3 min (Figure 2a), and unanimously agglomerated, so that nano features completely disappeared within 1 h (Figure 2b). A similar phenomenon was also observed at pH 2.5, but the extent of agglomeration was slightly attenuated, as compared to the case at pH 1.0 (data not shown). In sharp contrast, size-equivalent B-Se (average size of 60 nm; Figure 2c) did not suffer any destruction in the same acidic conditions (Figure 2d). We previously demonstrated that B-Se, with a size distribution of 20−60 nm and an average size of 36 nm, has comparable bioavailability relative to selenomethionine or methylseleno-cysteine.[8,9] The size of nanoparticles plays an important role in their biological activities, because smaller sized nanoparticles are more active than larger sized nanoparticles, in general. However, we revealed that B-Se with different sizes ranging from 10 to 200 nm did not show the expected size effect upon increasing selenoenzyme activities,[7] probably owing to the reason that the avidity of selenium uptake mechanisms in selenium-deficient cells may be largely increased to maintain the biosynthesis of selenoenzymes, which are highly funda-mental for redox homeostasis to override a potential entry advantage of smaller sized selenium nanoparticles.[23] Nonethe-less, we speculated that the bioavailability of E-Se would be
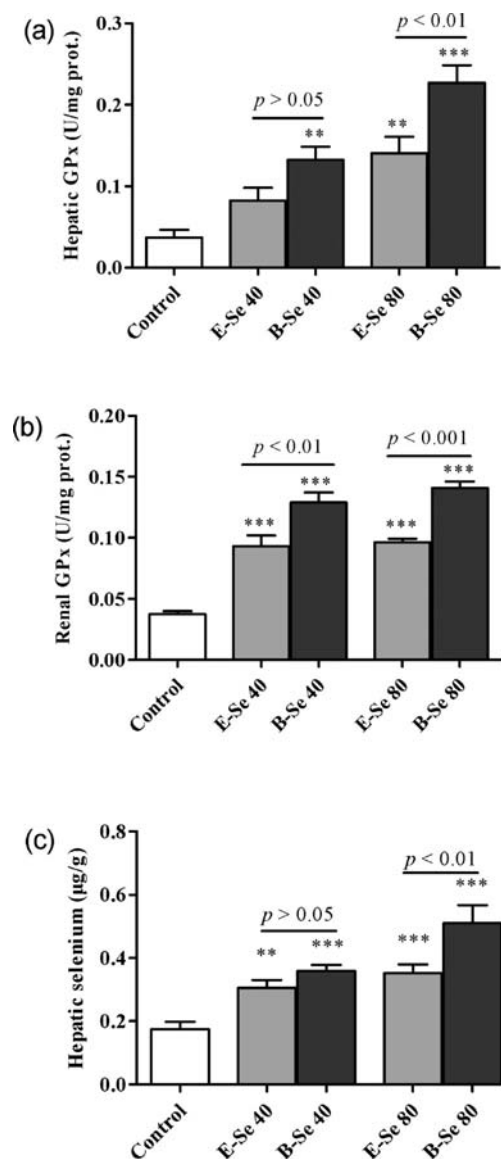
Figure 4. Bioavailability of E-Se and B-Se orally administered. Mice were orally administered with saline, E-Se, or B-Se at the dose of 40 or 80 μg of Se/kg once daily for 7 consecutive days. (a) Hepatic GPx activity. (b) Renal GPx activity. (c) Hepatic selenium level. Data are presented as the mean ± SEM ($n = 6$). (∗∗) $p < 0.01$ and (∗∗∗) $p < 0.001$ compared to the control.

reduced because the size of aggregated selenium nanoparticles in the investigated acidic environment, which is physiologically similar to gastric juice, was far greater than 200 nm, leading to the loss of nano features (Figure 2b). Therefore, we next examined this assumption using bioavailability experiments in mice.

Orally but Not Intraperitoneally Administered E-Se Has Reduced Bioavailability Compared to Size-Equiv-alent B-Se. To compare the bioavailability of different selenium forms, experimental animals must be selenium-deficient. To validate that the mice used in the present study were selenium-deficient, mice were orally administered with saline or sodium selenite at a high dose of 300 μg of Se/kg once daily for 7 consecutive days. Hepatic GPx and TrxR activities significantly increased after selenium treatment by 12- and 2-fold, respectively (panels a and b of Figure 3). The greater
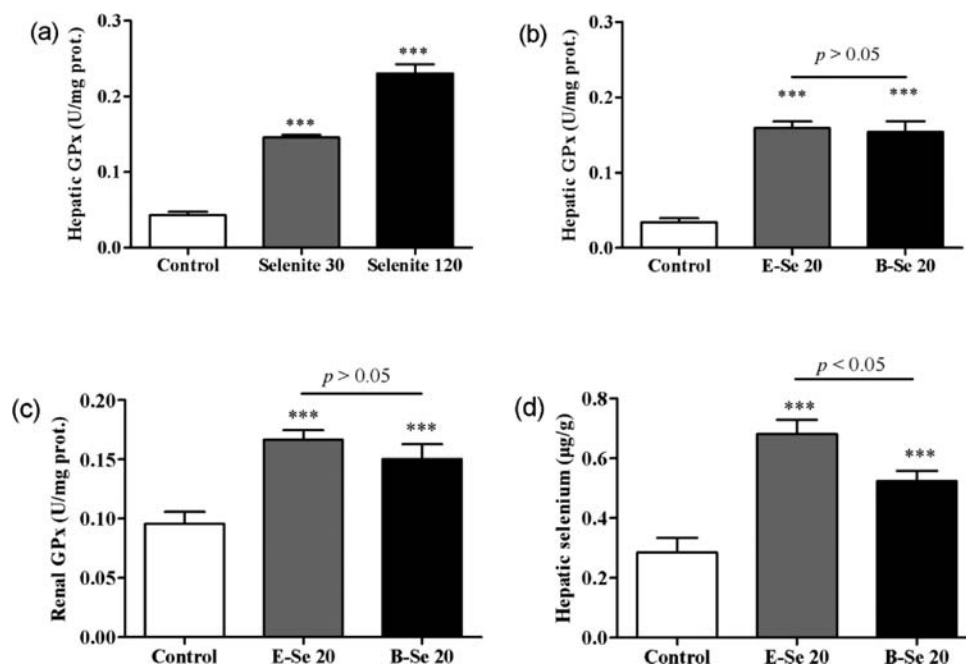
Figure 5. Bioavailability of E-Se and B-Se administered intraperitoneally. (a) First, mice were intraperitoneally injected with saline or sodium selenite at the dose of 30 or 120 μg of Se/kg once daily for 7 consecutive days and were sacrificed to measure hepatic GPx activity. Second, mice were intraperitoneally injected with saline, E-Se, or B-Se at a dose of 20 μg of Se/kg once daily for 7 consecutive days and then were sacrificed to measure (b) hepatic GPx activity, (c) renal GPx activity, and (d) hepatic selenium level. Data are presented as the mean ± SEM ($n = 6$). (∗∗∗) $p < 0.001$ compared to the control.

response of hepatic GPx and the lower response of hepatic TrxR to selenium supplementation agree with their inherent nature in the selenoprotein hierarchy.[24] Therefore, these commercially available mice could be employed for comparing the bioavailability of selenium without the need for additional selenium depletion treatment.

E-Se and B-Se were orally administered to selenium-deficient mice for 7 days at two lower nutritional dose levels, 40 and 80 μg of Se/kg, to avoid a high-dose engendered maximum of hepatic GPx activity that would override potential differences in bioavailability. B-Se caused dose-dependent increases in hepatic GPx activity and selenium retention (panels a and c of Figure 4). In comparison to B-Se at the same dose, E-Se at 80 μg of Se/kg showed a significantly reduced capacity for increasing hepatic and renal GPx activities as well as hepatic selenium retention (panels a−c of Figure 4). E-Se at 40 μg of Se/kg also exhibited a significantly reduced ability to increase renal GPx activity (Figure 4b). Although the two selenium forms did not display a significant difference in increasing hepatic GPx activity at 40 μg of Se/kg, only B-Se but not E-Se achieved a significant elevation compared to the control (Figure 4a). Therefore, E-Se indeed suffered from reduced bioavailability in general. The underlying mechanism is likely attributed to E-Se destruction in gastric fluid. Such a conjecture would be more convincing if E-Se and B-Se, provided through an administration route that bypasses gastric fluid, were able to achieve comparable bioavailability. Therefore, we next compared their bioavailability using the intraperitoneal injection route.

To find an appropriate intraperitoneally injected dose, which is able to increase but unable to saturate hepatic GPx activity, for the comparison of bioavailability, we performed a pilot study using sodium selenite. Intraperitoneal administration of selenite once daily for 7 consecutive days at doses of 30 and 120 μg of Se/kg caused a dose-dependent increase of hepatic

GPx activity (Figure 5a). Therefore, we compared E-Se and B-Se at a dose of 20 μg of Se/kg, which would be unlikely to induce maximum hepatic GPx activity. E-Se and B-Se were equally able to increase hepatic and renal GPx activities (panels b and c of Figure 5), and E-Se appeared to be more efficient than B-Se in increasing hepatic selenium retention (Figure 5d). Therefore, these results support the conjecture that E-Se destruction in gastric fluid causes reduced bioavailability.

To enhance this conclusion, a potential confounding factor that EGCG may reduce selenium bioavailability should be excluded. Therefore, we next investigated the impact of orally administered EGCG on the bioavailability of orally administered B-Se. While B-Se at a dose of 50 μg of Se/kg was able to effectively increase hepatic and renal GPx activities as well as hepatic selenium retention (panels a−c of Figure 6), the co-administration of EGCG at a dose of 1.25 mg/kg, which is equivalent to the ratio of selenium and EGCG in E-Se, or at a dose of 125 mg/kg, which amounts to the ratio of daily dietary selenium intake and average tea consumption in humans, did not generate a significant impact on the examined biomarkers (panels a−c of Figure 6). Therefore, it could be concluded that EGCG per se does not interfere with selenium uptake and selenoenzyme biosynthesis.

■ DISCUSSION

It has been demonstrated that pH has a tremendous impact on the protonation status and consequent electron-donating ability of EGCG.[25] In strong acidic pH conditions, the 3′,4′,4″-phenolic hydroxyl groups of EGCG, as shown in the right panel of Scheme 1, have no electron-donating ability. At neutral to alkaline pH conditions, the 3′,4′,4″-phenolic hydroxyl groups are transformed through deprotonation into phenolic anions with strong electron-donating ability (left panel in Scheme 1).[25] In the present study, selenium nanoparticles were successfully
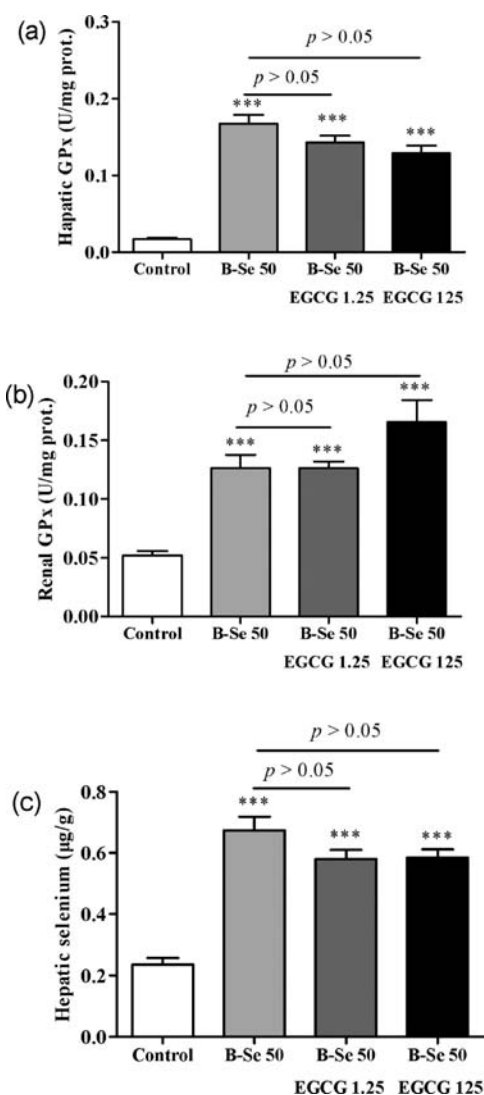
Figure 6. Impact of EGCG on the bioavailability of selenium nanoparticles. Mice were orally administered with saline or B-Se at a dose of 50 μg of Se/kg or the same amount of B-Se plus EGCG at a dose of 1.25 or 125 mg/kg once daily for 7 consecutive days. (a) Hepatic GPx activity. (b) Renal GPx activity. (c) Hepatic selenium level. Data are presented as the mean ± SEM ($n = 6$). (∗∗∗) $p < 0.001$ compared to the control.

prepared using EGCG at pH 8.0, wherein the 3′,4′,4″-phenolic hydroxyl groups of EGCG exhibited deprotonation with strong electron-donating ability. Nonetheless, EGCG and selenium did not form a covalent link because selenium nanoparticles could be almost fully precipitated by centrifugation (Figure 1d), whereas EGCG levels in the supernatant did not change (panels e and f of Figure 1). Because E-Se suffered rapid destruction at pH 1.0 (panels a and b of Figure 2), it could be inferred that the deprotonated 3′,4′,4″-phenolic anions with a strong electron-donating capacity in the EGCG molecule played a pivotal role in maintaining the stability of selenium nanoparticles. EGCG, as a disperser for selenium nanoparticles, relies on not only higher pH but also higher EGCG concentrations. At a molar ratio of 4:1 (EGCG/selenium), the resultant selenium nanoparticles exhibited a narrow size distribution without an obvious conglutination phenomenon (Figure 1a). However, if the molar ratio was reduced to 1:4, aggregation became noticeable (data not shown).

Not only can EGCG act as a disperser for fabricating gold nanoparticles, but it can also play a role in reducing sodium tetrachloroaurate ($NaAuCl_4$) into Au; EGCG with a redox potential of +420 mV and $AuCl_4^-$/Au with a redox potential of +990 mV constitute a thermodynamically favorable redox couple.[20] Although EGCG may act as a disperser of selenium nanoparticles, EGCG was unable to reduce selenite because the redox potential of SeO_3^{2-}/Se is +320 mV,[26] which is more negative than that of EGCG. Therefore, glutathione, whose GSH/GSSG redox potential is −240 mV, has been employed for reducing selenite to selenium atoms.[27]

In the present study, the size of E-Se was almost identical to that of B-Se. As expected, they showed comparable bioavailability when they were administered intraperitoneally (panels b−d of Figure 5). However, E-Se displayed reduced oral bioavailability compared to B-Se (panels a−c of Figure 4). Because EGCG did not interfere with the oral bioavailability of selenium nanoparticles (panels a−c of Figure 6), it could be conceived that the pronounced aggregation of selenium nanoparticles coated with EGCG seen at pH 1.0 in vitro (panels a and b of Figure 2) would occur in gastric fluid with a strong acidic pH as low as 1−2. It should be mentioned that E-Se, although it suffered from reduced oral bioavailability compared to B-Se, still had the capacity to increase selenium-related biomarkers (Figure 4), thereby suggesting that some proportion of selenium nanoparticles can be absorbed once they pass through the gastric fluid. The time-dependent aggregation profile of E-Se at pH 1.0 (panels a and b of Figure 2) and the fine materials in the massive aggregation atmosphere (Figure 2b) support this assumption.

At neutral or slightly alkaline pH, EGCG has a trend for auto-oxidation, leading to the formation of superoxygen anions, hydrogen peroxide, EGCG dimers, or polymers depending upon the temperature and time.[28] To avoid EGCG auto-oxidation, the E-Se solution was immediately pipetted into dozens of tubes after preparation, which were rapidly stored in a −80 °C freezer before daily treatments. The E-Se solution was withdrawn from each tube after thawing and instantly administered to mice. Notwithstanding these careful procedures for preventing EGCG auto-oxidation, we could not exclude that weak auto-oxidation of EGCG would occur. It is known that both EGCG and the oxidant polymers of catechins in black tea are able to stabilize gold nanoparticles,[29] and the oxidant polymers of catechins in pu'er tea can efficiently stabilize silver nanoparticles.[30] Consistently, we have observed that both EGCG and its auto-oxidation products, namely, EGCG polymers, have comparable capacity in terms of dispersing selenium nanoparticles (data not shown). Overall, the potential but very modest auto-oxidation of EGCG in our experiments did not destroy selenium nanoparticles. Therefore, the major factor leading to the reduced bioavailability of E-Se should be attributed to the protonation of EGCG in acidic pH conditions that cause massive aggregation of selenium nanoparticles.

According to the current results, two important implications are worth noting: (1) EGCG has been employed as a stabilizer for fabricating single-walled carbon nanotubes[19] and gold nanoparticles.[20] In conjunction with the present finding that EGCG is able to disperse selenium nanoparticles, it could be anticipated that EGCG, as a U.S. Food and Drug Administration (FDA)-approved biocompatible and bioactive compound, may also be employed for fabricating other types of nanomaterials. The present results suggest that nano-sized

Scheme 1. (Left Panel) E-Se Formation at Neutral to Alkaline pH and (Right Panel) E-Se Destruction at Strong Acidic pH
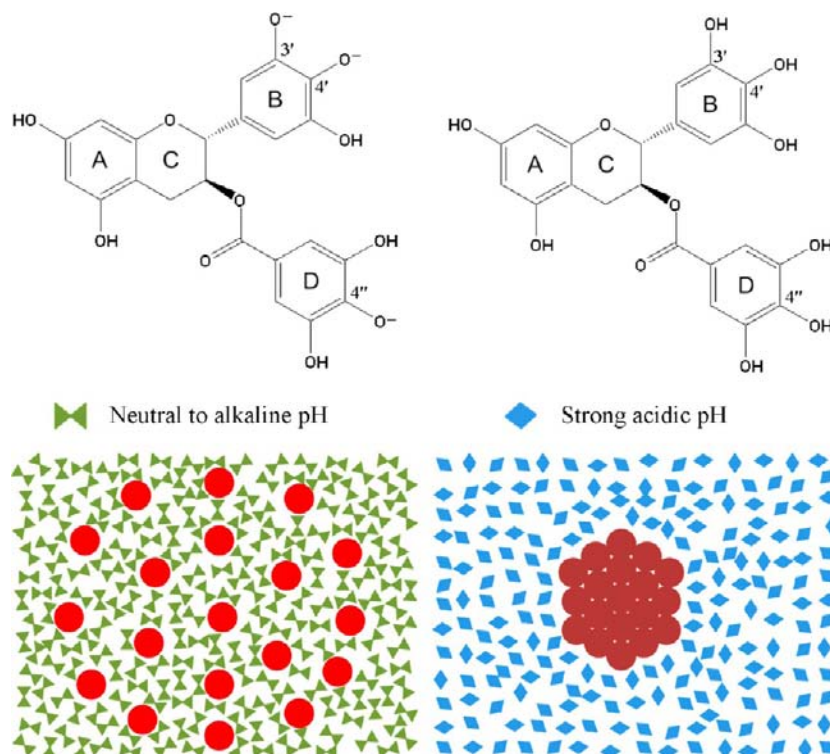
therapeutic or nutritional payloads coated with EGCG may suffer from reduced oral efficacy. (2) Hitherto, selenium nanoparticles have been prepared using different stabilizers, such as polysaccharides,[10−12] melatonin,[13] polyethylene glycol,[14] adenosine triphosphate,[15] BSA,[5] and herein EGCG; in view of bioavailability, none of these selenium nanoparticles, except B-Se[5−9] and E-Se (shown here), has been evaluated. The present results suggest that size-equivalent selenium nanoparticles prepared by different dispersers do not necessarily guarantee equivalent oral bioavailability.

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Author Contributions

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

BSA, bovine serum albumin; B-Se, BSA-dispersed selenium nanoparticles; EGCG, epigallocatechin-3-gallate; E-Se, EGCG-dispersed selenium nanoparticles; GPx, glutathione peroxidase; TrxR, thioredoxin reductase

REFERENCES

(1) Papp, L. V.; Lu, J.; Holmgren, A.; Khanna, K. K. From selenium to selenoproteins: Synthesis, identity, and their role in human health. *Antioxid. Redox Signaling* **2007**, *9*, 775−806.

(2) Rayman, M. P. The importance of selenium to human health. *Lancet* **2000**, *356*, 233−241.

(3) Whanger, P. D. Selenocompounds in plants and animals and their biological significance. *J. Am. Coll. Nutr.* **2002**, *21*, 223−232.

(4) Combs, G. F., Jr.; Garbisu, C.; Yee, B. C.; Yee, A.; Carlson, D. E.; Smith, N. R.; Magyarosy, A. C.; Leighton, T.; Buchanan, B. B. Bioavailability of selenium accumulated by selenite-reducing bacteria. *Biol. Trace Elem. Res.* **1996**, *52*, 209−225.

(5) Zhang, J. S.; Gao, X. Y.; Zhang, L. D.; Bao, Y. P. Biological effects of a nano red elemental selenium. *BioFactors* **2001**, *15*, 27−38.

(6) Huang, B.; Zhang, J.; Hou, J.; Chen, C. Free radical scavenging efficiency of Nano-Se in vitro. *Free Radical Biol. Med.* **2003**, *35*, 805−813.

(7) Zhang, J.; Wang, H.; Bao, Y.; Zhang, L. Nano red elemental selenium has no size effect in the induction of seleno-enzymes in both cultured cells and mice. *Life Sci.* **2004**, *75*, 237−244.

(8) Wang, H.; Zhang, J.; Yu, H. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. *Free Radical Biol. Med.* **2007**, *42*, 1524−1533.

(9) Zhang, J.; Wang, X.; Xu, T. Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: Comparison with Se-methylselenocysteine in mice. *Toxicol. Sci.* **2008**, *101*, 22−31.

(10) Chen, T.; Wong, Y. S.; Zheng, W.; Bai, Y.; Huang, L. Selenium nanoparticles fabricated in *Undaria pinnatifida* polysaccharide solutions induce mitochondria-mediated apoptosis in A375 human melanoma cells. *Colloids Surf., B* **2008**, *67*, 26−31.

(11) Zhang, Y.; Wang, J.; Zhang, L. Creation of highly stable selenium nanoparticles capped with hyperbranched polysaccharide in water. *Langmuir* **2010**, *26*, 17617−17623.

(12) Han, J.; Guo, X.; Lei, Y.; Dennis, B. S.; Wu, S.; Wu, C. Synthesis and characterization of selenium−chondroitin sulfate nanoparticles. *Carbohydr. Polym.* **2012**, *90*, 122−126.

7274

dx.doi.org/10.1021/jf4000083 | *J. Agric. Food Chem.* 2013, 61, 7268−7275

(13) Wang, H.; Wei, W.; Zhang, S. Y.; Shen, Y. X.; Yue, L.; Wang, N. P.; Xu, S. Y. Melatonin−selenium nanoparticles inhibit oxidative stress and protect against hepatic injury induced by Bacillus Calmette−Guérin/lipopolysaccharide in mice. *J. Pineal Res.* **2005**, *39*, 156−163.

(14) Zheng, S.; Li, X.; Zhang, Y.; Xie, Q.; Wong, Y. S.; Zheng, W.; Chen, T. PEG-nanolized ultrasmall selenium nanoparticles overcome drug resistance in hepatocellular carcinoma HepG2 cells through induction of mitochondria dysfunction. *Int. J. Nanomed.* **2012**, *7*, 3939−3949.

(15) Zhang, Y.; Li, X.; Huang, Z.; Zheng, W.; Fan, C.; Chen, T. Enhancement of cell permeabilization apoptosis-inducing activity of selenium nanoparticles by ATP surface decoration. *Nanomedicine* **2013**, *9*, 74−84.

(16) Jain, A. K.; Goyal, A. K.; Mishra, N.; Vaidya, B.; Mangal, S.; Vyas, S. P. PEG−PLA−PEG block copolymeric nanoparticles for oral immunization against hepatitis B. *Int. J. Pharm.* **2010**, *387*, 253−262.

(17) Li, B.; Du, W.; Jin, J.; Du, Q. Preservation of (−)-epigallocatechin-3-gallate antioxidant properties loaded in heat treated β-lactoglobulin nanoparticles. *J. Agric. Food Chem.* **2012**, *60*, 3477−3484.

(18) Hu, B.; Ting, Y.; Zeng, X.; Huang, Q. Bioactive peptides/chitosan nanoparticles enhance cellular antioxidant activity of (−)-epigallocatechin-3-gallate. *J. Agric. Food Chem.* **2013**, *61*, 875−881.

(19) Chen, Y.; Lee, Y. D.; Vedala, H.; Allen, B. L.; Star, A. Exploring the chemical sensitivity of a carbon nanotube/green tea composite. *ACS Nano* **2010**, *4*, 6854−6862.

(20) Shukla, R.; Chanda, N.; Zambre, A.; Upendran, A.; Katti, K.; Kulkarni, R. R.; Nune, S. K.; Casteel, S. W.; Smith, C. J.; Vimal, J.; Boote, E.; Robertson, J. D.; Kan, P.; Engelbrecht, H.; Watkinson, L. D.; Carmack, T. L.; Lever, J. R.; Cutler, C. S.; Caldwell, C.; Kannan, R.; Katti, K. V. Laminin receptor specific therapeutic gold nanoparticles (^{198}AuNP-EGCg) show efficacy in treating prostate cancer. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 12426−12431.

(21) Smith, A. D.; Levander, O. A. High-throughput 96-well microplate assays for determining specific activities of glutathione peroxidase and thioredoxin reductase. *Methods Enzymol.* **2002**, *347*, 113−121.

(22) Olson, O. E.; Palmer, I. S.; Cary, E. E. Modification of the official fluorometric method for selenium in plants. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 117−121.

(23) Peng, D.; Zhang, J.; Liu, Q.; Taylor, E. W. Size effect of elemental selenium nanoparticles (Nano-Se) at supranutritional levels on selenium accumulation and glutathione S-transferase activity. *J. Inorg. Biochem.* **2007**, *101*, 1457−1463.

(24) Berry, M. J. Insights into the hierarchy of selenium incorporation. *Nat. Genet.* **2005**, *37*, 1162−1163.

(25) Muzolf, M.; Szymusiak, H.; Gliszczyńska, S. A.; Rietjens, I. M.; Tyrakowska, B. pH-Dependent radical scavenging capacity of green tea catechins. *J. Agric. Food Chem.* **2008**, *56*, 816−823.

(26) Nickerson, W. J.; Falcone, G. Enzymatic reduction of selenite. *J. Bacteriol.* **1963**, *85*, 763−771.

(27) Watson, W. H.; Pohl, J.; Montfort, W. R.; Stuchlik, O.; Reed, M. S; Powis, G.; Jones, D. P. Redox potential of human thioredoxin 1 and identification of a second dithiol/disulfide motif. *J. Biol. Chem.* **2003**, *278*, 33408−33415.

(28) Lambert, J. D.; Elias, R. J. The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Arch. Biochem. Biophys.* **2010**, *501*, 65−72.

(29) Nune, S. K.; Chanda, N.; Shukla, R.; Katti, K.; Kulkarni, R. R.; Thilakavathi, S.; Mekapothula, S.; Kannan, R.; Katti, K. V. Green nanotechnology from tea: Phytochemicals in tea as building blocks for production of biocompatible gold nanoparticles. *J. Mater. Chem.* **2009**, *19*, 2912−2920.

(30) Loo, Y. Y.; Chieng, B. W.; Nishibuchi, M.; Radu, S. Synthesis of silver nanoparticles by using tea leaf extract from *Camellia sinensis*. *Int. J. Nanomed.* **2012**, *7*, 4263−4267.