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Remodeling of Selenium Metabolism through Adduct Formation of Selenoprotein P with Epigallocatechin Gallate

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Abstract

Selenoprotein P (SeP) is the major selenium transport protein in the blood and plays a central role in selenium metabolism by being involved in selenoprotein synthesis via selenium supply in various tissues. On the other hand, excess selenoprotein P, which is increased in patients with diabetes and other diseases, can be a malignant protein that causes metabolic disorders in various tissues through disruption of redox homeostasis. Therefore, developing methods to control selenium metabolism in physiological and pathological conditions are significant. In this study, we focused on epigallocatechin gallate (EGCg), an electrophilic plant component, and newly found that modification of the cysteine residue in SeP by this molecule inhibits its cellular uptake in SH-SY5Ycells. SeP-EGCg adduct failed to induce the expression of glutathione peroxidase, which is synthesized in cells by selenium supply through SeP. These results suggest that EGCg can be a candidate molecule to induce negative remodeling of selenium metabolism by inhibiting SeP incorporation into the cells.

Keywords: Selenium, Selenoprotein P, Epigallocatechin Gallate, Glutathione peroxidase

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Introduction

Selenium is an essential trace element and is incorporated into our bodies through a variety of dietary sources, including grains and seafood. The incorporated selenium is metabolized to inorganic selenide by multiple steps and the selenocysteine, which is a resulting product of this metabolic cascade, is translated into selenoprotein P (SeP) in the liver. SeP, a secreted protein, is released into the plasma and transports selenium to organs throughout the body[1]. It has been reported that approximately 53% of total plasma selenium is accounted for by SeP[2]. SeP is recognized by low-density lipoprotein (LDL) receptor-related proteins (LRPs) such as ApoER2 and taken up by endocytosis, then degraded by lysosomes to provide selenocysteine to the cytosol[3]. Selenocysteine undergoes further

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metabolism by selenocysteine lyase (Scly) to produce inorganic selenide and alanine[4]. This inorganic selenide is used for the synthesis of selenocysteine-tRNA^{sec} and translation of selenoproteins (glutathione peroxidase, etc.), which are important for redox homeostasis[5]. SeP is particularly important for selenium transport to the central nervous system[6]. It is known that radioisotope-labeled SeP is rapidly translocated to the brain when administered to selenium-deficient rat[6]. In addition, the selenium levels and selenoprotein expression in the brain are reduced in SeP KO mice [7, 8]. When SeP KO mice are fed with low-selenium diet, severe neurological deficits are observed[9]. On the other hand, we have found that SeP production is increased in type 2 diabetes patients and have found that inhibition of SeP by neutralizing antibodies improves diabetic pathology in mice[10]. Epidemiological studies revealed that plasma SeP levels increased in type 2 diabetes, and this increase has a significant correlation with insulin resistance[11]. Excess SeP is also related to the decrease in insulin secretion of pancreatic β cells[12]. Furthermore, recent evidence demonstrated the role of SeP expression in ferroptosis sensitivity using patient-derived primary glioblastoma cells[13]. Therefore, excess SeP may have adverse health effects, and it is important to suppress SeP in excessive conditions.

Metabolic remodeling is well understood, especially in the context of cancer studies, by examining metabolomic changes at the level of altered expression of metabolic enzymes. In contrast, its variation in selenium metabolism from selenium uptake to translation to selenoproteins, i.e., metabolic remodeling of selenium, is not well understood. We have recently found that sulforaphane, a phytochemical of broccoli sprouts, suppresses the expression of SeP by promoting its degradation in lysosomes[14]. We also reported that epigallocatechin gallate (EGCg) in tea suppresses its translation via induction of a long noncoding RNA CCDC152, which is located in the antisense region of the SeP gene, in the liver[15]. Therefore, such factors may contribute to the improvement of metabolic pathologies such as diabetes via inhibition of SeP, and in fact, the consumption of sulforaphane and tea has been reported to reduce the risk of type 2 diabetes[16, 17]. However, the details of the mechanism are unknown.

A common chemical property found in sulforaphane and EGCg is electrophilicity. Sulforaphane is covalently bound to cysteine residues in proteins via isothiocyanate groups. Epigallocatechin gallate, on the other hand, has an unsaturated carbonyl group and is known to covalently bind to cysteine and lysine residues in proteins in the same as sulforaphane[18, 19]. Since SeP has 16 cysteine and 10 selenocysteine residues in its polypeptide, the electrophilic molecule may target these residues. Therefore, we considered the possibility that these molecules could functionally inhibit SeP by forming adducts against SeP, thereby inhibiting not only its expression but also its selenium transport function. In this study, we applied the biotin-PEAC$_5$ maleimide labeling (BPML) assay to verify the formation of EGCg adducts on SeP and to elucidate their effects on selenium utilization[20].

Materials and methods

Chemicals

Biotin-PEAC5 maleimide (BPM) was obtained from Dojindo (Kumamoto, Japan) and EGCg was from BioVerde (E5737, Kyoto, Japan). All other chemicals used were of the highest quality commercially available.

SDS-PAGE and western blotting

The protein sample or cells were dissolved in 1% sodium dodecyl sulfate (SDS) buffer, and the protein concentration was determined using a DC protein assay kit (BioRad, CA, USA) with bovine serum albumin as the standard. The protein samples were separated by SDS-PAGE and subjected to Western blotting with the indicated antibodies. The antibodies used in this study are as follows. Anti-Glutathione peroxidase 1 (GPx1) is obtained from abcam (ab108427, Cambridge, UK), anti-GAPDH from (015-25473, WAKO, Osaka, Japan), and anti-SeP monoclonal antibody (BD1) was previously developed and validated[10].

Purification of human SeP

Human SeP was purified from human plasma as we previously reported[21]. Human frozen plasma was provided from the Japanese Red Cross Tohoku Block Blood Center (Human experiment approved No. 25J0012).

BPML and acidic-BPML assay

Biotin-PEAC5 maleimide labeling (BPML) assay and acidic-BPML (aBPML) were performed with slight modifications of the previous report (**Figure 1A**) [20]. Briefly, purified human SeP (20 μg/ml) was reacted with EGCg in 200 mM Tris-HCl (pH 7.0) and incubated at 37°C for 30 min to bind the compound to the protein. Then BPM (30 μM) was added and reacted at 37°C for 30 min for BPML. In the case of acidic-BPML, citric acid buffer (pH 3,200 mM) was added and incubated with BPN at 37°C for 30 min. The sample was desalted by the methanol/chloroform extraction. The desalted precipitate was dissolved by 1×Sample buffer [30 mM Tris-HCl (pH6.8), 5% glycerol, 1% SDS, 2.5% 2-ME] and heated at 95°C for 5 minutes. The sample solution was subjected to SDS-PAGE and detected by Avidin-HRP.

Cell culture

Human neuroblastoma SH-SY5Y cells were obtained from KAC (Kyoto, Japan). The cells were cultured in high glucose DMEM with 10% fetal bovine serum (FBS), 100 U/mL and 100 μg/mL penicillin–streptomycin in a humidified incubator under the conditions of 37 °C, 5% CO_2, and 95% ambient air. Before the experiments, the cells were seeded and pre-cultured for 24 hours in a culture plate. Selenium-deficient medium was prepared by adding 2 μM α-tocopherol, 2.5 mg/mL BSA, 5 μg/mL insulin, and 5 μg/mL transferrin to the DMEM without serum.

Data analysis

Western blotting images were obtained by chemiluminescent imaging system Lumino Graph (Atto, Tokyo, Japan). The bands corresponding to each protein molecular weight were cropped and shown in the figure.

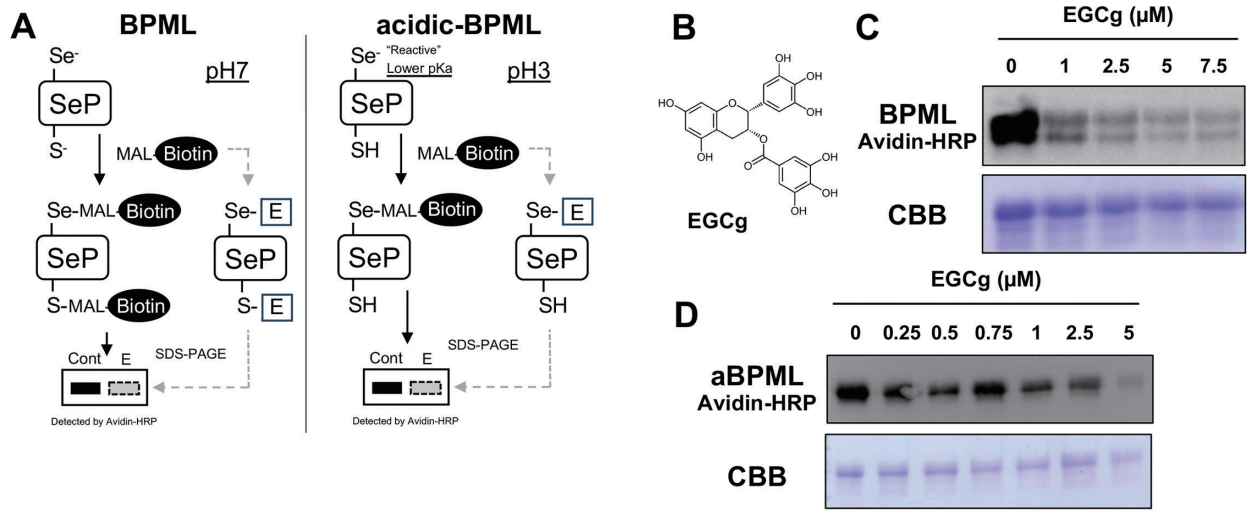

Figure 1. Detection of the binding of EGCg to SeP.
A. Scheme of BPML and acidic-BPML (aBPML).
B. Chemical structure of EGCg.
C. Purified SeP (50 nM) that had been precultured in the indicated concentration of EGCg was subjected to BPML assay.
D. Purified SeP (50 nM) was reacted with an indicated concentration of EGCg and subjected to an aBPML assay. Coomassie brilliant blue (CBB) stain was shown as the loading control.

Results and discussion

EGCg has an unsaturated carbonyl group in its structure and is reported to react with cysteine residues in proteins through covalent bonds (**Figure 1B**). First, to validate the adduct formation of EGCg to SeP, we verified with the BPML assay that detects modification of cysteine or selenocysteine residues. This assay uses biotin-PEAC5-maleimide (BPM), biotin bound to *N*-ethylmaleimide, which selectively reacts with cysteine or selenocysteine, thereby validating the competitive reduction of biotin labeling by the EGCg adduct[20]. The p*K*a value of free cysteine is around 8.3-8.6 and that of free selenocysteine is estimated at approximately 5.2[22]. Thus, under neutral pH conditions, the binding to cysteine and selenocysteine can be examined, while under acidic conditions (acidic-BPML; aBPML), the adduct to selenocysteine can be verified (**Figure 1A**). The BPML result indicates that purified SeP protein was modified by EGCg in a concentration-dependent manner at a concentration of more or less than 1 μM (**Figure 1C**), while in the aBPML, the biotin labeling was inhibited at concentrations more than 5 μM (**Figure 1D**). Under these conditions, the mol ratio of EGCg binding to SeP 50 nM was 1:20 for Cys and 1:100 or higher for selenocysteine. These results indicate that modification of SeP by EGCg occurs for both cysteine and selenocysteine residues but is more likely to occur at low concentrations for cysteine residues. Blood levels of EGCg in humans are known to reach approximately 0.6 μM after drinking 2-3 cup of green tea [23], thus the modifications of cysteine residues of SeP could occur *in vivo*.

Next, the selenium transport activity of SeP modified by EGCg was examined using SH-SY5Y cells, a human neuroblastoma, because this cell line is known to easily take up SeP and use it for selenium metabolism via ApoER2, a major SeP receptor[3]. First, SeP-EGCg conjugates were prepared *in vitro*, and their endocytosis was verified by adding them to the culture medium. The levels of SeP in whole cell lysates were used as an indicator of incorporated SeP. Interestingly, their incorporation was inhibited in a concentration-dependent manner by EGCg at the concentration where modifications to cysteine residues are detected and modifications to selenocysteine residues are not detected (**Figure 2A**). Furthermore, it was verified as the induction of the selenoprotein GPx1 as an indicator of selenium utilization after cellular uptake of SeP. Although SeP induced GPx1 expression of more than 1 nM, this induction was similarly suppressed by pre-incubation with EGCg with a 1:10 mol ratio (**Figure 2B**). Under these conditions, the amount of intracellular SeP was similarly reduced, suggesting that GPx induction was reduced due to uptake inhibition (**Figure 2A, B**). The domain of SeP recognized by ApoER2 is not the N-terminal domain but by the selenium-rich C-terminal domain, and binding to the YWTD β-propeller domain of ApoER2. The

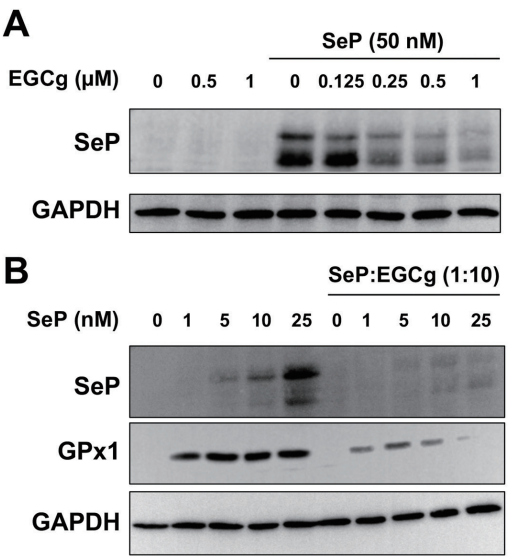

Figure 2. Inhibition of SeP uptake and production of GPx by EGCg.

A. Purified SeP was incubated with the indicated concentration of EGCg and then added to the incubation medium of SH-SY5Y cells that had been preculture in serum free medium for 24 hr. After 24 hr, the cells were harvested and subjected to Western blotting.

B. The cells were preculture in selenium deficient medium for 24 hr. Purified SeP was incubated with EGCg at the indicated 10 times higher mol ratio (SeP:EGCg = 1:10) and added to the medium and further incubated for 24 hr. Then the cell lysate was subjected to Western blotting. Control cells with EGCg are in the presence of EGCg (250 nM).

C-terminal domain of SeP contains 9 Cys residues and it is thought that the binding of ApoER2 to SeP is inhibited by modification of these Cys residues. For example, the CQC residue in the C-terminal domain is essential for the binding of SeP to ApoER2, and it has been reported that point mutations at C324 in CQC residue abolish the binding activity[24]. Therefore, it is possible that EGCg binds to the residue, thereby inhibiting uptake of SeP. The bindings of EGCg to Lys residues of SeP are also taken into account, and detailed molecular mechanisms will be elucidated by analyzing binding sites by LC/MS. In addition, when EGCg-SeP containing EGCg-bound selenocysteine is taken up to the cells and degraded by lysosomes, however, it is also possible that the EGCg-modified selenocysteine may not pass through the amino acid transporter of lysosome-cytosol. If it moves to the cytosol, it will be recognized by selenocysteine lyase (SCL), which is essential for selenium metabolism. Thus, the mechanism involved in inhibiting selenium metabolism might not only be the inhibition of uptake of SeP.

In summary, the present study indicates that EGCg forms an adduct to the Cys residues of SeP, presumably by which cellular uptake is inhibited and selenium metabolism is suppressed (**Figure 3**). In this study we used neuronal cells as a model, which show highly efficient SeP uptake, however, it will be necessary to examine the effects in hepatic cells in studies aimed at suppressing diabetes and other diseases that depend on the over-production of SeP. In addition, identifying which Cys residues at which sites are important is an open query, and these are the limitations of this study. At least, the results of this study would indicate that the development of covalent inhibitors that form targeted adducts at the Cys residues of SeP would be promising to trigger metabolic remodeling of selenium, and this could be a piece of important information.

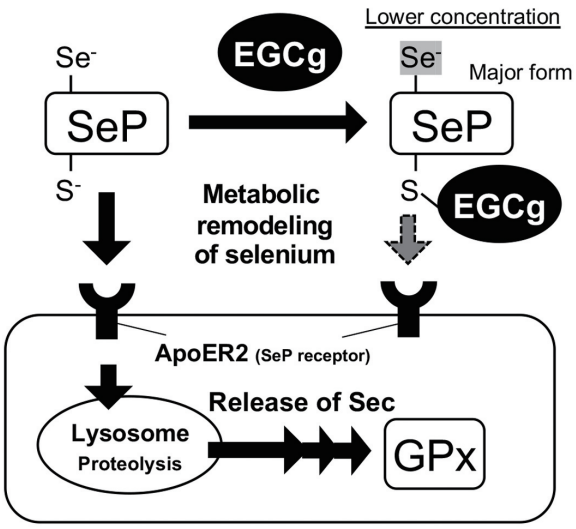

Figure 3. Metabolic remodeling of selenium by EGCg. EGCg covalently binds to cysteine rather than selenocysteine residues of SeP, thereby inhibiting its cellular uptake and suppressing GPx expression, an intracellular selenoprotein. These cascades evoked by EGCg are assumed to be an example of the metabolic remodeling of selenium.

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