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Review articles

Relationship between selenoprotein P and selenocysteine lyase: Insights into selenium metabolism

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ABSTRACT

Selenoprotein P (SelenoP) functions as a plasma transporter of selenium (Se) from liver to other tissues via incorporation into multiple selenocysteine (Sec) residues. Selenocysteine lyase (Scly) is an intracellular enzyme that decomposes Sec into selenide, providing Se for the synthesis of new selenoproteins. Both SelenoP and Scly are mostly produced by the liver. Previous studies demonstrated that male mice lacking SelenoP (SelenoP KO) or Scly (Scly KO) had increased or decreased total hepatic Se, respectively. While SelenoP regulation by Se is well-studied, Scly regulation by Se has not been reported. We hypothesize that Scly is negatively regulated by Se levels, and that absence of SelenoP jeopardizes Scly-dependent Se recycling. Using *in vitro* and *in vivo* models, we unveiled a tissue-specific Se regulation of Scly gene expression. We also determined that SelenoP, a considered source of intracellular Se, affects Scly expression and activity *in vitro* but not *in vivo*, as in the absence of SelenoP, Scly levels and activity remain normal. We also showed that absence of SelenoP does not increase levels of transsulfuration pathway enzymes, which would result in available selenocompounds being decomposed by the actions of cystathionine γ-lyase (CGL or CTH) and cystathionine β-synthase (CBS). Instead, it affects levels of thioredoxin reductase 1 (Txnrd1), an enzyme that can reduce selenite to selenide to be used in selenoprotein production. This study evaluates a potential interplay between SelenoP and Scly, providing further insights into the regulation of selenium metabolism.

1. Introduction

Selenium (Se) is a trace element acquired through the diet. Once it is absorbed by the digestive tract, it is carried mostly to the liver where it is metabolized, used for selenoprotein production, and redistributed to other tissues via the bloodstream. Hence, the liver is a central organ in Se metabolism. Se is used to synthesize the amino acid selenocysteine (Sec), found in the active site of selenoenzymes, but of unknown function in other selenoproteins. Sec is co-translationally incorporated into a peptide chain via highly specific incorporation machinery, requiring certain cis and trans factors to function properly.

Sec lyase (Scly) is an enzyme that specifically recognizes the Sec residue [1] and breaks it down into L-alanine and selenide using pyridoxal 5′-phosphate (PLP) as a cofactor for the reaction [2]. The Sec sources that feed into this reaction are believed to be dietary, by-products of Se metabolism, or following selenoprotein degradation. Decomposition of Sec releases selenide which re-enters Sec synthesis by binding to a phosphate to generate selenophosphate [3,4]. Selenophosphate is utilized to synthesize Sec on its cognate $tRNA^{[Ser]Sec}$, and incorporated into newly translated selenoproteins. Hence, Scly recycles the Se atom.

Tissue-specific Se distribution and recycling mechanisms that compensate for limited Se availability would be advantageous for viability and optimal health, especially in those tissues where the requirement for Se is high. Given its potential importance as a provider of Se for the selenoprotein biosynthesis pathway [5], it is surprising that the regulation of the Scly gene by Se is unknown. Interestingly, Se was promptly ruled out as a regulator of Scly activity [6,7]. In humans, no correlation was found between Se levels and Scly activity in the liver, kidney, heart, adrenal gland and muscle [7]. Nevertheless, Scly activity was undetected in the human brain but was found to be active in the brains of rodents. This discrepancy suggests either methodological differences or specific characteristic of the enzyme in human brain. In rats, diets with different chemical forms of Se (selenite, selenomethionine or selenocystine) did not alter Scly activity in the liver, kidney, testis and muscle, even though those Se diets contained concentrations of Se that ranged from 100-fold lower than the basal torula-yeast diet to borderline toxic (0.02 *vs.* 2 ppm) [6,8].

Despite the above reported Scly activity regulation, Scly mRNA levels could be negatively regulated by Se levels, a possibility that was

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our starting point for this study. We included two separate tissues, the liver, and the brain, in our analysis. The regulation of Scly by Se levels was addressed by *in vivo* analysis of Scly mRNA expression and activity in mice and *in vitro* in representative cell lines of these tissues treated with varying Se concentrations.

It is conceivable that protein degradation is one of the Sec sources for Scly action. The plasma selenoprotein P (SelenoP), that contains several Sec residues is, therefore, a possible candidate source of Sec to Scly. To assess the relationship between SelenoP and Scly, we evaluated the expression and activity of Scly in CRISPR-Cas9 SelenoP-depleted hepatic cells and a mouse model of whole-body Se deficiency, the SelenoP KO mouse. Moreover, we assessed the expression of enzymes from alternative pathways such as the transsulfuration pathway and selenite reduction pathways. These pathways metabolize selenocompounds into either selenide for selenoprotein synthesis or selenosugars for excretion and could be compensating for the disruption of SelenoP as a source of Se and consequently maintaining Se metabolism.

2. Materials & methods

2.1. Materials

All chemicals used were from either Sigma-Aldrich or Fisher Scientific unless otherwise noted.

2.2. Animals

SelenoP KO heterozygote mice were a kind gift from Dr. Raymond F. Burk from Vanderbilt University (Nashville, TN). Due to the fertility issues [9], heterozygote mice are used as breeders to obtain homozygote SelenoP KO for experiments. The development and characterization of SelenoP KO mice were previously described in detail [9]. SelenoP and C57BL/6J wild-type (WT) from The Jackson Laboratory (Bar Harbor, ME) were bred, born and raised in the Vivarium at the JABSOM – University of Hawaii. Animals were weaned at 18–21 days and euthanized at ten weeks old. All experiments were conducted in male mice and following the principles and procedures approved by the Institutional Animal Care and Use Committee of the University of Hawaii (protocol #04–017). After experiments, tissues were harvested including brain, lungs, liver, kidneys, testes, and ~5 cm segments of the small intestine. Fecal matter was removed from intestinal tissue samples, and tissues were rinsed in 1× phosphate-buffered saline (PBS) before immediate transfer to liquid nitrogen and subsequent storage at −80 °C.

2.3. Diets

Mice were placed on standard AIN-76A rodent diet (Research Diets, Inc.) containing adequate (0.25 ppm) Se until assigned for experiments. Special diets formulated for precise Se content (Research Diets, Inc.) have been previously described [10]. Briefly, pellets contained 12% kcal fat, 68% kcal carbohydrate, plus 0.08 ppm of Se in casein (low), or were supplemented with sodium selenite to contain a total of 0.25 ppm (adequate) or 1 ppm (high) of Se. Mice were maintained in specific Se diets for two months. SelenoP KO mice were maintained in standard mouse chow containing 0.25–0.3 ppm Se.

2.4. Cell culture

Human hepatocellular carcinoma (HepG2), murine hepatocellular carcinoma (Hepa1–6), and murine hippocampal neuronal (Ht22) immortal cell lines from American Type Culture Collection (ATCC) were maintained in 1× Dulbecco's Modified Eagle's Medium (DMEM; Gibco - Invitrogen Life Technologies) with 10% fetal bovine serum (FBS; Gibco - Invitrogen Life Technologies) and 1% penicillin-streptomycin-glutamine (PSG; Gibco - Invitrogen Life Technologies) at 37 °C with a 5% CO_2 atmosphere. Each batch of FBS was tested for its Se content by mass spectrometry, and the same lot used throughout the study. The lot for our experiments had ~30 nM total Se content. Sodium selenite was added to media, reaching final concentrations required for experiments. Cells were grown to 70–80% confluency and harvested after 48 h of selenite supplementation.

2.5. Development of SelenoP$^{-/-}$ Hepa1–6 cells

Hepa1–6 cells with 70–80% confluency had their media changed 24 h before transfection into antibiotic-free DMEM with 10% FBS. Cells were then transfected with either 5 μg of a mouse SelenoP CRISPR/Cas9-GFP plasmid or 1 μg of a scrambled (SCR)-GFP CRISPR/Cas9 plasmid (Santa Cruz Biotechnology) using Lipofectamine 2000 (Invitrogen Life Technologies) and following manufacturer's protocol. Upon transfection with the SelenoP CRISPR/Cas9-GFP plasmid, cells were cultivated with 20% FBS and supplemented with 40 nM sodium selenite, bringing the final media selenite concentration to 100 nM. After a 48 h incubation period, transfection was confirmed by fluorescent microscopy in an IX71 reflected fluorescence microscope (Olympus). Cell sorting was performed at the University of Hawaii COBRE Molecular and Cellular Immunology Core using a BD FACSAria apparatus (BD Biosciences). Prior to sorting, cells were trypsinized, centrifuged for 4 min at 200 rpm, and resuspended in a 400 μl of RPMI-1640 (Life Technologies) with no Phenol Red. Live cell discrimination was achieved by the addition of propidium iodide. Single cell sorting and single double discrimination were carried out by the BD FACSAria into 96-well plates containing 200 μl of DMEM with 20% FBS and 1% PSG. Single cells were cultured for three weeks after which successful colonies were transferred to larger plates containing DMEM with 10% FBS and 1% PSG. Cells sorted for the presence of the SelenoP CRISPR/Cas9-GFP plasmid and those sorted for the presence of the control SCR plasmid were grown to 70–80% confluency on 10 cm cell culture plates. DNA from cells was isolated using the DNeasy Blood & Tissue Kit (Qiagen). PCR was then performed using a HotStarTaq DNA Polymerase (Qiagen) following the recommended protocol. Primers flanked the 3′ TGA-rich region of the SelenoP gene. The PCR product was purified using an E.Z.N.A.® Gel Extraction Kit (Omega Bio-Tek) and sequenced at the Advanced Studies in Genomics, Proteomics and Bioinformatics Core Facility at the University of Hawaii to confirm successful SelenoP deletion.

2.6. RNA and quantitative PCR (qPCR) analysis

Cells were collected, and total RNA was extracted using Trizol (Invitrogen Life Technologies) followed by treatment with the RNeasy Clean-Up kit (Qiagen). Frozen tissues were pulverized on liquid nitrogen, and total RNA was also extracted by the Trizol method. One microgram of total RNA was then reverse-transcribed (High Capacity cDNA Reverse Transcription kit, Life Technologies/Applied Biosystems) and 10 ng cDNA used in qPCR with SybrGreen (Invitrogen Life Technologies). qPCR results were calculated using the Δ^{-Ct} method, normalized to 18S rRNA or GAPD mRNA as housekeeping genes. PCR primers were used at 100 nM and are listed in Table 1.

2.7. Western blot and antibodies

Protein was collected in CelLytic (Sigma) with a 1:100 ratio of Protease Inhibitor Cocktail Set III, EDTA-Free (EMD Millipore) for both tissue and cell lines. Tissues were sonicated 30–90 pulses, depending on the tissue, using a Sonic Dismembrator Model 100 (Fisher Scientific). Cell lines were placed on shakers for 15 min before scraping for protein. Both tissue and cell line samples were centrifuged at 12,000 rpm at 4 °C for 10 mins, where after supernatant containing protein was collected. Protein concentration was measured via Bradford assay using the Protein Assay Dye Reagent Concentrate (BioRad). 10–20 μg protein was