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Original Article

# Possible Role of Red Blood Cells in Selenocysteine Metabolism

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## Summary

Selenium, an essential trace element, is incorporated in the form of selenocysteine in selenoproteins. In mammals, inorganic and organic selenium compounds are the 2 sources of selenium. Generally, organic selenium compounds, such as selenocysteine and selenomethionine, are the main selenium sources. These selenoamino acids are obtained from the diet, which contains selenium-containing proteins, and absorbed by duodenum. Then, these selenoamino acids enter the blood through the duodenal capillaries. On the other hand, selenite, an inorganic selenium compound, is taken up by red blood cells (RBCs). However, the relation between organic selenium compounds and their uptake by RBCs is not yet examined. In this study, we showed that selenocysteine is taken up by RBCs. We also showed that selenide, which is produced by the reduction of selenite, binds to hemoglobin.

## Introduction

Selenium, an essential trace element, is cotranslationally incorporated as the 21st amino acid selenocysteine in selenoproteins. Selenoproteins, such as thioredoxin reductase[1)] and glutathione peroxidase[2)], contain a selenocysteine residue at the active site. These proteins are essential for normal embryonic development[3)], DNA biosynthesis[4)], redox regulation of certain transcription factors, regeneration of several antioxidants, and cellular redox control. The selenocysteine residue is directed by the UGA codon and $tRNA^{[Ser]Sec}$ and cotranslationally incorporated into the nascent polypeptide chain of selenoproteins. On the other hand, selenomethionine is nonspecifically incorporated in proteins. Both selenocysteine and selenomethionine in food proteins serve as the main sources of selenium for mammals ; these amino acids are absorbed by duodenal capillaries after digestion in the same manner as that of other amino acids[5,6)]. However, the subsequent fate of selenium-containing amino acids in blood is unclear. In contrast, it has already been established that selenite is taken up by the red blood cells (RBCs) through the band 3 protein (anion exchanger 1, AE1) and is subsequently released into plasma after reduction[7)]. In RBCs, selenite undergoes reduction to form selenide that is bound to hemoglobin (Hb)[7,8)]. However, little is known about the interaction between Hb and selenium-containing amino acids. In this study, we examined whether selenium-containing amino acids (selenocysteine, selenomethionine, and selenocystine) are absorbed by RBCs. The interaction between Hb and abovementioned selenium-containing amino acids was also investigated.

## Materials and Methods

### 1. Materials

L-Selenocystine was purchased from Sigma (St. Louis, MO). L-Selenomethionine, selenite, dithiothreitol (DTT), bovine hemoglobin, and 4,4'-diisothiocyano-2,2'-stilbene disulfonate (DIDS) were purchased from Nacalai Tesque (Kyoto, Japan). L-Selenocysteine was obtained by reduction of L-selenocystine by DTT. All other reagents used in this study were of analytical grade.

### 2. Preparation of blood and RBCs

Male Std : Wistar rats (8-week-old ; Shimizu Laboratory Supplies, Kyoto, Japan) were killed by cervical dislocation. Blood was collected from the abdominal aorta by using a heparinized syringe. RBCs were separated from plasma by centrifugation at $400 \times g$ for 15 min and washed with

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phosphate-buffered saline (PBS, pH 7.4) twice.

### 3. Uptake of selenite and selenocysteine by RBCs in plasma

A blood sample was incubated with selenocysteine (5 μM) or selenite (5 μM) at 37℃ for various periods. RBCs were separated from plasma by centrifugation at 8,000 × *g* for 10 sec and washed with phosphate-buffered saline (PBS, pH 7.4) twice. After the separation of the RBCs from plasma, the amount of total selenium in the RBCs was determined using 2,3-diaminonaphthalene as described by Watkinson[9]. Selenium concentration was expressed as the amount of selenium in RBCs divided by the volume of RBCs.

### 4. Uptake of selenite and selenocysteine by RBCs in PBS

RBCs were incubated with an equal volume of 10 μM selenocysteine and 10 μM selenite in PBS at 37℃ for various periods. After washing, the amount of total selenium in the RBCs was determined as described above. Selenium concentration was expressed as the amount of selenium in RBCs divided by the volume of RBCs.

### 5. Binding of selenium compound to Hb

Hb (20 nmol) was incubated in 1 mL of PBS (pH 7.4) containing either of selenite (20 nmol), selenocystine (20 nmol), selenide (20 nmol), or selenocysteine (20 nmol). After incubation for 1 min at 37℃, Hb was washed and isolated by ultrafiltration. The amount of Hb-bound selenium was determined 2,3-diaminonaphthalene.

## Results and Discussion

### 1. Uptake of selenite and selenocysteine by RBCs

The amount of selenium in the selenium-treated RBCs was plotted against the incubation time; selenocysteine uptake by RBCs was found to be more significant than that of other selenium-containing amino acids (Fig. 1A). Consistent with the previous reports[7], we found that selenite was taken up by RBCs within few minutes and then rapidly excreted into plasma after reduction (Fig. 1A). Since the excretion of reduced selenium from RBCs is known to depend on the presence of plasma[7,8], we attempted to examine the uptake of selenocysteine by RBCs in PBS instead of plasma. As expected, incubation of selenite with RBCs in the absence of plasma resulted in the accumulation of selenium in RBCs (Fig. 1B). Selenocysteine was also taken up by RBCs without significant subsequent return into PBS (Fig. 1B). The rate of uptake of selenocysteine by RBCs was lower in PBS than in plasma. This implies that an unidentified factor in plasma facilitates the uptake of selenocysteine by RBCs. In addition, the lag in the uptake of selenocysteine during the initial stages, as observed in Fig. 1A and 1B, might be due to a factor in plasma that competes with the uptake of selenocysteine. It has been reported that selenite is reduced by glutathione (GSH)[10] and thioredoxin reductase (TrxR)[11]. In a nonenzymatic reaction with selenite, GSH produces GSSeH and GSSeSG[10], which are then reduced to selenide ($Se^{2-}$) by glutathione reductase and TrxR[12,13]. The intracellular production of selenide might be associated with the efflux of selenite-derived selenium from RBCs. In contrast, the selenium in selenocysteine is in the most reduced form (R-SeH), thus it would not be affected by reductants and reductases such as GSH and TrxR, respectively. Hence, no efflux of selenocysteine-derived selenium was observed.

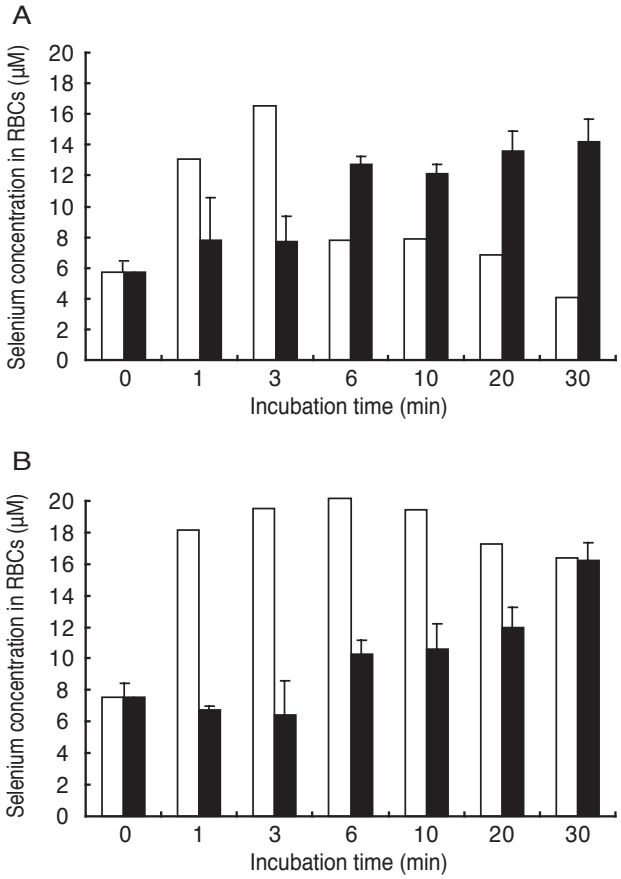


**Fig. 1** Uptake of selenite and selenocysteine by RBCs. (A) A blood sample was incubated with 5 μM selenocysteine (closed bar) or 5 μM selenite (open bar) for various periods. After the separation of the RBCs from plasma, the amount of total selenium in the RBCs was determined. (B) RBCs were incubated with an equal volume of 10 μM selenocysteine (closed bar) and 10 μM selenite (open bar) in PBS for various periods. After washing, the amount of total selenium in the RBCs was determined. The data of selenocysteine was presented as mean ± SEM from three independent experiments.

## 2. Accumulation of selenocysteine-derived selenium in RBCs

To further examine the accumulation of selenocysteine in RBCs without efflux, we incubated RBCs with selenocysteine in PBS, as described previously, and collected and resuspended them in plasma at 50 % (v/v). After incubation in plasma, we determined the amount of selenium in the RBCs. The result clearly shows that selenocysteine-derived selenium did not efflux from RBCs in plasma (Fig. 2). AE1, which is the most abundant anion transporter in RBCs, is responsible for the uptake of selenite by RBCs[7], and 4,4'-diisothiocyano-2,2'-stilbene disulfonate can inhibit this uptake. In general, amino acids are taken up by cells through a amino acid-specific transport system. As expected, 4,4'-diisothiocyano-2,2'-stilbene disulfonate had no effect on the uptake of selenocysteine by the RBCs (data not shown).

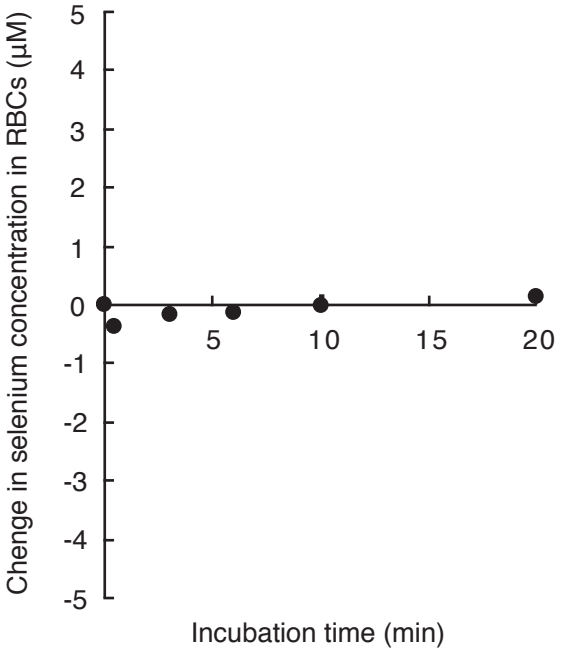


**Fig. 2** Time course of change in the amount of selenocysteine-derived selenium in RBCs. A blood sample was incubated with 10 μM selenocysteine for 10 min. The RBCs were separated from plasma. The selenium-treated RBCs were suspended in plasma for various times (0, 0.5, 3, 6, 10 and 20 min). After washing, the amount of selenium in the RBCs was determined.

## 3. Binding of selenium compounds to Hb

Hb is the most abundant protein in RBCs. In mammals, Hb constitutes about 97 % (about 5 mM) of dry weight of RBCs[14,15], which is comparable to the GSH level (about 2 mM)[16], and possesses iron in the heme-containing oxygen-binding sites[17]. The heme molecule at these sites also binds to carbon monoxide, cyanide, nitrogen dioxide, and sulfide[18,19]—a homolog of selenide. We examined whether selenocysteine binds to Hb. The results of our experiments revealed that selenide significantly binds to Hb (Fig. 3).

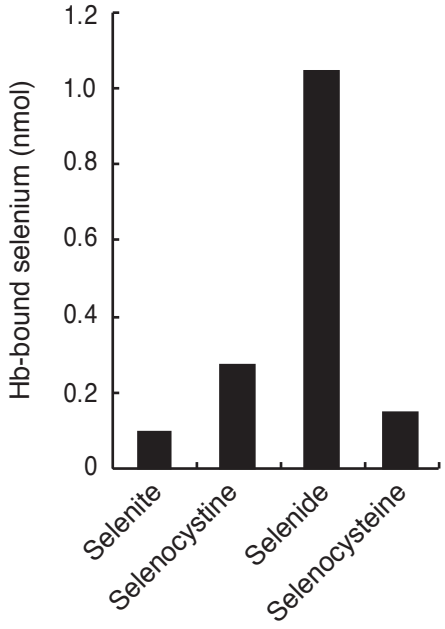


**Fig. 3** Binding of selenium compound to Hb. Hb (20 nmol) was incubated in PBS (pH 7.4) containing either of selenite (20 nmol), selenocystine (20 nmol), selenide (20 nmol), or selenocysteine (20 nmol). After Hb was isolated, the amount of Hb-bound selenium was determined.

Meanwhile, the affinity of selenocysteine toward Hb was lower than that of selenide (Fig. 3). In general, selenocysteine is degraded by selenocysteine lyase (SCL)[20,21] and released as elemental selenium that is reduced to selenide by GSH. However, no SCL activity was detected in the extracts of RBCs (data not shown). Thus, selenocysteine probably exists in a free form after being taken up by RBCs.

## Conclusion

In this study, we demonstrated that selenocysteine was taken up by RBCs. Our results also indicate that selenocysteine is not decomposed to selenide in RBCs ; therefore, selenocysteine does not excrete from RBCs. Although it is generally believed that both selenite and selenocysteine are converted into selenide, our data suggest that the fate of selenocysteine in RBCs is different from that of selenite. This study may provide a clue to the metabolism of selenocysteine in blood.

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