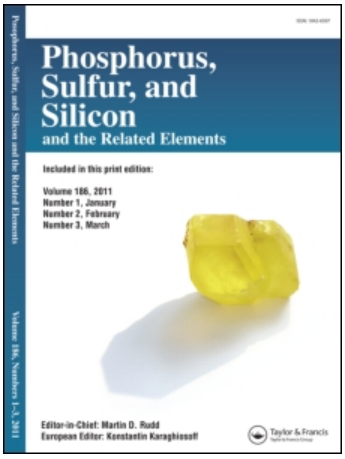

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Mechanisms of Mammalian Selenocysteyl-tRNA Synthesis

Takaharu Mizutani[a]; Hiroshi Kurata[a]; Kenichiro Yamada[a]
[a] Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan

To cite this Article Mizutani, Takaharu , Kurata, Hiroshi and Yamada, Kenichiro(1992) 'Mechanisms of Mammalian Selenocysteyl-tRNA Synthesis', Phosphorus, Sulfur, and Silicon and the Related Elements, 67: 1, 425 — 428
To link to this Article: DOI: 10.1080/10426509208045865
URL: http://dx.doi.org/10.1080/10426509208045865

Phosphorus, Sulfur, and Silicon, 1992, Vol. 67, pp. 425–428
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Printed in the United Kingdom

MECHANISMS OF MAMMALIAN SELENOCYSTEYL-tRNA SYNTHESIS

TAKAHARU MIZUTANI, HIROSHI KURATA & KENICHIRO YAMADA
Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467 Japan

Abstract The mechnisms of synthesis of mammalian selenocysteyl-tRNA were studied with murine active enzyme fractions. (^{75}Se)Selenocysteyl-tRNA was synthesized *in vitro* by 500kDa protein from seryl-tRNA and (^{75}Se)HSe^-, which should be activated with ATP by 20kDa protein. This and other factors in mamalian systems are compared with those in *Escherichia coli*.

INTRODUCTION

Some mammalian selenoproteins, such as glutathione peroxidase (GSHPx) (1) and type-I iodothyronine deiodinase (ID-I) (2), contain selenocysteine (Scy) in the active site. Scy corresponds to the opal nonsense codon UGA (1, 2). This Scy is co-translationally incorporated into these proteins. Scy is synthesized on natural suppressor seryl-tRNA and Scy on the tRNA is transferred to peptides on ribosomes. In order to clarify the mechanisms for synthesis of Scy-tRNA from seryl-tRNA, we partially purified some enzymes from murine liver. In this report, we show some properties of the enzymes.

RESULTS AND DISCUSSION

We first searched the activities in the liver extract. The activitiy was not found in organella, such as nuclei, microsomes and mitochondria, but found in a supernatant at 105,000 x *g*. A pattern of chromatography of the supernatant showed that the active fraction was eluted near the

position of Ser-tRNA synthetase (SerRS) on DEAE-cellulose chromatography. Then, we clarified a Se donor to synthesize Scy-tRNA using (^{75}Se). In the previous report (3), we used a mixture of glutathione selenotrisulfide and HSe^- as a Se-donor. Then, we found that HSe^- fraction was active, but glutathione selenotrisulfide was inactive in the *in vitro* Scy-tRNA synthesis. Therefore, in the following experiments, we used (^{75}Se)HSe^- as a Se-donor (4). The reaction product using (^{75}Se)HSe^- showed a clear spot of (^{75}Se)Scy on two dimensional TLC plates. This Scy on the tRNA was confirmed by five TLC systems.

The initial assay mixture contained supprressor tRNA, serine, ATP and SerRS. These are necessary to synthesize seryl-tRNA. Then, (^{75}Se)HSe^- and the enzyme fraction were added to the above mixture and incubated for 2hr at 30 ℃. (^{75}Se)Scy-tRNA synthesized was precipitated by addition of ethanol. The amino acid on the tRNA was liberated by weak alkaline treatment and analyzed by TLC with a solvent system, n-butanol-acetic acid-water (4:1:1). The materials, such as seryl-tRNA, ATP, HSe^- and the active enzyme fraction, in the reaction mixture are all essential to synthesize (^{75}Se)Scy-tRNA. In another experiment of the *in vitro* Scy synthesis, (^{3}H)serine or (^{14}C)serine were used as a precursor of Scy. A part of those radioactivity was found in the spot of Scy on two dimensional TLC plates. It was presumed that Scy-tRNA was produced from seryl-tRNA through phosphoseryl-tRNA (3, 5, 6). However, Scy-tRNA was synthesized in the absence of tRNA kinase. Therefore, we considered that Scy-tRNA was not synthesized through phosphoseryl-tRNA, but directly synthesized from seryl-tRNA. The apparent Km value of HSe^- in this reaction was 40nM. This value indicated that this enzyme had a high affinity to HSe^-. Se concentration in our body is 2.5μM and most Se in our body is a bound state. Free Se must be less. So, this low value (40nM) is reasonable.

The active fraction obtained from DEAE-cellulose was chromatographed on Sephacryl S-300, but the activity was

TABLE Comparison of some factors relating to the Scy incorporation into selenoproteins between mammalian and *E. coli*

Factors	Mammalian#	*E. coli*##
Codon	UGA	UGA
Precursor of Scy	Serine	Serine
tRNA for Scy	Opal sup $tRNA_{CmCA}$	SELC ($tRNA_{UCA}$)
	(90 nucleotides)	(95 nucleotides)
	A bulge on TFC arm	8 bps of AA-arm
		Long extra arm
SerRS	65.5kDa	48.4kDa
	(α_2 type)	(α_2 type)
Scy synthase	500kDa protein	SELA (650kDa)
	(labile with NH_2OH)	(PLP enzyme)
Se donor	HSe^-	HSe^-
HSe^- activator	20kDa protein	SELD (38kDa)
	with ATP	with ATP
Translation factor	(anti-RF)	SELB (68kDa)
		EF-like protein
Scy incorporation	Co-translational	Co-translational
Context mechanism	Unknown	A stem-loop at 3'-site of UGA
Final products	GSHPx	FDH
	ID-I	

From this report and the results by D. Hatfield.
From the results by A. Böck.

disapeared. However, the activity was recovered by mixing 500kDa protein and 20kDa protein eluted from S-300. The 20kDa protein bound the equimolar (^{75}Se)HSe^- and (^3H)ATP. This result suggests that ATP is necessary to a step of incorporation of HSe^-. But we have not confirmed the active intermediate from HSe^- and ATP. As a conclusion,

we considered from these results that mammalian Scy-tRNA was synthesized by 500kDa protein from seryl-$tRNA_{CmCA}$ and HSe^--ATP, which is activated by 20kDa protein. These two roles of 500kDa and 20kDa proteins are similar to the SELA and SELD in *E. coli*, respectively. Some mammalian factors relating to the Se incorporation into selenoproteins are compared with those in *E. coli*, as shown in TABLE.

The UGA codon is normally a termination codon, but the codon in mRNAs of selenoproteins corresponds to Scy. It is important to clarify the mechanisms to recognize the UGA codon on the mRNAs as Scy. On the standpoint of context effect near Scy UGA codon, bacterial mRNAs of formate dehydrogenase (FDH)(7) and glycine reductase (8) took a specific secondary structure at downstream of the UGA codon. But we could not find any similar structure on mammalian mRNAs of GSHPx (1)and ID-1 (2) at those downstream of UGA codon by computor analysis. The expression of human GSHPx in *E. coli* was unsuccessful (Sukenaga, personal communication) and this also suggested that the context mechanism near Scy UGA codon in mammalian was different from that on bacterial selenoprotein mRNAs.

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