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Insights into the chemical biology of selenium

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The long-sought pathway by which selenocysteyl-tRNA$^{[Ser]Sec}$ is synthesized in eukaryotes has been revealed. Seryl-tRNA$^{[Ser]Sec}$ is O-phosphorylated and SecS,

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a pyridoxal phosphate-dependent protein, catalyzes the reaction of O-phosphoseryl-tRNA$^{[Ser]Sec}$ with monoselenophosphate to give selenocysteyl-tRNA$^{[Ser]Sec}$. ^{1}H-{^{77}Se} HMQC-TOCSY NMR spectroscopy has been developed to detect the selenium-containing amino acids present in selenized yeast after protease XIV digestion. An archived selenized yeast sample is found to contain the novel amino acid S-(methylseleno)cysteine in addition to selenomethionine. Arsenite and selenite react with GSH to form $(GS)_2AsSe^-$. The structure of this compound has been determined by EXAFS, ^{77}Se NMR and Raman spectroscopic and chromatographic studies. Its formation under biological conditions has been demonstrated.

INTRODUCTION

Selenium plays an important role in biology. It has long been recognized as an essential mineral. Selenocysteine is the twenty-first amino acid incorporated cotranslationally into proteins.[1] Selenium administered in 200 μg doses per day as selenized yeast has been shown to decrease the onset of colon, prostate and lung cancer in humans by as much as 50%.[2] However, selenium also shows a dark side. It is toxic and the margin of safety is relatively modest. This paper presents vignettes relevant to selenium's role in each of these areas in which chemical studies contributed significantly to revealing the role of selenium.

Selenoproteins occur widely in archaea, prokaryotes and eukaryotes.[3] A number of them have been isolated and characterized. Typically the selenium occurs as selenocysteine in these proteins. It was discovered that in the genes for two of these proteins; glutathione peroxidase[4] and formate dehydrogenase,[5] the codon UGA was found in the position corresponding to that for selenocysteine in the protein. Since UGA is a well-known stop codon, how does the biological machinery know when to incorporate selenocysteine and when to terminate protein synthesis? It was discovered that three additional factors were required for selenocysteine incorporation. A selenocysteine insertion sequence (SECIS) in the mRNA is needed. In prokaryotes this is a stem-loop structure next to the UGA codon on the 3′-side. In eukaryotes the SECIS is in the 3′-untranslated region of selenoprotein mRNA. A special elongation factor is also required. Finally, a special tRNA charged with selenocysteine is essential. An unusual quaternary complex forms at the ribosome ensuring selenocysteine incorporation.[6]

The selenocysteyl-tRNA is unusual not only for its tRNA structure, but also because it is the only known tRNA in eukaryotes that is not aminoacylated directly with the amino acid which it delivers to the ribosome. Rather, it is aminoacylated with serine which is then converted to selenocysteine while on the tRNA. In prokaryotes the attached serine is dehydrated by the pyridoxal phosphate-dependent Sec synthase

to afford the dehydroalanyl moiety. A selenium donor then converts the dehydroalanyl moiety into selenocysteyl-tRNA.

RESULTS AND DISCUSSION

The selenium donor which converts dehydroalanyl-tRNA into selenocysteyl-tRNA is monoselenophosphate **1**. This species is biosynthesized in a reaction catalyzed by seleno-

$$\underset{}{HSe^- + ATP} \longrightarrow \underset{\mathbf{1}}{SePO_3H_x^{3-x}} + AMP + OPO_3H_x^{3-X} \quad (1)$$

phosphate synthase as shown in Equation (1). The identity of monoselenophosphate was established with certainty by comparison with the authentic chemical compound.[7] Monoselenophosphate was chemically synthesized in the following way. Reaction of the known *O*,*O*,*O*-trimethylselenophosphate **2** with trimethylsilyl iodide gave *O*,*O*,*O*-tris(trimethylsilyl)selenophosphate **3** as shown in Equation (2). Although **3** hydrolyzes in water to

$$\underset{\mathbf{2}}{(MeO)_3PSe} + 3TMSI \longrightarrow \underset{\mathbf{3}}{(TMSO)_3PSe} + 3MeI \quad (2)$$

give monoselenophosphate, this procedure is inconvenient because **3** is not water soluble. A more preferable procedure is first to cleave one trimethylsilyl group as shown in Equation (3) to give

$$(TMSO)_3PSe \xrightarrow[i Pr_2NEt \\ CH_2Cl_2]{(CH_3)_2CHOH} \underset{\mathbf{4}}{i Pr_2NHEt^+ OP(Se)(OTMS)_2} \quad (3)$$

water-soluble salt **4**. Addition of **4** to aqueous buffers affords monoselenophosphate **1** which must be rigorously protected from air since it is easily decomposed on oxidation [8]. Monoselenophosphate 1 could be characterized spectroscopically and by elemental analysis of its precipitated barium salt. As expected, salt **4** selectively methylates on selenium, not oxygen, on treatment with methyl iodide. Further chemical reactions of monoseleno-phosphate were studied as well.[9] Chemically prepared monoselenophosphate and the enzymatically synthesized selenium donor compound were shown to be identical by ^{31}P NMR spectroscopy, chromatographic behavior and biological activity. Monoselenophosphate was also shown to be the source of the selenium in the metalloid modified base (2-seleno-5-methylaminouracil) that occurs in some tRNAs found in anaerobic bacteria.[10]

Although this biosynthesis of selenocysteyl-tRNA in prokaryotes was worked out many years ago, that in eukaryotes and archaea was uncovered only very recently.[11,12] The enzymes that catalyzed the conversion of the serine attached to the tRNA remained elusive. A comparative genomics analysis revealed genes that co-occur with selenoproteins. The hydroxyl group of seryl-tRNA$^{[Ser]Sec}$ is first phosphorylated to give *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ in a reaction catalyzed by *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ kinase. The selenium donor is biosynthesized in a reaction catalyzed by the enzyme SPS2. Then the enzyme SecS catalyzes the reaction of *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ and the selenium donor to form selenocysteyl-tRNA$^{[Ser]Sec}$. SecS is a pyridoxal phosphate-dependent protein that was previously identified in mammals as SLA (soluble liver antigen). That the selenium donor is monoselenophosphate was shown by the formation of Sec from phosphoseryl-tRNA$^{[Ser]Sec}$, chemically generated monoselenophosphate and mouse SecS.[11]

As pointed out in the Introduction, daily administration of selenized yeast to humans dramatically reduced the onset of three important types of cancer. Identification of the chemical forms of selenium (selenium speciation) in biological material is a challenging task because of the complexity of the system. Typically the material is enzymatically hydrolyzed. The complex aqueous solution thus obtained is separated into its components by HPLC or GC after derivatization. The components are analyzed by selenium specific ICP-MS (inductively coupled plasma-mass spectrometry) or GC-AED-MS (gas chromatography-atomic emission detection-mass spectrometry). As an alternative to these established methodologies we applied ^{77}Se NMR spectroscopic analysis. The advantage to this method is that it could be applied directly to the complex aqueous solution after enzymatic hydrolysis without the need for separation. Furthermore, NMR spectroscopic data are rich in information about chemical structure. Thus deduction of chemical structures of unknown compounds by NMR spectroscopic methods is feasible.

Selenium-77 is an attractive nucleus for NMR spectroscopy for several reasons. It has spin = ½ and occurs in 7.6% natural isotopic abundance. Although it is not as sensitive as ^{1}H, its sensitivity is comparable to ^{13}C. Furthermore, it has a chemical shift range of 3000 ppm.[13] Measuring ^{77}Se by inverse ^{1}H detection using ^{1}H-{^{77}Se}HMQC spectroscopy is advantageous because this results in an increase in sensitivity by a factor of almost 70.[14] A further advantage to this methodology is that only those ^{1}H nuclei spin-spin coupled to ^{77}Se are detected. Consequently, in a complex mixture of ^{77}Se-containing compounds and those not containing ^{77}Se, only the ^{77}Se-containing compounds are detected. This results in a dramatic simplification of the ^{1}H NMR spectrum of

the complex mixture obtained on proteolysis of the selenized yeast. The 2D ^{1}H-{^{77}Se}HMQC spectrum obtained with a protease XIV digestion of a recent selenized yeast tablet prepared for use in a current colon cancer study showed both ^{1}H and ^{77}Se chemical shifts corresponding to selenomethionine. Similar studies on an archived sample from the Clark studies showed the presence of a selenium compound in addition to selenomethionine. To ascertain the structure of this additional component, more NMR information was required. Since only the protons J-coupled to ^{77}Se are observed in these spectra and their 1D counterparts, a TOCSY sequence was added.[15] With authentic selenomethionine, signals were now observed for not only the Me and γ-hydrogens but α- and β-, as well. This methodology was also applied to synthetic selenoamino acid derivatives **5a** and **6a** that might be related to the unidentified seleno compound in the selenized yeast extracts. Interestingly, only the Me signal for **5a** was

$MeSeSCH_2CH(NHR^1)CO_2R$	$MeSSeSCH_2CH(NHR^1)CO_2R$
5a, R = Et, $R^1 = CO_2Et$	**6a**, R = Et, $R^1 = CO_2Et$
b, $R = R^1 = H$	**b**, $R = R^1 = H$

detected. This could mean that the coupling constant between ^{77}Se and the β-hydrogens through sulfur is very small. However, **6a** showed α, β and Me hydrogen signals. Indeed the coupling of ^{77}Se with the Me hydrogens through sulfur in this case is about 4.7 Hz. Consequently, further optimization of the spectrum of **5a** is expected to reveal the signals for the α, β and γ-hydrogens. Analysis of the spectra of the unknown component in the hydrolysate obtained from the Clark sample revealed that it is *S*-(methylseleno)cysteine **5b** and not the isomeric **6b**.

It has already been mentioned that selenium is toxic. It is also well-known that arsenic is toxic as well. Indeed this is evidenced by the arsenocosis found in Bangladesh due to the presence of inorganic arsenic in the public water supply. Remarkably, mixtures of selenite and arsenite simultaneously administered to mammals ameliorate each others' toxicity. Furthermore, this effect is greatest with a 1:1 ratio of Se:As which suggests the in vivo formation of a stoichiometric compound. To explore this possibility chemically, glutathione (GSH), which is the most prevalent reducing agent in cells, was mixed in varying ratios with selenite and arsenite. When the ratio was that shown in Equation (4), a new compound **7** was formed in which there is a 1:1 ratio of Se:As. The

structure of this compound was elucidated by

$$8\mathrm{GSH} + \mathrm{As(OH)_3} + \mathrm{SeO_3H^-} \longrightarrow \underset{\mathbf{7}}{(\mathrm{GS})_2\mathrm{AsSe^-}} + 3\mathrm{GSSG} + 6\mathrm{H_2O} \quad (4)$$

EXAFS, ^{77}Se NMR, Raman spectroscopic and chromatographic studies.[16] Although this compound is formed chemically, is it formed biologically? Indeed the bile from rabbits injected with selenite and arsenite is found by EXAFS studies to contain substantial amounts of **7**. Furthermore, **7** is assembled in erythrocyte lysate.[17] The mechanism for formation of **7** has been studied.[18] An exciting application of the beneficial effect of selenium on arsenic toxicity is the use of dietary selenium supplements, or other forms of selenium, to counter arsenic toxicity.[19]

In summary, the use of chemical synthesis, spectroscopic methods and chemical insight contributed significantly to our present understanding of the role of selenium in biology. Achievement of the results presented above required the collaboration of a diverse group of scientists in the U.S., Canada and Poland.

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