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Monoselenophosphate: Synthesis, Characterization, and Identity with the Prokaryotic Biological Selenium Donor, Compound SePX†

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ABSTRACT: A labile, selenium donor compound required for synthesis of selenium-dependent enzymes and seleno-tRNAs is formed from ATP and selenide by the SELD enzyme. This compound, tentatively identified as a selenophosphate [Veres, Z., Tsai, L., Scholz, T. D., Politino, M., Balaban, R. S., & Stadtman, T. C. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 2975–2979], is indistinguishable from chemically prepared monoselenophosphate by ^{31}P NMR spectroscopy and ion pairing HPLC. Furthermore, addition of chemically prepared monoselenophosphate caused a dose-dependent decrease in the amount of ^{75}Se incorporated into tRNAs from ^{75}SePX generated *in situ* by SELD enzyme. A procedure is described for the chemical synthesis of monoselenophosphate in which the readily prepared $(MeO)_3PSe$ is converted in quantitative yield to $(TMSO)_3PSe$ followed by complete cleavage of the latter to monoselenophosphate in oxygen-free aqueous buffer. The chemical properties of chemically synthesized monoselenophosphate are described.

The specific insertion of selenium into certain Se-dependent enzymes and Se-tRNAs in prokaryotes requires the formation of a highly reactive, reduced selenium donor compound. This selenium compound is required for addition to the double bond of 2,3-aminoacrylyl-tRNA$_{UCA}$ to form selenocystyl-tRNA$_{UCA}$ (Leinfelder et al., 1990; Forchhammer & Böck, 1991) and for the replacement of the sulfur atom of the 5-methylaminomethyl-2-thiouridine moiety of tRNAs with selenium to form 5-methylaminomethyl-2-selenouridine (Wittwer & Stadtman, 1986; Veres et al., 1990, 1992). An enzyme that is the product of the *sel*D gene catalyzes the formation of this reactive selenium donor compound from selenide and ATP (Ehrenreich et al., 1992; Kim et al., 1992; Veres et al., 1992). On the basis of ^{31}P NMR[1] spectroscopic studies, it was shown that the labile selenium donor is a compound containing a P–Se bond, and it was suggested that this SePX compound is a selenophosphate (Veres et al., 1992). An enzyme preparation derived from a mutant strain (selA1) of *Salmonella typhimurium* (Kramer & Ames, 1988) having a defective *sel*D gene (Stadtman et al., 1989) served as an assay system for determination of the biological effectiveness of SePX as donor for seleno-tRNA synthesis (Veres et al., 1992). In the present paper, we show that this compound is identical with chemically prepared monoselenophosphate,

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[1] Abbreviations: DCM, dichloromethane; DIPEA, diisopropylethylamine; DIPEAH, diisopropylethylammonium; DTT, dithiothreitol; IPA, isopropyl alcohol; IR, infrared; MTFMS, methyl trifluoromethanesulfonate; NMR, nuclear magnetic resonance; TBAH, tetrabutylammonium hydroxide; TEA, triethylamine; THF, tetrahydrofuran; TMS, trimethylsilyl; TPP, triphenylphosphate.

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$SePO_3^{3-}$. Since monoselenophosphate has not been previously prepared and characterized, these details also are reported.

MATERIALS AND METHODS

Materials

[^{75}Se]Selenite (1000 Ci/mmol) was obtained from the Research Reactor Facility, University of Missouri. DIPEA, MTFMS, TMSI, and Se were obtained from Aldrich Chemical Co. and used as received. TEA was purchased from Aldrich Chemical Co. and distilled from anhydrous KOH prior to use. "OmniSolv" IPA was obtained from EM Science and used without further purification. Reagent grade DCM from EM Science was distilled from P_4O_{10} prior to use. Reagent grade THF from Fisher Scientific was distilled from sodium and benzophenone before using. Tetrabutylammonium hydroxide (40%) was from Janssen Chimica. DTT was from Gibco BRL.

Methods

Spectroscopy and Elemental Analysis. ^{1}H NMR spectra were measured at 250 MHz using a Bruker WM-250 spectrometer on samples dissolved in deuterochloroform containing tetramethylsilane as the internal standard. ^{13}C NMR spectra were measured at 62.89 MHz using a Bruker WM-250 spectrometer on samples dissolved in deuterochloroform. ^{31}P NMR spectra were measured at 161.98 MHz using a Bruker AMX-400 spectrometer on samples dissolved in deuterochloroform containing TPP as the internal standard and referenced to 85% H_3PO_4. Other samples dissolved in aqueous buffers were studied at 121.5 MHz using a Bruker AM-300 NMR spectrometer equipped with a 5-mm broadband probe. All data were acquired using a 6-μs pulse width (45° flip angle), 1.5-s recycle time, sweep width of 10 000 Hz, and a block size of 8192 points. Varying numbers of free induction decays (FIDs) were averaged depending on the concentration of the compound under interrogation (see Figure legends). An exponential filter of 5–10 Hz was applied to all FIDs before Fourier transformation. Peaks were plotted relative to 85% phosphoric acid. IR spectra were obtained on a Perkin-Elmer Model 983 spectrometer. Elemental analyses were done at Desert Analytics, Tucson, AZ or Huffmann Laboratories, Golden, CO.

Synthesis of $(TMSO)_3PSe$. A 1.4-g (6.9-mmol) sample of $(MeO)_3PSe$ prepared as previously reported (Bhardwaj & Davidson, 1987), and 5 g (21 mmol) of TMSI were heated at 95 °C for 20 h. The solution was then fractionally distilled to afford 2.59 g (97% yield) of $(TMSO)_3PSe$, bp 80 °C (0.6 mm), whose spectra and properties were identical with those reported previously (Borecka et al., 1979).

Synthesis of $(DIPEAH)^+[(TMSO)_2P(Se)(O)]^-$. A degassed solution of 390 mg (3.0 mmol) of DIPEA and 180 mg (3.0 mmol) of IPA dissolved in 2 mL of anhydrous DCM was cannulated into a degassed solution of 188 mg (0.50 mmol) of $(TMSO)_3PSe$ dissolved in 2 mL of anhydrous DCM under an argon atmosphere and cooled in a dry ice–acetone bath. After being stirred for 5 min, the reaction mixture was warmed to room temperature. After 15 min at room temperature, the mixture was concentrated under vacuum to ~0.5 mL and cooled to 0 °C, and hexane was added dropwise until 150 mg (70% yield) of $(DIPEAH)^+[(TMSO)_2P(Se)(O)]^-$ precipitated as a colorless solid: IR 3000, 2950, 2664, 1426, 1393, 1159, 938 (PO), 778, 579 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.18 (s, 18 H), 1.32 (m, 15 H), 2.99 (q, 2 H, J = 7.3 Hz), 3.55 (septet, 2 H, J = 6.6 Hz); ^{13}C NMR (62.89 MHz, $CDCl_3$) δ 1.5 (q), 11.7 (q), 18.0 (q), 41.6 (t), 52.8 (d); ^{31}P NMR (161.98 MHz, $CDCl_3$) δ 18[2] (J = 768 Hz). Anal. Calcd for $C_{14}H_{38}NPO_3SeSi_2$: C, 38.69; H, 8.81. Found: C, 39.17; H, 9.27.

Methylation of $(DIPEAH)^+[(TMSO)_2P(Se)(O)]^-$. A sample of $(DIPEAH)^+[(TMSO)_2P(Se)(O)]^-$ was prepared as above from 754 mg (2.0 mmol) of $(TMSO)_3PSe$, 1.56 g (12 mmol) of DIPEA, and 720 mg (12 mmol) of IPA in 5 mL of DCM. To this solution cooled in a dry ice–acetone bath was added 0.5 mL (5.6 mmol) of MTFMS. The reaction mixture was warmed to room temperature, stirred for 15 min at this temperature, and then concentrated under vacuum. The residue was dissolved in 20 mL of anhydrous THF, and 2 mL (15 mmol) of TMSCl was added dropwise followed by the addition of a solution of 2.2 mL (15 mmol) of TEA in 5 mL of anhydrous THF. The reaction mixture was stirred at room temperature for 20 h and then concentrated under vacuum. The residue was fractionally distilled to give 380 mg (48% yield) of $MeSeP(O)(OTMS)_2$, bp 67 °C (2 mm), identical with the reported data (Borecka et al., 1979).

Hydrolysis of $(DIPEAH)^+[(TMSO)_2P(Se)(O)]^-$. A solution of 377 mg (1.0 mmol) of $(TMSO)_3PSe$ in 10 mL of anhydrous DCM was treated with a solution of 750 mg (6.0 mmol) of DIPEA and 360 mg (6.0 mmol) of IPA in 2 mL of DCM as described above. The reaction mixture was concentrated under reduced pressure. The remainder of the procedure was done in a dry box under an atmosphere of O_2-free nitrogen. The residue was treated with a solution of 750 mg (3.0 mmol) of $BaCl_2$ in freshly prepared 0.1 M LiOH solution.

The mixture was filtered, washed with 5 mL of degassed water, and dried at room temperature under vacuum, in the dark, for 24 h to give 310 mg (50% yield) of $Ba_3(SePO_3)_2$: IR 3320 (H_2O), 1438 ($BaCO_3$), 1031 (PO), 936, 590 cm^{-1}. Anal. Calcd for $Ba_3O_6P_2Se_2 \cdot 0.70\ BaCO_3 \cdot 11H_2O$:[3] Ba, 47.7; P, 5.8; Se, 14.8. Found: Ba, 47.7; P, 5.9; Se, 14.8.

Hydrolysis of $(TMSO)_3PSe$. $(TMSO)_3PSe$ dissolved in chloroform to a final concentration of 60 mM was mixed with an equal volume of 0.1 M Tricine·KOH buffer, pH 7.2, containing 20 mM DTT and 60 mM $MgCl_2$. The separation of phases was enforced by centrifugation. The pH of the water phase was adjusted by adding 6 M KOH or HCl before analyzing it by ^{31}P NMR spectroscopy. All experiments were carried out using argon to protect the selenium compounds against oxidation.

Selenophosphate synthetase (SELD protein) purification was carried out as described earlier (Veres et al., 1992).

Selenophosphate Synthetase Reaction and Sephadex G-10 Chromatography of the Products. The reaction mixture (1 mL) contained 100 mM Tricine·KOH, pH 7.2, 2 mM DTT, 3 mM $MgCl_2$, 1.5 mM ATP, 1.5 mM $Na^{75}SeH$ (2 μCi), and 40 μM enzyme. $Na_2{}^{75}SeO_3$ was reduced with DTT at pH 9 under argon before addition to the reaction mixture. After a 2-h incubation at 37 °C under argon, 40 μL of 0.5 M DTT was added and the whole reaction mixture was applied to a Sephadex G-10 column (1 × 120 cm). The column was

[2] The reference used was TPP, which absorbs at 16.1 ppm upfield of 85% phosphoric acid. The chemical shift reported is relative to 85% phosphoric acid as 0 ppm.

[3] Satisfactory elemental analysis was difficult to obtain owing to the extreme sensitivity of the material to air oxidation. Under rigorously anaerobic conditions without extensive drying material giving the analysis shown was obtained. The presence of water and $BaCO_3$ are confirmed by their absorptions in the IR spectrum (Miller & Wilkins, 1952) of this sample. The presence of $BaCO_3$ in otherwise analytically pure samples of $Ba_2O_4P_2S$ and Li_2CO_3 in $Li_4O_4P_2S$ has been reported before (Loewus & Eckstein, 1983).

equilibrated and eluted with 100 mM Tricine·KOH, pH 7.2, containing 20 mM DTT and 1 mM MgEDTA. Two radioactive peaks were eluted from the column. The first one is the ^{75}Se-labeled enzyme product and the second one is unreacted Na^{75}SeH. No radioactivity was present in the excluded protein peak. For some experiments the ^{75}Se-labeled seleno-compound eluted in the first radioactive peak was further chromatographed on a reversed-phase HPLC column in the presence of TBAH.

Ion-Pairing HPLC of the ^{75}Se-Labeled Enzyme Product. An aliquot of the combined fractions from the first radioactive peak from the Sephadex G-10 column was chromatographed on an octadecyl HPLC column using a modification of the method described by Lazzarino et al. (1991). For this procedure an Apex octadecyl 15 cm × 4.6 mm, 5-μm particle size column (Jones Chromatography) and tetrabutylammonium as the pairing ion were used. The starting buffer (buffer A) consisted of 10 mM TBAH, 10 mM KH_2PO_4, 1% methanol, and 2 mM DTT, pH 7.0. The second buffer (buffer B) contained 2.8 mM TBAH, 100 mM KH_2PO_4, 30% methanol, and 2 mM DTT, pH 5.5. The column was isocratically eluted with buffer A for 3 min followed by a linear gradient of 0–40% buffer B between 3 and 5 min and then 40–44% buffer B between 5 and 16 min. At 18 min buffer B reached 100%, and the column was eluted for 15 min with buffer B before returning to the initial conditions. The chromatography was performed at room temperature with a flow rate of 0.8 mL/min. Radioactivity in the fractions (0.4 mL) was measured with a Beckman model 5500 γ counter. The fractions containing the radioactive compound were analyzed by ^{31}P NMR spectroscopy. In another experiment, an aliquot of the combined fractions from the first radioactive peak from the Sephadex G-10 column was mixed with synthetic monoselenophosphate before application to the HPLC column. The recovery of radioactivity from the column was 95% in each experiment.

The Effect of Synthetic Selenophosphate on the Incorporation of ^{75}Se into tRNAs. French-pressure-cell extracts from the selA1 mutant *Salmonella* strain were prepared as described (Veres et al., 1990). Ammonium sulfate was added to 80% saturation, and the protein precipitate was collected by centrifugation and desalted on PD-10 columns for use as the enzyme source. The reaction mixture (0.5 mL) for studying ^{75}Se incorporation into tRNA contained 100 mM Tricine·KOH buffer, pH 7.2, 100 μL of crude selA1 enzyme preparation, 5 mM ATP, 10 mM $MgCl_2$, 10–20 A_{260} units of *Escherichia coli* bulk tRNA, 3.8 μM selenophosphate synthetase, 50 μM Na^{75}SeH (23 μCi), and 0–880 μM synthetic selenophosphate as indicated. Incubations were carried out at 37 °C under argon for 25 min. The reaction was stopped by adding 0.5 mL of 88% (vol/vol) phenol. Bulk tRNA was isolated by phenol extraction and DEAE-cellulose chromatography as described (Wittwer et al., 1984) except that phenol extraction and ethanol precipitation were repeated twice.

RESULTS AND DISCUSSION

$(TMSO)_3PSe$ has been synthesized previously (Borecka et al., 1979) by the reaction of $(TMSO)_3P$ with selenium. However, a more convenient synthesis of this material was sought by the reaction of TMSI with the known and readily available $(MeO)_3PSe$ (Bhardwaj & Davidson, 1987). Such cleavage of the MeO group with concomitant formation of the TMSO moiety is expected because TMSI dealkylates phosphonate diesters (Blackburn & Ingleson, 1978; Morita et al., 1978), produces $(TMSO)_3PSe$ among other products

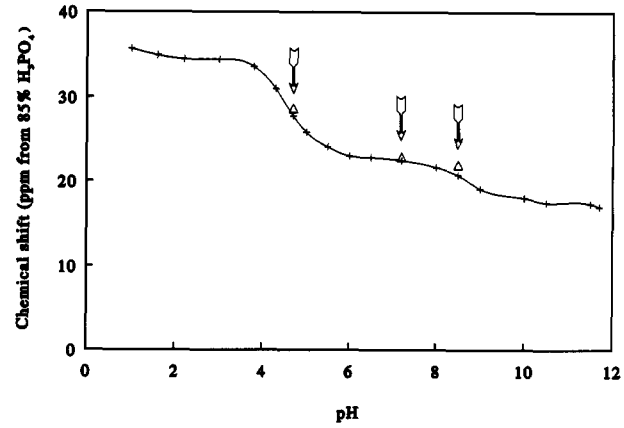

FIGURE 1: Plot of ^{31}P chemical shift obtained by adding $(TMSO)_3PSe$ to 0.1 M Tricine·KOH buffer, pH 7.2, containing 20 mM DTT and 60 mM $MgCl_2$ and adjusting the pH with 6 M KOH or HCl versus pH. (Δ and arrows) Enzymic products. The ^{31}P chemical shift measured at each pH value is plotted, and the points are connected by a hand-drawn curve.

from $(EtO)_3PSe$ (Borecka et al., 1979), and dealkylates tetramethyl symmetrical monothiopyrophosphate without cleavage or isomerization of the labile P–S–P bond (Loewus & Eckstein, 1983). Indeed, conditions were found under which $(TMSO)_3PSe$ is formed in quantitative yield from $(MeO)_3PSe$ and TMSI.

Cleavage of TMSO groups is facile and selective. However, in forming monoselenophosphates on such cleavage of $(TMSO)_3PSe$, degassed solvents and *oxygen-free atmospheres* must be used owing to the great sensitivity of these materials to air oxidation. Treatment of $(TMSO)_3PSe$ with DIPEA and IPA (Loewus & Eckstein, 1983) resulted in selective cleavage of one of the TMS groups to produce $(DIPEAH)^+$-$[(TMSO)_2P(Se)(O)]^-$. Alkylation of this ambident monoanion with MTFMS occurred exclusively on Se rather than O to produce the known $MeSeP(O)(OTMS)_2$ (Borecka et al., 1979). Monitoring the reaction of $(TMSO)_3PSe$ with excess DIPEA and IPA by ^{31}P NMR spectroscopy showed that cleavage of one TMS group occurs rapidly and selectively. Further cleavage does not occur under these conditions. However, complete cleavage of the TMS groups occurs with IPA and BF_3–Et_2O or in water. Treatment of the monocleaved product $(DIPEAH)^+[(TMSO)_2P(Se)(O)]^-$ with aqueous barium hydroxide resulted in the precipitation of $Ba_3(SePO_3)_2$ in 50% yield. Addition of $(TMSO)_3PSe$ to aqueous buffers resulted in the cleavage of all of the TMS groups as judged by ^{1}H NMR spectroscopy. The ^{31}P signal for monoselenophosphate thus prepared depended on the pH of the solution. A plot of the ^{31}P chemical shift versus pH is shown in Figure 1. As shown by the triangles at the arrows on this figure, the same chemical shifts as a function of pH were exhibited by the enzyme product, SePX, at the three indicated pH values. The reason for the dependence of ^{31}P chemical shift on pH is that the species changes from tribasic acid to monoanion to dianion to trianion as the pH is raised and the ^{31}P chemical shift depends on the charge. This dependence was reported for phosphoric acid (Jones & Katritzky, 1960; Crutchfield et al., 1962), condensed phosphates (Crutchfield et al., 1962), adenine nucleotides (Cohn & Hughes, 1960; Jaffe & Cohn, 1978), and thiamine diphosphate (Chauvet-Monges et al., 1978). The magnitude of the change in chemical shift between monoanion and dianion is considerably greater for selenophosphate than for orthophosphate. Similar differences for thiophosphate ions as compared to orthophosphate ions have been reported (Jaffe & Cohn, 1978). The ^{31}P chemical shift

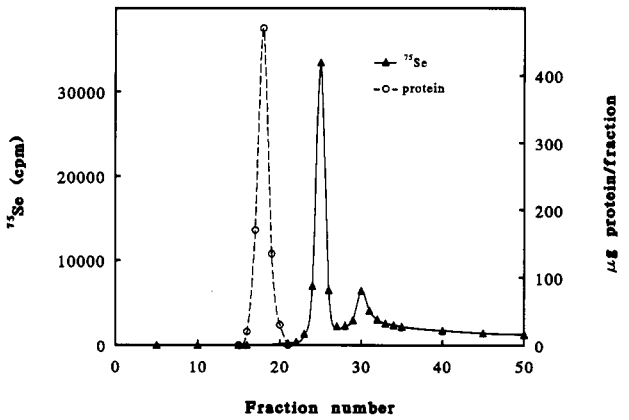

FIGURE 2: Sephadex G-10 chromatography of the reaction mixture containing ATP and selenide as described in Materials and Methods. Fractions (2 mL) were collected.

dependence on pH gives titration curves which enable one to determine pK values (Phillips, 1966). Similarly, the dependence of ^{17}O chemical shifts in phosphates on pH has also been used to determine pKs (Gerlt et al., 1982). Consequently, from Figure 1, pK_2 and pK_3 for H_3PO_3Se may be estimated as 4.6 and 8.8, respectively. The corresponding values for H_3PO_4 are 7.2 and 12.3, respectively (Jencks & Regenstein, 1966), and for H_3PO_3S are 5.6 and 10.3, respectively (Dittmer & Ramsay, 1963), 5.40 and 10.14, respectively (Peacock & Nickless, 1969), and 5.4 and 10.2, respectively (Gerlt et al., 1982). It has been suggested (Frey & Sammons, 1985) that the increased acidity of H_3PO_3S compared with H_3PO_4 is due to localization of negative charge on sulfur in thiophosphate anions. Thus, owing to the larger size and greater polarizability of sulfur relative to oxygen, thiophosphate anions are more stable than the corresponding phosphate anions. Such effects account for the greater acidity of thiols compared with alcohols. Selenium is larger and more polarizable than sulfur and can thereby stabilize negative charge better than sulfur. As a consequence of this effect, selenols are more acidic than thiols (Fringuelli & Taticchi, 1986). Therefore, one expects H_3PO_3Se to be more acidic than H_3PO_3S as is observed.

Radioactive compound SePX was prepared from Na^{75}SeH and ATP using purified selenophosphate synthetase (SELD enzyme) as described (Materials and Methods; Veres et al., 1992). The ^{75}Se-labeled product was completely separated from residual labeled selenide and protein by passage over a long, small diameter Sephadex G-10 column under strictly anaerobic conditions (Figure 2). The first radioactive peak of the profile contained the labeled SePX product together with AMP and residual ATP. Compound SePX prepared in this way was compared by ^{31}P NMR spectroscopy with authentic monoselenophosphate made by hydrolysis of $(TMSO)_3PSe$. The results are shown in Figure 3. The ^{31}P chemical shifts for the two compounds are identical as are the ^{31}P–^{77}Se coupling constants (551 Hz). This value for $^1J_{^{31}P-^{77}Se}$ is in the reported range (–200 to –1100 Hz) for such one-bond coupling constants (Verkade & Mosbo, 1987).

Further purification of SePX and separation from the nucleotides following Sephadex G-10 chromatography (Figure 2) were achieved by ion-pairing HPLC on an Apex C_{18} column as described under Materials and Methods. With known mixtures of adenylates, the elution times were AMP, 13.4 min, ADP, 21.6, min, and ATP, 22.9 min, under these conditions. The order of elution and extent of separation of these nucleotides are similar to those reported (Lazzarini et al., 1991). In a typical elution profile of the Sephadex G-10 sample, compound SePX emerged at 10.7 min, AMP at 13.1 min, and ATP at 23 min. Chromatography of radioactive SePX with carrier authentic monoselenophosphate resulted in the coelution of the two compounds as monitored by ^{31}P NMR analysis and γ counting of individual fractions for ^{75}Se detection. Recoveries of radioactivity in compound SePX were 95%.

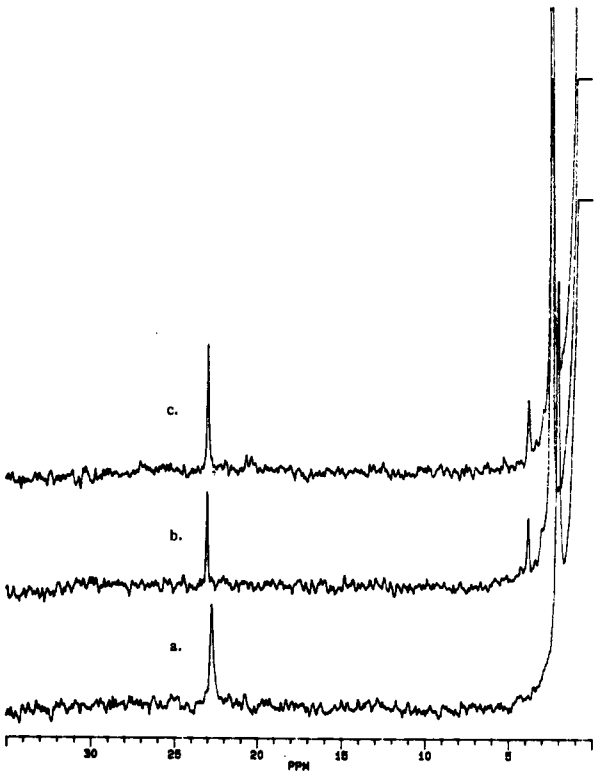

FIGURE 3: ^{31}P NMR spectra of selenophosphate compounds. (a) Authentic monoselenophosphate (ca. 2 mM) prepared by adding $(TMSO)_3PSe$ to 0.1 M Tricine·KOH buffer, pH 7.2, containing 20 mM DTT, and 60 mM $MgCl_2$; (b) compound SePX (1.1 mM) prepared as described under Materials and Methods and partially purified by Sephadex G-10 chromatography; (c) mixture of authentic monoselenophosphate (ca. 2 mM) and compound SePX (1.1 mM). ^{31}P NMR spectra were collected at 121.5 MHz. Each spectrum is an average of 1024 FIDs and is referenced to an external standard of 85% phosphoric acid (0 ppm). The spectra of samples containing SePX also show resonance for AMP, at about 4 ppm.

The ability of SePX to serve as selenium donor for synthesis of 2-selenouridine in tRNAs was tested using as catalyst a partially purified enzyme from the *Salmonella* (SelA1) mutant that is unable to synthesize selenophosphate (Veres et al., 1990). SePX was generated *in situ* from Na^{75}SeH and ATP by added purified selenophosphate synthetase. Addition of unlabeled authentic monoselenophosphate decreased the amount of ^{75}Se incorporated into the added thio-tRNA substrate in a dose-dependent manner (Figure 4). This observed decrease in ^{75}Se incorporation into tRNAs is due almost entirely to dilution of ^{75}SePX by the added unlabeled $SePO_3^{2-}$. No product inhibition of selenophosphate synthetase activity was observed when reaction mixtures containing the same concentrations of ATP, selenide, and enzyme were supplemented with 0.2 and 0.5 mM selenophosphate and only 7% inhibition was observed with 1.0 mM added selenophosphate. In contrast, as shown in Figure 4, ^{75}Se incorporation into tRNAs was decreased by about 95% when 500 μM unlabeled selenophosphate was added.

In a separate experiment, selenophosphate synthetase, [^{75}Se]selenide, and ATP were omitted, and ^{75}Se-labeled SePX compound freed of ATP by ion-pairing HPLC was used as substrate. In this system, the mutant enzyme preparation

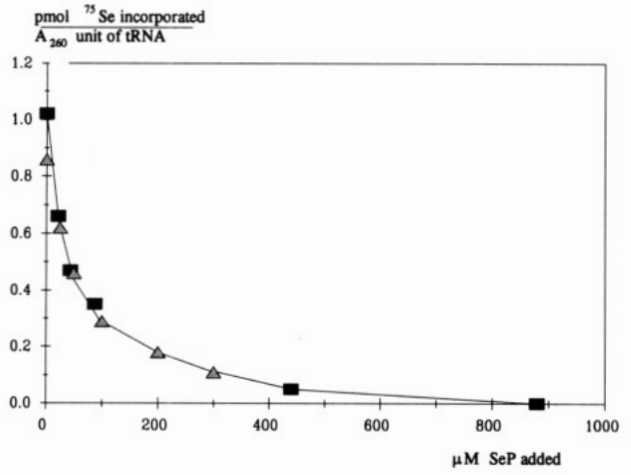

FIGURE 4: Dose-dependent decrease in incorporation of ^{75}Se into tRNAs in the presence of added nonlabeled monoselenophosphate. Reaction mixtures containing an enzyme preparation from *Salmonella* mutant selA1, purified selenophosphate synthetase, Na^{75}SeH, ATP, and tRNAs were supplemented with authentic monoselenophosphate as described under Materials and Methods. The symbols Δ and ■ indicate two different sets of experiments.

catalyzed the conversion of 5-methylaminomethyl-2-thiouridine in the added thio-tRNA to radioactive 5-methylaminomethyl-2-selenouridine with ^{75}SePX as the sole selenium donor (data not shown). Since this reaction involving substitution of sulfur with selenium occurred in the absence of added ATP, direct attack of selenophosphate on the 2-thio moiety of the uridine residue in the "wobble position" of the tRNAs is indicated. The fact that addition of the β,γ-methylene diphosphonate analogue (10 mM) of ATP had no effect on the conversion of the 2-thiouridine residues to 2-selenouridine residues with ^{75}SePX as the sole selenium donor is further evidence that an initial activation of the 2-thio moiety by phosphorylation is not required.

On the basis of the above experimental evidence showing the identity of compound SePX and chemically prepared monoselenophosphate by ^{31}P NMR spectroscopy, ion pairing HPLC, and enzymic analysis, it is concluded that compound SePX is monoselenophosphate, a general selenium donor compound synthesized by selenophosphate synthetase.

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REFERENCES

Bhardwaj, R. K., & Davidson, R. S. (1987) *Tetrahedron 43*, 4473–4479.

Blackburn, G. M., & Ingleson, D. (1978) *J. Chem. Soc., Chem. Commun.*, 870–871.

Borecka, B., Chojnowski, J., Cypryk, M., Michalski, J., & Zielinska, J. (1979) *J. Organomet. Chem. 171*, 17–34.

Chauvet-Monges, A.-M., Hadida, M., & Crevat, A. (1978) *C. R. Hebd. Seances Acad. Sci., Ser. C: 286*, 489–492.

Cohn, M., & Hughes, T. R., Jr. (1960) *J. Biol. Chem. 235*, 3250–3253.

Crutchfield, M. M., Callis, C. F., Irani, R. R., & Groth, G. C. (1962) *Inorg. Chem. 1*, 813–817.

Dittmer, D. C., & Ramsay, O. B. (1963) *J. Org. Chem. 28*, 1268–1272.

Ehrenreich, A., Forchhammer, K., Tormay, P., Veprek, B., & Böck, A. (1992) *Eur. J. Biochem. 206*, 767–773.

Forchhammer, K., & Böck, A. (1991) *J. Biol. Chem. 266*, 6324–6328.

Frey, P. A., & Sammons, R. D. (1985) *Science 228*, 541–545.

Fringuelli, F., & Taticchi, A. (1986) in *The Chemistry of Organic Selenium and Tellurium* (Patai, S., & Rappoport, Z., Eds.) Vol. 1, pp 600–601, Wiley, Chichester.

Gerlt, J. A., Demou, P. C., & Mehdi, S. (1982) *J. Am. Chem. Soc. 104*, 2848–2856.

Jaffe, E. K., & Cohn, M. (1978) *Biochemistry 17*, 652–657.

Jencks, W. P., & Regenstein, J. (1976) in *Handbook of Biochemistry and Molecular Biology* (Fasman, G. D., Ed.) 3rd ed., Vol. 1, p 305, CRC Press, Cleveland, OH.

Jones, R. A. Y., & Katritzky, A. R. (1960) *J. Inorg. Nucl. Chem. 15*, 193–194.

Kim, I. Y., Veres, Z., & Stadtman, T. C. (1992) *J. Biol. Chem. 267*, 19650–19654.

Kramer, G. F., & Ames, B. N. (1988) *J. Bacteriol. 170*, 736–743.

Lazzarino, G., Di Pierro, D., Tavazzi, B., Cerroni, L., & Giardina, B. (1991) *Anal. Biochem. 197*, 191–196.

Leinfelder, W., Forchhammer, K., Veprek, B., Zehelein, E., & Böck, A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 543–547.

Loewus, D. I., & Eckstein, F. (1983) *J. Am. Chem. Soc. 105*, 3287–3292.

Miller, F. A., & Wilkins, C. H. (1952) *Anal. Chem. 24*, 1253–1294.

Morita, T., Okamoto, Y., & Sakurai, H. (1978) *Tetrahedron Lett.*, 2523–2526.

Peacock, C. J., & Nickless, G. (1969) *Z. Naturforsch. A24*, 245–247.

Phillips, R. (1966) *Chem. Rev. 66*, 501–527.

Stadtman, T. C., Davis, J. N., Zehelein, E., & Böck, A. (1989) *BioFactors 2*, 35–44.

Veres, Z., Tsai, L., Politino, M., & Stadtman, T. C. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 6341–6344.

Veres, Z., Tsai, L., Scholz, T. D., Politino, M., Balaban, R. S., & Stadtman, T. C. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 2975–2979.

Verkade, J. G., & Mosbo, J. A. (1987) in *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis* (Verkade, J. G., & Quin, L. D., Eds.) pp 453–455, VCH Publishers, Inc., New York.

Wittwer, A. J., & Stadtman, T. C. (1986) *Arch. Biochem. Biophys. 248*, 540–550.

Wittwer, A. J., Tsai, L., Ching, W.-M., & Stadtman, T. C. (1984) *Biochemistry 23*, 4650–4655.