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Monoselenophosphate: Its Hydrolysis and Its Ability to Phosphorylate Alcohols and Amines

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The rate of hydrolysis of monoselenophosphate, the labile selenium donor compound required for the synthesis of selenium-dependent enzymes and seleno-tRNAs, was determined by ^{31}P NMR spectroscopy. The rate depended on the pH of the solution and was maximal at a pH ~7. This suggests that the dianion is the species that reacts fastest. Added alcohols and amines do not significantly affect the rate of hydrolysis but are phosphorylated. The entropy of activation is positive for the hydrolysis of monoselenophosphate. These data suggest a dissociative in nature mechanism for the hydrolysis of monoselenophosphate involving a monomeric metaphosphate-like transition state in the rate-determining step.

INTRODUCTION

Monoselenophosphate is a labile selenium donor compound required for the synthesis of selenium-dependent enzymes and seleno-tRNAs and it is formed from ATP and selenide by selenophosphate synthetase (SELD enzyme). Selenophosphate synthetase from *Escherichia coli* has been isolated and some of its physical and catalytic properties determined (*1*). Cys-17 (*2*) and Lys-20 (*3*) are essential for catalytic activity as shown by studies on mutant enzymes. Human selenophosphate synthetase has also been cloned and shown to be an essential component of selenoprotein synthesis (*4*). Selenocystyl-$tRNA_{UCA}$ is formed from 2,3-aminoacryl-$tRNA_{UCA}$ by the addition of monoselenophosphate to the double bond (*5*, *6*). In addition, monoselenophosphate is required for replacement of the sulfur atom in the 5-methylaminomethyl-2-thiouridine residues in the anticodon of certain prokaryotic tRNAs by selenium to give 2-methylaminomethyl-2-selenouridine residues (*7–9*). Monoselenophosphate has been chemically synthesized, characterized, and shown to be identical with the biological selenium donor (*10*). In the present paper, the hydrolysis of monoselenophosphate is reported as well as its ability to phosphorylate alcohols and amines.

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EXPERIMENTAL

MSP[3] was prepared as reported previously (*10*, *11*). AE, AHP, EDA, EG, G, TMSC, and TMP were purchased from Aldrich Chemical Co. and used as received. Ches, Mes, Pipes, and Tris were Sigma Ultra grade obtained from Sigma Chemical Co. Hepes was bought from Serva and Mops was from Calbiochem and were used without further purification. TB (40%) was obtained from Janssen Chimica. ^{31}P NMR spectra were measured at 121.424 MHz using a Varian Unity 300 spectrometer.

Kinetic Studies

A stock solution of monoselenophosphate was prepared by adding rigorously deoxygenated water (0.6 mL) under an argon atmosphere to MSP (54.3 mg, 0.125 mmol) shortly before use. An aliquot of this stock solution (0.12 mL) was mixed with deoxygenated buffer (0.38 mL) under an argon atmosphere. This solution was transferred via a gas-tight syringe to an evacuated 5-mm NMR tube equipped with a coaxial tube containing D_2O for a lock signal. In some cases an insert containing 1 M H_3PO_4 was added as an ^{31}P NMR standard. The NMR tubes were sealed under argon and placed in a constant temperature bath. The progress of the reaction was monitored by ^{31}P NMR spectroscopy after removing the NMR tube from the bath and cooling. The NMR spectroscopic parameters used were: acquisition time (1.6 s), pulse width (10.0), recycle delay (0), number of scans (128), and spectral width (10 kHz). After measuring the ^{31}P NMR spectrum, the sample was returned to the constant temperature bath. For each kinetic run, the spectrum was measured at least seven times through at least two half-lives. The relative concentration of monoselenophosphate was determined by integration of its ^{31}P NMR signal relative to the sum of the integration of all of the ^{31}P NMR signals observed. The decay of monoselenophosphate obeyed irreversible first order kinetics because the time course was well-fit by a first order model. In some cases the reaction was followed for over three half-lives and the first order plot remained linear throughout the reaction.

The rate data reported in Table 1 and pictured in Fig. 1 were obtained using 50–100 mM selenophosphate initial concentrations and buffer concentrations for Good buffers of 500 mM except for Pipes (one run at 360 mM two at 470 mM at an ionic strength of 1.3) and up to 900 mM or less for the other buffers and Ches. C buffer concentrations were 300–450 mM in order to maintain an ionic strength of 0.9–1. The ionic strength was maintained at 0.7–1.0 by addition of the required

[3] Abbreviations used: A, acetate; AE, 2-aminoethanol; AHP, ammonium hydrogen phosphoramidate; B, KOH/KCl; C, carbonate; Ches, 2-(*N*-cyclohexylamino)ethanesulfonic acid; EDA, ethylene diamine; EG, ethylene glycol; G, glycerol; GC–MS, gas chromatography–mass spectroscopy; GP, glycerophosphate; H, potassium chloride/hydrochloric acid; Hepes, *N*-(2-hydroxyethyl) piperazine-*N*-2-ethanesulfonic acid; HMDS, hexamethyldisilazane; M, morpholine; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Mops, (3-*N*-morpholino)propanesulfonic acid; MSP, diisopropylethylammonium *O*,*O*-bis(trimethylsilyl) monoselenophosphate; NMR, nuclear magnetic resonance; PH, phthalate; P_i, orthophosphate; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); TB, tetra-*n*-butylammonium hydroxide; TMSC, trimethylchlorosilane; TMP, 2,4,6-trimethylpyridine; Tris, tris(hydroxymethylaminomethane).

amount of KCl or NaCl.[4] The pH of the solution was determined by measuring the pH of the solution with an Orion digital analyzer 1501 at room temperature immediately after the NMR tubes were opened on completion of the monitoring of the reaction. The pH at room temperature was then corrected to that at the temperature of the reaction using the known pH dependence on temperature of the buffer (*12*).[5] The error limits for the rate constants, shown in Table 1, are the product of the standard deviation and Student's factor at 0.95 confidence.

The rate data at different buffer concentrations were obtained as above and are reported in Table 2.

Phosphorylation of EG

A solution of monoselenophosphate prepared by dissolving MSP (44.4 mg, 0.102 mmol) in deoxygenated water (0.3 mL) was cannulated into an ampoule containing EG (806 mg, 13.0 mmol). The ampoule was sealed under argon and placed in a constant temperature bath maintained at 54°C for 4 h. The resulting solution was lyophilized and the residue was treated with anhydrous TMP (0.6 mL), HMDS (0.3 mL), and TMSC (0.15 mL). This reaction mixture was heated at reflux for 1 h. After cooling, the solution was analyzed by GC–MS (*13*, *14*) and found to contain silylated O-phosphorylated EG whose MS was identical with authentic compound.

Phosphorylation of G

A solution of monoselenophosphate prepared by dissolving MSP (15 mg, 0.030 mmol) in deoxygenated water (0.1 mL) and G (390 mg, 4.3 mmol) was sealed in an ampoule under argon and heated in a constant temperature bath maintained at 54°C for 10 h. After cooling to room temperature, the solution was passed through an ion exchange column (Dowex 50W-X8, 50–100 mesh) and the eluate lyophilized. The residue was dissolved in anhydrous TMP (10 mL) containing TMSC (1.30 g, 12.0 mmol) and HMDS (2.30 g, 14.0 mmol) and heated at reflux for 1 h. After cooling, the solution was analyzed by GC–MS (*13*, *14*) and found to contain silylated α- and β-GP whose mass spectra were identical with authentic compounds.

RESULTS

The hydrolysis of monoselenophosphate was monitored by ^{31}P NMR spectroscopy in deoxygenated aqueous buffers. The signal due to monoselenophosphate, whose chemical shift depends on pH, was well-separated from that of P_i, whose chemical shift also depends on pH. The solutions were sealed under inert gas in NMR tubes and kept in a refluxing solvent chosen to maintain the desired temperature (generally

[4] The counterion has no effect on the reaction rates. No differences were observed between KCl or NaCl.

[5] Handbook of Chemistry and Physics (1990) 71st ed., pp. 829–831, CRC Press, Boca Raton.

53.7 ± 0.1°C). The rates of reaction obey first order kinetics and were determined by monitoring the decrease in the signal of monoselenophosphate with time.

pH-Rate Profile

The rate of hydrolysis of monoselenophosphate depended on the pH of the solution. These results are listed in Table 1. The pH-rate profile for this reaction, except for the data in Tris and AE buffers (as explained below), is shown in Fig. 1. To accurately determine the pH of the solution at the reaction temperature, the pH of the buffer solution was measured at room temperature and then extrapolated to the reaction temperature using the known (*12*) (see footnote 5) pH–temperature dependence of the buffer. The dependence of pH on temperature for acetate buffer is small but it is significant for Tris, AE, EDA, and M buffers. To provide adequate buffer capacity over the range of pH studied, a variety of buffers were used. Overlap between the ranges of buffers was obtained and there was no evidence for significant catalysis by specific buffers. That is, the rates were the same at a given pH within experimental error. Increasing the buffer concentration at a given pH modestly reduced the rate of hydrolysis as shown in Table 2. The rates of reaction using Tris or AE buffers were significantly lower than those using Good buffers at the same pH. In the Tris and AE solutions $J_{^{31}P-^{77}Se}$ are significantly lower than those values in Good buffers of the same pH. This suggests that there may be specific solvation due to hydrogen bonding between Tris and AE or their conjugate acids with selenophosphate. Similarly the hydrolysis of μ-monothiopyrophosphate is inhibited by monoprotonated EDA (*15*). Consequently, the rate data for Tris and AE are not included in Fig. 1.

The pH-rate profile shown in Fig. 1 can be fitted using Eqs. [1]–[2].

$$\text{rate} = k_{\text{obs}}[H_{3-n}O_3PSe^{n-}] \qquad [1]$$

$$k_{\text{obs}} = \frac{k_0 + k_1 f_1 + k_2 f_1 f_2 + k_3 f_1 f_2 f_3}{1 + f_1 + f_1 f_2 + f_1 f_2 f_3},$$

where

$$f_i = \frac{K_i}{[H^+]} \text{ and } \frac{K_i}{[H^+]} = 10^{(\text{pH}-\text{p}K_i)} \qquad [2]$$

A best least-squares fit of the data at 53.7°C provides the following rate constants: $k_0 < 1 \times 10^{-6}$ s^{-1} (free acid), $k_1 = 0.3 \times 10^{-5}$ s^{-1} (monoanion), $k_2 = 45.1 \times 10^{-5}$ s^{-1} (dianion), $k_3 < 1.0 \times 10^{-6}$ s^{-1} (trianion) and equilibrium constants: p$K_2 = 4.61$ and p$K_3 = 9.1$ (p$K_1 = 0$ was assumed because the data do not permit its accurate assessment). These kinetically determined p*K*s may be compared with the thermodynamic p*K*s at room temperature determined from the dependence of the ^{31}P chemical shift on pH (*10*) at an ionic strength of 0.9: p$K_2 = 4.5$ and p$K_3 = 9.3$. These values are in reasonable agreement, although they refer to p*K*s at different temperatures.

TABLE 1
Rate Constants for the Hydrolysis of Monoselenophosphate as a Function of pH at 53.6°C

Buffer	pH	$k_{hyd} \times 10^5$, s^{-1}
H	ca. 1.7	0.363 ± 0.039
PH	3.17	2.53 ± 0.39
PH	3.22	1.31 ± 0.15
PH	3.72	6.83 ± 0.16
A	3.65	5.30 ± 0.28
A	4.03	10.4 ± 0.60
A	4.75	25.7 ± 3.3
A	5.50	37.3 ± 3.0
Mes	5.94	42.9 ± 1.1
Mes	6.49	45.0 ± 2.6
Mes	6.64	46.0 ± 2.1
Pipes	6.63	47.3 ± 2.0
Mops	6.75	44.0 ± 1.7
Mops	7.02	46.4 ± 1.3
Mops	7.42	47.5 ± 1.5
Hepes	7.2	44.3 ± 2.1
Hepes	7.4	44.6 ± 2.2
Hepes	7.78	40.9 ± 1.4
Tris	7.16	26.8 ± 3.1
Tris	7.20	30.1 ± 1.5
Tris	7.78	24.8 ± 1.6
Ches	8.61	35.7 ± 2.2
Ches	8.75	28.0 ± 4.4
Ches	8.87	29.2 ± 3.5
AE	8.27	23.1 ± 2
AE	8.42	21.9 ± 1.4
EDA	8.97	26.8 ± 4.9
EDA	9.29	17 ± 1.6
AE	9.40	9.29 ± 1.34
EDA	9.40	11.8 ± 0.9
EDA	9.56	10.7 ± 0.7
EDA	9.66	8.03 ± 0.48
EDA	9.87	8.27 ± 1.2
C	9.55	13.9 ± 2.3
C	9.65	9.59 ± 0.93
C	9.66	11.7 ± 1.7
C	9.67	10.8 ± 1.1
C	9.84	8.26 ± 0.70
C	9.84	8.90 ± 0.73
B	12.5	0.0385 ± 0.0105
TB	12.6	0.0631 ± 0.0133
TB	13.0	0.0505 ± 0.0111

Activation Parameters

The activation parameters for the hydrolysis of monoselenophosphate were determined at various pHs by measuring the rate constants at five different temperatures over a range of 23–68°C. The results are presented in Tables 3 and 4.

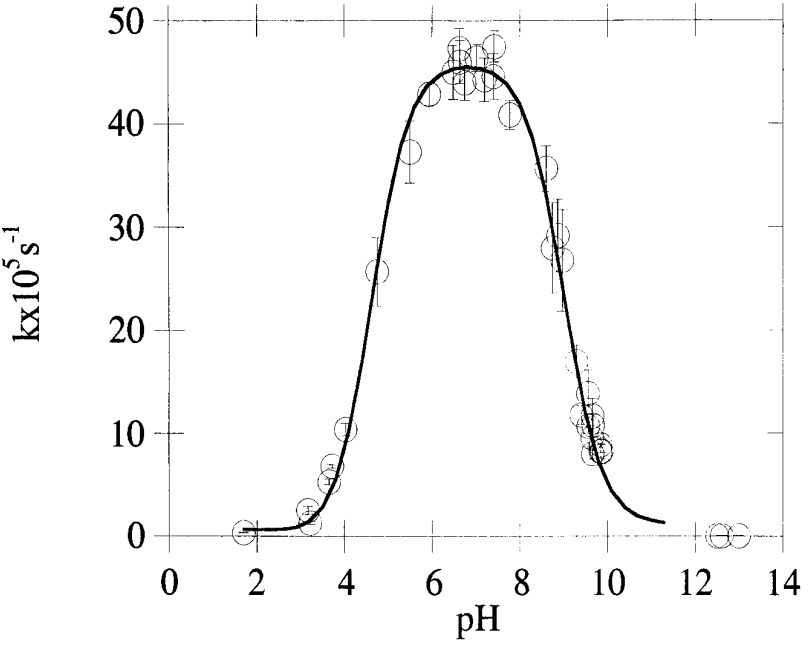

FIG. 1. Graph of the rate constant versus pH for hydrolysis of monoselenophosphate at 53.6°C.

TABLE 2
Rate Constants for the Hydrolysis of Monoselenophosphate as a Function of Buffer Concentration at 53.6°C

Buffer	Concentration, mM	pH	k_{obs}/k_{calc}[a]
A	0.3	4.14	1.96 ± 0.15
A	0.6	4.47	1.27 ± 0.11
A	0.9	4.80	1.03 ± 0.12
A	0.9	5.50	0.92 ± 0.07
A	1.2	4.89	1.14 ± 0.21
A	1.5	4.90	1.05 ± 0.23
Tris	0.3	6.36	0.76 ± 0.05
Tris	0.6	6.90	0.63 ± 0.05
Tris	0.9	7.29	0.67 ± 0.03
Tris	0.9	7.30	0.58 ± 0.09
Tris	1.2	7.16	0.59 ± 0.07
Tris	1.5	7.30	0.46 ± 0.05
Hepes[b]	0.9	6.89	0.92 ± 0.03
Hepes[b]	1.8	7.02	0.94 ± 0.03
Hepes[b]	2.7	6.89	0.85 ± 0.02

[a] k_{calc} is obtained at the given pH by the mathematical fitting of the curve shown in Fig. 1.
[b] These solutions were also 0.9 mM in G.