Preparation and Determination of Sodiumhydrogen S-(2-aminoethyl) Phosphorothioate (Sodiumhydrogen Cysteamine-S-phosphate)

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In a study of S-phosphorylation in biological systems it became of interest to have access to S-(2-aminoethyl) phosphorothioic acid (cysteamine-S-phosphoric acid *). This substance could possibly be formed by enzymic S-phosphorylation of 2-aminoethanethiol (cysteamine).

The sodiumhydrogen salt of cysteamine-S-phosphoric acid has now been prepared from trisodium phosphorothioate and 2-bromoethylammonium bromide with N,N-dimethylformamide as catalyst according to the following scheme:

$$BrNH_3CH_2CH_2Br + Na_3SPO_3 \longrightarrow NH_2CH_2CH_2SPO_3HNa + 2\ NaBr$$

A slight excess of 2-bromoethylammonium bromide was used to ascertain complete reaction of the phosphorothioate. This reaction gave directly an analytically pure product in good yield (95 %).

From the above scheme it follows that the 2-aminoethyl residue could possibly be bound either to an oxygen or to the sulfur atom of the phosphorothioic acid residue (or to both in a mixture). Therefore, the actual position of this group in the molecule had to be established. From the following experiments it could be concluded that the 2-aminoethyl group in the synthesized substance is exclusively bound to the sulfur atom of the phosphorothioic acid residue: a) unlike phosphorothioate [1] the substance does not reduce iodine in strongly acid solution; b) hydrolysis in 1 M perchloric acid at 100° gave *ortho*-phosphate and a nitroprussiate positive substance. Hydrogen sulfide or phosphorothioate could not be detected. The hydrolysate consumed 98.2 atom-% iodine per mole of compound hydrolyzed *; c) paper chromatographic analysis of untreated and iodine oxidized hydrolysate ** (*n*-butanol: acetic acid: water, 4:1:1, and *n*-propanol: 29 % ammonia: water, 6:3:1) gave the same result as was obtained with authentical cysteamine treated the same way as the hydrolysate. No trace of 2-aminoethanol could be detected.

Mercury(II)ions (and 6-chloromercuri-2-nitrophenol [2]) were found to catalyze the hydrolysis of cysteamine-S-phosphate in acid solution. This fact has been utilized in the colorimetric determination of the substance. The optimum mercury(II)ion concentration was found to be about 45 μM (higher concentrations develop cloudiness in the samples). The same color yield was obtained with this method as is obtained with *ortho*-phosphate alone.

Methods and results. Trisodium phosphorothioate was prepared according to Yasuda and Lambert[3]. Phosphate determinations were based on the method by Gomori[4]. 2-Bromoethylammonium bromide was obtained from Eastman Kodak Company.

Synthesis of sodiumhydrogen cysteamine-S-phosphate. 9.0 g (50 mmole) of trisodium phosphorothioate were dissolved in 50 ml of water. 10.9 g (53 mmole) of 2-bromoethylammonium bromide and 25 ml of N,N-dimethylformamide were added. The solution was vigorously stirred for 40 min during which time a large amount of white crystals separated. (The reaction is conveniently followed by the disappearence of phosphorothioate, which is tested for by the addition of silver ions, whith which a black precipitate is obtained). 300 ml of ethanol were then added and the precipitated crystals (Found: P 12.3; H_2O 28.7***. Calc. for $NH_2CH_2CH_2SPO_3HNa, 4H_2O$: P 12.3; H_2O 28.7) were collected by filtration and washed thoroughly with ethanol. The crystals were dehydrated by stirring them in

* This is in line with the terminology adopted by *Chemical Abstracts* for certain biologically important phosphate esters, *e.g.*, creatine phosphate *etc.* However, to prevent confusion with the isomer cysteamine-N-phosphate the letter S is used to denote the position of the phosphate residue.

* This is about the same yield as was obtained with pure cysteamine after similar treatment.

** To avoid multiple spot formation, which was observed in the presence of perchlorate, hydrochloric acid was used for the hydrolysis in this experiment.

*** Water of crystallization was determined by drying to constant weight at 105°.

200 ml of dry methanol for 1 h. 8.5 g (95 %) of substance were thus obtained after drying *in vacuo*. (Found: C 13.5; H 3.9; P 17.3. Calc. for $NH_4CH_2CH_2SPO_3HNa$ (179.13):C 13.4; H 3.9; P 17.3). The dehydrated product was found to be more stable upon storage than the originally precipitated crystals, which easily lose water of crystallization.

Hydrolysis of cysteamine-S-phosphate. 2.668 mmole of cysteamine-S-phosphate were dissolved in 10 ml of 1 M perchloric acid and heated on a boiling water bath for 30 min in a stream of nitrogen. After hydrolysis this solution consumed 2.62 matom of iodine (98.2 atom-% per mole of cysteamine-S-phosphate) as titrated with a standardized iodine solution.

A similar procedure of hydrolysis was used in the early experiments to determine *ortho*-phosphate.

Determination of cysteamine-S-phosphate by mercury(II)ion catalyzed hydrolysis. To 0.50 ml of sample (containing 0—1 μmole cysteamine-S-phosphate) were added: 0.10 ml mercury(II) acetate (70 mg mercury(II)acetate dissolved in 100 ml of 5 % acetic acid), 2.90 ml of water, 0.50 ml of "elon"[4] and 1.00 ml of molybdate[4]. The solution was set aside for 1 h at room temperature and read against a similarily treated blank (water instead of sample) at 660 mμ in a 1 cm cuvette. One μmole of cysteamine-S-phosphate in the sample was found to give an absorbancy of 0.712. Some quantitative data are presented in Table 1.

Table 1. Recovery experiment for determination of cysteamine-S-phosphate.

Cysteamine-S-phosphate, mmole		% error
Added	Found	
0.213	0.213	0
0.425	0.423	—0.5
0.638	0.643	+0.8
0.851	0.847	—0.5
1.06	1.05	—0.9

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