SYNTHESIS OF CYSTEINE-^{35}S-SULFATE*

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SUMMARY

A short and very convenient method is described for the preparation of cysteine-^{35}S-sulfate using unlabeled cysteine-S-sulfate and L-cysteine-^{35}S. This method provides an 80% yield and can be used for microquantity synthesis.

Key words: Cysteine-S-sulfate, L-cysteine-^{35}S, Exchange reaction

INTRODUCTION

Cysteine-S-sulfate is an abnormal urinary and plasma metabolite associated with sulfite oxidase deficiency (1), a rare childhood neurodegenerative disease (2). Recently we demonstrated that this compound destroys neurons in the rat central nervous system when administered either subcutaneously or intracerebrally (3). In order to establish the metabolic fate of cysteine-S-sulfate and trace its regional distribution in brain after *in vivo* administration, we explored methods for preparing ^{35}S - labeled cysteine-S-sulfate. Here we describe a simple method for preparing cysteine-^{35}S-sulfate from cold cysteine-S-sulfate and cysteine-^{35}S.

MATERIAL AND METHODS

Cysteine-^{35}S HCl was purchased from the Radiochemical Center, Amersham/Searle Corp., Chicago, Illinois. Cysteine-S-sulfate was prepared in our laboratory by

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the Segel and Johnson method (4). Purity of the compound was confirmed by I.R. and elemental analysis. The percentages calculated for $C_3H_6NO_5S_2Na \cdot H_2O$ were: C, 14.93; H, 3.35; N, 5.81; S, 26.59; Na 9.53. The percentages found were: C, 14.87; H, 3.24; N, 5.65; S, 26.39; Na, 9.42.

The method employed for obtaining ^{35}S labeled cysteine-S-sulfate takes advantage of an exchange reaction which occurs chemically when cysteine and cysteine-S-sulfate are incubated in aqueous solutions together.

$$\overset{*}{R\text{-}SH} + R\text{-}S\text{-}SO_3^- \longrightarrow R\text{-}\overset{*}{S}\text{-}S\text{-}R + HSO_3^-$$

$$R\text{-}\overset{*}{S}\text{-}S\text{-}R + R\text{-}S\text{-}SO_3^- \longrightarrow R\text{-}\overset{*}{S}\text{-}SO_3^- + R\text{-}S\text{-}S\text{-}R$$

$$\overset{*}{R\text{-}SH} + 2\ R\text{-}S\text{-}SO_3^- \longrightarrow R\text{-}S\text{-}S\text{-}R + R\text{-}\overset{*}{S}\text{-}SO_3^- + HSO_3^-$$

Cysteine-^{35}S, 25 μmoles and sodium cysteine-S-sulfate, 100 μmoles were incubated in a total volume of 2.0 ml distilled deionized water for 30 min. at room temperature with constant shaking under nitrogen. After incubation the mixture was diluted to 3.0 ml by addition of 1 ml distilled deionized water and centrifuged to remove solid cystine. The supernatant was passed through Dowex-50W H^+ (200-400 mesh) resin in an 0.8 X 8.0 cm chromatography column with 20 mg of water used as eluant. To study the eluted material for radioactivity, 1 ml of eluate was mixed with 10.0 ml Scintiverse (Fisher Scientific Co.) and counted with a scintillation spectrometer. The eluate was concentrated by evaporation and its content precipitated by the addition of acetone. This was further recrystallized by treatment with hot water and aqueous ethanol (4). The product was identified as cysteine-S-sulfate and evaluated for purity by thin layer chromatography (TLC) on 2.5 X 10 cm glass plates coated with a 250 micron thick layer of cellulose, in the following solvent systems: (a) n-butanol:acetic acid: water (70:20:20); (b) isopropyl alcohol:formic acid:water (75:12.5:12.5) and (c) n-butanol:pyridine: 1N HCl (1:1:1), with authentic unlabeled cysteine-S-sulfate used as a standard. A radioactive scanner established localization of radioactivity to the cysteine-S-sulfate spot. Further confirmation was achieved by paper electrophoresis at pH 3.5 using a high voltage of 5 kv for 2 1/2 hours.

RESULTS AND DISCUSSION

Since several oxidation products of cysteine other than cysteine-S-sulfate (e.g., cysteine sulfinic acid or cysteic acid) might have formed in our preparation and would have been adsorbed on Dowex-50W and eluted with cysteine-S-sulfate, we ruled this out in early experiments. Before crystalization, the water eluate was analyzed on TLC using all three solvent systems mentioned in methods. Only one ninhydrin-positive spot was observed in any of the three systems. This was further confirmed by incubating cysteine-^{35}S alone without adding cysteine-S-sulfate. Analysis of the water eluate revealed only 2% as much radioactivity as was obtained when cysteine-S-sulfate was included in the incubation. Moreover on TLC there was no ninhydrin spot, which suggests that the slight amount of radioactivity in the eluate was due to inorganic $^{35}SO_4$.

By performing the incubation anaerobically (under nitrogen) we obtained a 15% enhancement of yield apparently due to inhibition of the conversion of cysteine to cystine which normally occurs under aerobic conditions. We were consistently able to obtain a high yield (80%) of cysteine-^{35}S-sulfate by anaerobic incubation provided the ratio of cysteine-^{35}S to cysteine-S-sulfate was 1:4 in the reaction mixture. Of course, the presence of excess amounts of non-labeled cysteine-S-sulfate lowered the specific activity of the final product. However, this difficulty could be overcome by using cysteine of a high specific activity as a starting material.

The radioactive product of the reaction was confined to one peak corresponding to a unique ninhydrin spot in the same position as non-labeled authentic cysteine-S-sulfate. Identification of the reaction product as cysteine-S-sulfate was reinforced by the fact that it behaved exactly as a sample of authentic cysteine-S-sulfate on TLC in three different solvent systems. By the procedure described herein a microquantity of cysteine-^{35} sulfate can be obtained, the final concentration of which could be determined by ninhydrin reaction (5), using a standard curve of the ninhydrin reaction obtained with known amounts of non-labeled cysteine-S-sulfate.

It is concluded that the incubation of cysteine-^{35}S with unlabeled cysteine-S-sulfate, as described here, affords a rapid and easy method of obtaining chromatographically pure cysteine-^{35}S-sulfate.

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