New Chemo-Enzymic Synthesis of Very High Specific Radioactivity [^{35}S] (S) Methionine [1].

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SUMMARY

[^{35}S] H_2SO_4 was quantitatively reduced to [^{35}S] H_2S with a pretreated mixture of $HI/HCl/H_3PO_2$. [^{35}S] H_2S was used in a (S) homoserine sulfhydrylase [EC.4.2.99.10] catalysed thiol exchange reaction with O-acetyl-(S) homoserine to afford [^{35}S] (S) homocysteine. The latter was methylated (CH_3I/NaOH) in situ giving rise to [^{35}S] (S) methionine which was purified by HPLC. Overall yield 10-15%; specific radioactivity : > 46 Tbq/mmol e.g. > 1243 Ci/mmol; chemical and radiochemical purities: > 98%.

Key words: [^{35}S], [^{35}S] (S) homocysteine, [^{35}S] H_2S, O-acetyl-(S) homoserine sulfhydrylase (OAHS)

INTRODUCTION

(S) Methionine is widely used to study biological processes and its labelling is important for these investigations [2]. [^{35}S] (S) Methionine 4 can be prepared by chemical or biological methods. Chemical methods require mmole scale, leading to 4 with low specific activity (1-10 Ci/mmol) [3]. The biological

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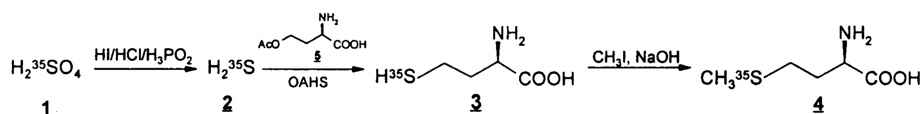
method, using µmole scale, is more adapted to prepare 4 with high specific activity (> 40 TBq/mmol). This process uses microorganisms (yeast or bacteria) which are grown on minimum media in the presence of [^{35}S] sulphate salts as the exclusive source of sulphur. After hydrolysis of the proteins, the labelled [^{35}S] amino acids are purified by HPLC. However, [^{35}S] (S) methionine is contaminated by unlabelled amino acids (e.g. (S) Valine, (S) Leucine) and by [^{35}S] (R) methionine in the range of 1-3% [5] and, this method requires numerous steps. In our laboratory, we have developed a new method using an isolated enzyme for the routine preparation of pure high specific activity [^{35}S] (S) methionine 4 involving very few steps.

RESULTS AND DISCUSSION

The strategy of our work was to use an isolated enzyme: O-acetyl (S) homoserine sulfhydrylase [E.C.4.2.99.10] obtained from Saccharomyces cerevisiae [6], which catalyzed the exchange of the O-acetyl group of O-acetyl-(S) homoserine 5 or O-acetyl-(S) serine by the thiol group to afford (S) homocysteine or (S) cysteine respectively. The labelled thiol was prepared from the commercially available [^{35}S] H_2SO_4 1. The methylation of (S) homocysteine 3 gave (S) methionine 4 according to the following scheme:



To avoid an isotopic dilution of [^{35}S] H_2SO_4 1 by the traces of sulfates present in the reducing mixture $HI/HCl/H_3PO_2$, the latter was pretreated by heating at 110°C for 1h [1]. These conditions have been determined by using a [^{35}S] H_2SO_4 radiotracer. After heating no radioactivity was found in the mixture by liquid scintillation counting. Thus, sulfates were quantitatively reduced to H_2S which was removed under a stream of helium. After cooling, [^{35}S] H_2SO_4 1 was added to the pretreated reducing mixture and heated at 110°C for 1h. [^{35}S] H_2S 2 formed was trapped in a solution of NaOH. The basic solution was adjusted to pH 7.8 by addition of 1N HCl and O-acetyl-(S) homoserine 5 [7], O-acetyl-(S) homoserine sulfhydrylase [E.C.4.2.99.10] purified according to [6] and the cofactors necessary for the enzymatic reaction were added. After 1 h at 20°C, [^{35}S] (S) homocysteine 3 was methylated by addition of CH_3I and NaOH. The reaction mixture was stirred at 20°C for 30 min and injected onto a HPLC system. [^{35}S] (S) methionine was obtained with a chemical and radiochemical purities better than 98% (overall yield: 10-15%).

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EXPERIMENTAL

GENERAL

50% H_3PO_2, pyridoxal phosphate and CH_3I were purchased from Janssen, dithiothreitol from Sigma. HPLC was run on a Waters system equipped with a 510 pump and a 490E UV-multiwavelength detector and a Berthold LB 503 radioactivity detector. [^{35}S] H_2SO_4 (44.4-48.1 Tbq/mmol, 1200-1300 Ci/mmol) was prepared by Cis Bio Industrie (LAPIB) Saclay.

Pretreatment of the reducing mixture:

A mixture of $H_2O/HCl/HI/H_3PO_2$: 5.3/4.7/10/2.5 v/v (5 mL) was introduced in a reactor and heated at 110°C for 45 min while bubbling helium (0.9 L/h) then cooled at room temperature.

[^{35}S] H_2S **2**

To the pretreated reducing mixture, [^{35}S] H_2SO_4 **1** (400 mCi-14.8 Tbq, SA = 1243 mCi/mmol-46 Tbq/mmol, 200 μL) was added and heated at 110°C for 45 min while bubbling helium (0.4 L/h). [^{35}S] H_2S was trapped in a cooled solution of 1N NaOH (20 μmol). (reduction yield: > 99%).

[^{35}S] (S) homocysteine **3**

To the solution of [^{35}S] H_2S **2** was added a Tris-HCl buffer (5 mL, 1M). The solution was adjusted to pH 7.8 by 1N HCl and pyridoxal phosphate (1 mL, 5.3 mg/mL), O-acetyl (S) homoserine (100 μL, 16.1 mg/mL), dithiothreitol (2 μL, 154 mg/mL), and O-acetyl-(S) homoserine sulfhydrylase (5 μL, SA = 0.135 mmol/min/mg of proteins, C = 2.4 mg of proteins/mL) were successively added. The reaction medium was maintained at 20°C for 1h.

[^{35}S] (S) Methionine **4**

To the reaction mixture were added CH_3I (0.08 mmol, 5 μL) and a solution of 1N NaOH (25 μmol). The reaction mixture was stirred at 20°C for 1h and injected on a semi-preparative C18 column (Zorbax) fitted with a pre-column. The system was eluted with water, swept by a stream of helium, at a flow rate of 3 mL/min. 40 to 60 mCi of [^{35}S] (S) methionine (retention time: 8-10 min) were collected in a tube cooled at 0-5°C, under argon. The overall yield range from 10 to 15%.

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The radiochemical purity was better than 98%, checked by HPLC on a analytical Zorbax-NH_2 column, eluent: 0.1 M KH_2SO_4 (pH 4)/CH_3CN: 80/20. ([^{35}S] (S) methionine sulphoxide was the main by-product). It was also checked by autoradiography on TLC: cellulose: eluent: n-butanol/formic acid/water: 4/1/1 and found to be better than 98%.

The chemical purity was checked by TLC in the above conditions. No other amino acid was detected by ninhydrin revelation.

The optical purity was better than 98%, checked by chiral TLC (Macherey-Nagel): $CH_3CN/CH_3OH/H_2O$: 4/1/1, a single spot. (no radioactive spot corresponding to [^{35}S] (R) methionine was detected).

The specific radioactivity, measured on a Kontron amino acid analyzer and by liquid scintillation counting, was equal to that of the starting precursor [^{35}S] H_2SO_4 **1**.

The conditions of storage and the rate of decomposition of [^{35}S] (S) methionine were the same as described in ref. 5

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