

NOTE

SYNTHESIS OF L-[³⁵S]HOMOCYSTEINE THIOLACTONE HYDROCHLORIDE

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SUMMARY

L-[³⁵S]Homocysteine thiolactone has been synthesized by demethylation of L-[³⁵S]Methionine with sodium in liquid ammonia and subsequent lactonisation in acid solution. The radiochemical yield of the carrier added synthesis was in the range of 45 to 50 % with a radiochemical purity higher than 96 %.

KEY WORDS: Homocysteine thiolactone hydrochloride,
demethylation, S-35

INTRODUCTION

Homocysteine is an important metabolite involved in the methionine, cysteine and methylation metabolism. In the presence of SAH-hydrolase it can be condensed with adenosine to form S-adenosyl homocysteine which accumulates in ischemic heart regions due to an increased concentration of adenosine (1). Autoradiographic studies of ischemic and normoxic heart regions using [³⁵S] homocysteine have confirmed this approach (2). An efficient and simple method of recovering methionine-free L-[³⁵S]homocysteine thiolactone is described.

EXPERIMENTAL

Material and Methods

L-[³⁵S]Methionine in aqueous solution ($\sim 10^3$ Ci/mmol) was purchased from Amersham Buchler, Braunschweig, FRG. Methionine

and the other chemicals were obtained from MERCK, Darmstadt, FRG. The chemical purity of the prepared radioactive compound was checked by TLC (RP2 silica plates, MERCK) and the radiochemical purity by TL radioactivity scanning. Measurement of radioactivity was carried out with Beckmann LS2800 liquid scintillation counter using Insta-Gel scintillation cocktail. The results were corrected for quenching using an external standard method.

Demethylation of L-[³⁵S]methionine

L-[³⁵S]methionine solution (70 μ l, ~ 1 mCi) was added to L-methionine (10 mg) and dried in vacuum over phosphorus pentoxide. A stream of ammonia dried over molecular sieve (4Å) was then introduced into the reaction vessel. The flask was cooled to about -40°C and liquid ammonia (2 ml) was condensed in. Elemental sodium (6-7 mg) was added to the solution, and gaseous ammonia was bubbled through the deep blue reaction mixture. Within 10 to 15 min the blue colour disappeared completely and after additional 5 to 10 min at -40°C ammonium chloride (7 mg) was added to destroy the sodium amide formed. Subsequently, the cooling bath was removed and the liquid ammonia evaporated by a gentle flow of helium. The ammonia free residue was dissolved in water (0.2 ml) followed by the addition of concentrated hydrochloric acid (0.3 ml). The solution was heated under reflux for about 30 min in an inert atmosphere and evaporated to dryness under a stream of helium. The slightly yellow residue was suspended in ethanol (4 ml) and filtered over a SEP-PAK C-18 cartridge.

Isolation of methionine-free L-[³⁵S]homocysteine thiolactone

The ethanol solution was evaporated to dryness and the residue dissolved in methanol (2 ml). The solution was placed on a neutral SEP-PAK alumina cartridge, similar to a method described in the literature (3) and eluted with methanol (3 ml). The solution was acidified with concentrated hydrochloric acid (10 μ l) and evaporated to dryness.

An aliquot of the [³⁵S]homocysteine thiolactone was placed on a reversed phase thin layer plate (RP2 silica gel) alongside standards of methionine and homocysteine thiolactone. The chromatogram was developed using a mixture of equal parts by volume of isopentanol, dioxane, pyridine and water (3). The amino acids were detected by ninhydrin (Rf-values: homo-

cysteine 0.38, methionine 0.85). The activity distribution was measured by scanning the plate using a Geiger-Müller detection system.

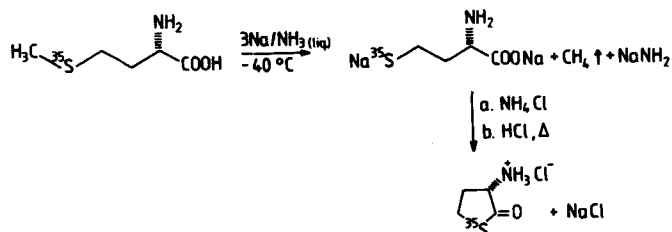


Fig. 1 Reaction scheme for the synthesis of L-[³⁵S]homocysteine thiolactone hydrochloride

Results and Discussion

As high specific activity L-[³⁵S]homocysteine thiolactone was not needed, the demethylation of L-[³⁵S]methionine was done in presence of a large excess of L-methionine. The presence of the carrier depressed side reactions and allowed the chemical reaction to proceed under equimolar conditions. The reductive thioether cleavage by elemental sodium liquid ammonia is a convenient method to prepare the homocysteine sodium salt in good yields (4). The subsequent lactonisation of the homocysteine by heating with 6 M hydrochloride acid led to nearly quantitative cyclisation of the thioamino acid. To prevent the oxidative dimerisation of homocysteine forming homocystine the lactonisation is done in an inert atmosphere.

TLC analysis of the crude product showed that about 70 % of the L-[³⁵S]methionine was demethylated yielding L-[³⁵S]homocysteine thiolactone. After the purification using an alumina SEP-PAK cartridge it was shown that the synthesized homocysteine was free from methionine. Methionine is held strongly by the alumina through its carboxyl group, and is not eluted by methanol. While homocysteine thiolactone, is eluted very rapidly, traces of homocysteine are also retained by the SEP-PAK alumina cartridge. The specific activity of the

purified product obtained was in the range of 6 to 8.5 Ci/mol and the radiochemical yield of the thiolactone was about 50 %. The purified homocysteine thiolactone should be kept under acidic conditions to minimize oxidation.

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