PREPARATION OF L-CYSTEINE-^{35}S HYDROCHLORIDE BY REDUCTION OF L-CYSTINE-^{35}S

M.B. Skakun-Todorović and S.R.Albahari
Radioisotope Laboratory
Boris Kidrič Institute of Nuclear Sciences - Vinča
11001 Beograd, P.O.B. 522,
Yugoslavia

S u m m a r y

The method for the synthesis of L-cysteine hydrochloride labelled with ^{35}S is described. L-Cystine-^{35}S obtained from baker's yeast was reduced with tin in hydrochloric acid and radiochemically pure L-cysteine-^{35}S hydrochloride was isolated by ion-exchange chromatography on a column.

The obtained L-cysteine-^{35}S hydrochloride was stable in aqueous solution, pH 2, for six months and had specific activity of 20-125 mCi/mmol and radiochemical purity better than 95%.

Key words: L-cysteine-^{35}S, L-cystine-^{35}S, sulphur-^{35}S

I N T R O D U C T I O N

Besides other sulphuric amino acids, such as methionine and cystine, cysteine is also widely applied in biology, chemistry and medicine.

Radiolabelled cysteine is very suitable for investigation of the mechanism of proteins synthesis, their metabolism etc.

Cysteine belongs to the group of chemoprotectors i.e. chemical compounds which, when applied before irradiation, provide certain protection during external and internal irradiation by

0362-4803/79/0516-0711$01.00 Received December 2, 1978

X-rays.[1-3] The study of distribution of L-cysteine-^{35}S in organs and tissues, as well as its metabolism, contribute to clarification of its protective effect.

Since penicillin is an important derivative of cysteine, L-cysteine-^{35}S is used for investigation of the formation mechanism of this antibiotic from Penicillia.[4]

Several methods for chemical preparation of DL-cysteine-^{35}S are described in the literature.[5-8] In the Institute "Boris Kidrič" Radioisotope Laboratory, DL-cysteine-^{35}S-HCl is produced from thiourea-^{35}S and α-chloroacrylic acid methyl ester by the modified method of Behringer and Zillikens.[6]

In our work L-isomer of the mentioned amino acid was obtained from L-cystine-^{35}S-HCl, isolated from baker's yeast,[9] by reduction with tin in hydrochloric acid.[5]

Specific activity of the obtained L-cysteine-^{35}S-HCl is 20-125 mCi/mmol and radiochemical purity is higher than 95%.

Traces of tin are eliminated by cationic ion-exchange chromatography on a column. Pure L-cysteine-^{35}S-HCl was obtained in a yield of 70-80%, radiochemically stable for six months in aqueous solution, pH 2.

EXPERIMENTAL

L-Cystine-^{35}S-HCl (50 mCi, specific activity of the order Ci/mmol) was diluted with inactive L-cystine (240 mg, 1 mmol) and dissolved in 3.3 ml of hydrochloric acid (1:2). Metallic tin (124 mg, 1.05 mmol) was added to the solution which was then refluxed in nitrogen stream for 4 hours by constant stirring up to complete dissolution of tin.

After completion of the reaction 5,5 ml of water was added to the solution and tin was precipitated by saturation with hydrogen sulphide. The filtrate was evaporated to dryness under reduced pressure and the residue was dissolved in water and adjusted to pH 2 with 10% NaOH.

Aqueous solution of L-cysteine-^{35}S-HCl was passed through an ion-exchange column (15x200 mm) of Dowex 50Wx 8, 200-400 mesh in H^+ form. The column was previously rinsed throughly with 20 ml of 0.2 N acetic acid. After application of L-cysteine-^{35}S-HCl, the column was washed with 15 ml of 0.2 N acetic acid, and L-cysteine-^{35}S-HCl was eluted with 1,5 N HCl at the flow rate of 1 ml/min in nitrogen stream. Portions of 5 ml each were collected. Fractions

of L-cysteine-^{35}S-HCl of radiochemical purity >95% were combined and evaporated to dryness under reduced pressure.

L-cysteine-^{35}S-HCl was stored in aqueous solution, at pH 2, specific activity 4-5 mCi/ml at - 20°C

Radiochemical purity was checked by using thin-layer chromatography on Silica Gel G (Merck), on glass plates (50 x 200 mm) and with 0.25 mm layer thickness in the following solvent systems: a) n-butanol/formic acid/water (75:15:10); n-butanol/propanol/ 0.1 N HCl (3:1:1) and c) n-butanol/acetic acid/water (4:1:1).

Radioactive components were detected by using Berthold-Dünnschicht und Papierchromatogramm Scanner LB-280.

RESULTS AND DISCUSSION

Reduction of L-cystine-^{35}S-HCl to the L-cysteine-^{35}S-HCl is quantitative (Fig. 1-2).

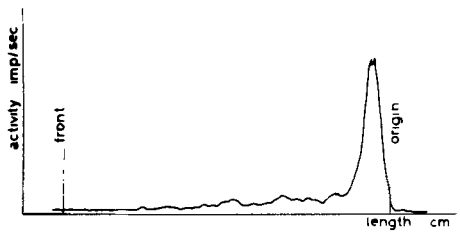

FIG. 1 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTINE-^{35}S-HCl BEFORE REDUCTION

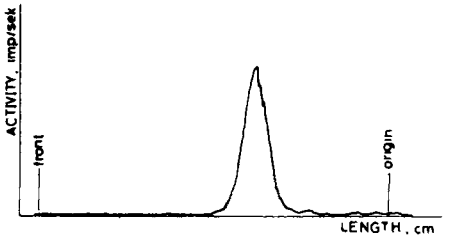

FIG. 2 THIN-LAYER RADIOCROMATOGRAM OF L-CYSTEINE-^{35}S-HCl AFTER REDUCTION

Radiochemical impurities of L-cystine-^{35}S-HCl as well as accompanying inactive amino acid, L-leucine, have no influence on the course of the reduction.

The obtained L-cysteine-^{35}S is of radiochemical purity >95%. Radioactive impurities are L-cystine-^{35}S and an unidentified component with Rf=0.47.

It is known from the literature[10-12] that L-isomer of cysteine-^{35}S is very unstable. Already after 48 hours storage, aqueous solution of L-cysteine-^{35}S at radioactive concentration of 4-5 mCi/ml has shown considerable increase of quantity of the mentioned impurities.

Radiochemical stability of L-cysteine-^{35}S was investigated under various conditions, by thin-layer chromatography. The results are shown in Figs. 3-7.

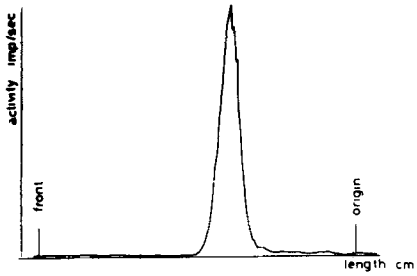

FIG. 3 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTEINE-^{35}S-HCl IN AQUEOUS SOLUTION, AFTER STORAGE FOR 24 HOURS AT -20°C

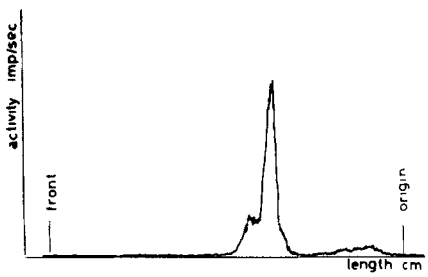

FIG. 4 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTEINE-^{35}S-HCl IN DILUTE SOLUTION OF HCl(1:2) AFTER STORAGE FOR 24 HOURS AT -20°C

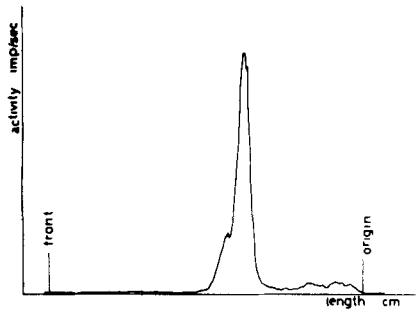

FIG. 5 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTEINE-^{35}S-HCl IN AQUEOUS SOLUTION AFTER STORAGE FOR 3 DAYS

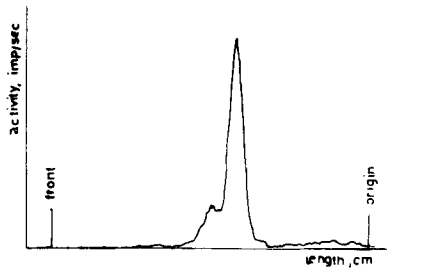

FIG. 6 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTEINE-^{35}S-HCl IN AQUEOUS SOLUTION UNDER VACUUM AFTER STORAGE FOR 3 DAYS AT - 20°C

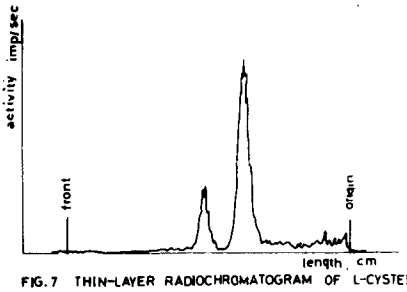

FIG. 7 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTEINE-^{35}S-HCl IN AQUEOUS SOLUTION WITH β-MERCAPTOETHANOL UNDER NITROGEN, STERILIZED, AFTER STORAGE FOR 3 DAYS AT - 20°C

It is evident from the shown radiochromatograms that considerable increase of radioactivities of L-cystine-^{35}S and of the radioactive components Rf=0.47 occured already after 3 days storage, in aqueous solution without previous purification.

After purification by ion-exchange on the column, L-cysteine-^{35}S-HCl is stable up to six months, in aqueous solution, pH 2, as shown in Figs. 8-12.

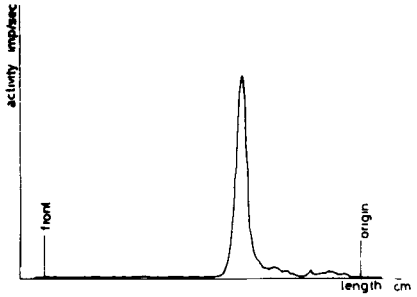

FIG. 8 THIN-LAYER RADIOCHROMATOGRAM OF L-CYSTEINE-^{35}S-HCl AFTER PURIFICATION TROUGH THE COLUMN

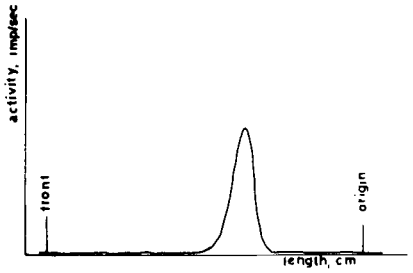

FIG.9 THIN-LAYER RADIOCHROMATOGRAM OF THE PURIFIED L-CYSTEINE-^{35}S-HCl AFTER STORAGE FOR 5 DAYS AT -20°C

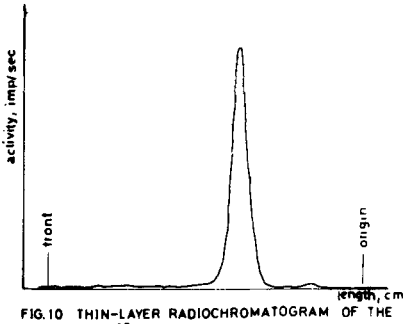

FIG.10 THIN-LAYER RADIOCHROMATOGRAM OF THE PURIFIED L-CYSTEINE-^{35}S-HCl AFTER STORAGE FOR 10 DAYS AT -20°C

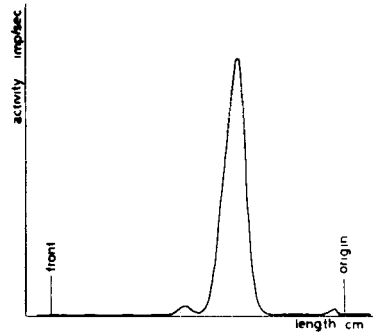

FIG. 11. THIN-LAYER RADIOCHROMATOGRAM OF THE PURIFIED L-CYSTEINE-^{35}S-HCl AFTER STORAGE FOR 30 DAYS AT -20°C

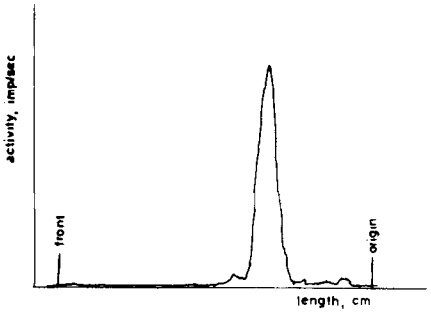

FIG. 12 THIN-LAYER RADIOCHROMATOGRAM OF THE PURIFIED L-CYSTEINE-^{35}S-HCl AFTER STORAGE FOR 6 MONTHS AT -20°C

REFERENCES

1. Brunborg, G.- Int. J. Radiat. Biol. 32: 285 (1977)
2. Bridges, B.A.- Adv. Radiat Biol. 19: 651 (1969)
3. Vos, O. and Budke, L.- Int. J. Radiat. Biol. 30: 433 (1976)
4. Fruton, J.S. and Simmonds, S.- General Biochemistry, New York- John Wiley and Sons, Inc. 1959, p. 798
5. Kogan, N.A. and Feldman, J.H.- Ž. Prikl. Chim. 44: 1438 (1971)
6. Behringer, H. and Zillikens, P.-Ann. 574: 140 (1951)
7. Emiliozzi, R., Pichat, L. and Herbert, M.- Bull. Soc. Chim. France, 1544 (1959)
8. Turner, R.B. and Voitle, D.- J. Am. Chem. Soc. 72: 628 (1950)
9. Albahari, R.S. and Skakun-Todorović, B.M.- J. Labelled Compounds and Radiopharmaceuticals 14:727 (1978)
10. Voelker, J., Schümann and Holt, C.V.- Biochem. Z. 335: 382 (1962)
11. Bonker, G.J. and Tonge, B.L.- J. Chromatog. 12: 52 (1963)
12. Wainer, A.- J. Chromatog. 21: 126 (1966)