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Synthesis of N^G-Mono[^{14}C-methyl]-L-arginine

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N^G-Mono[^{14}C-methyl]-L-arginine의 합성

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국문초록

N^G-Mono[^{14}C-Methyl]-L-arginine을 방사선화학적 방법으로 mono[^{14}C]-Methylamine으로부터 합성한후 양이온 교환수지에 흡착시킨 다음 암모니아수로 용출시켜 정제하였으며 flavianic acid를 사용하여 결정상태로 얻었다. 한편 flavianate와 음이온 교환수지를 함께 실온 이하의 온도에서 교반혼합함으로서 유리 상태의 amino acid를 쉽게 만들 수 있으며 박층크로마토그라피, 박층전기영동 및 섬광분광분석법으로 순도를 조사하였다.

ABSTRACT

Radiochemical synthesis of N^G-mono[^{14}C-methyl]-L-arginine is described. The compound was synthesized from radio-active mono[^{14}C]-methylamine as easily and purified by strong cation-exchange resin (NH_4^+ form) liquid chromatography using a gradient of ammonium hydroxide, and crystallized as flavianate. The free amino acid was successfully prepared by strirring its flavianate and strong anion-exchange resin (OH^- form), which could remove the flavianic acid from its salt in water below room temperature.

Purity of the compound was tested by thin-layer chromatography, thin-layer electrophoresis, and scintillation spectrometry.

INTRODUCTION

N^G-Monomethyl-L-arginine is a naturally occurring amino acid in several proteins, among which are myelin basic preotein[1] and histones[2]. An enzyme which is responsible for the methylation of arginine residue in protein has been

characterized[3]. Appreciable quantities of N^G-monomethyl-L-arginine are present in humane urine[4], and synthesized by using N, O-dimethylpseudouronium sulfate and the copper complex of ornithine[5]. However, the reaction was slow (3 days) and the yield was low (17 %). Another procedure using the copper complex of ornithine and N, S-dimethylthiopseudouronium iodide prepared from monomethylthiourea and methyl iodide, was reported[6,7]. But the compound synthesized by following the above methods, even though recrystallization several times, is always contaminated with a few percent of ornithine[8], which will be able to inhibit any biological reaction of N^G-monomethyl-L-arginine.

The lack of commercially available and racemically pure N^G-mono[^{14}C-methyl]-L-arginine has limited its experimental applications, such as the metabolic fates of the amino acid *in vitro* and *in vivo*. To overcome the deficiency, a procedure has been developed for the synthesis of the various N^G-alkyl derivatives of arginine[9,10]. For the convenience of the other workers in this field, author wishs to report a symplified method for preparation of N^G-mono [^{14}C-methyl]-L-arginine.

EXPERIMENT

Materials

[^{14}C]Methylamine hydrochloride (46.0 mCi/mmole) was purchased from New England Nuclear. L-Ornithine hydrochloride, methylamine hydrochloride, carbon disulfide, ethyl chloroformate, methyl iodide, flavianic acid, Dowex 1, and Dowex 50 were purchased from Sigma Chemical Co., and the remaining chemicals were of the highest grade.

Synthesis

N-Mono[^{14}C-methyl]-thiourea

Carbon disulfide (7.6 g, 0.1 mole), a solution of methylamine hydrochloride (6.75 g, 0.1 mole) in 15 ml of water, and a solution of [^{14}C]methylamine hydrochloride (46.0 mCi/mmole, 1.0 mCi) in 5 ml of water were placed in a 100 ml three-neck flask with a reflux condenser, a dropping funnel, and thermometer, and then was cooled to 0-5°C in an ice-water bath.

A solution of sodium hydroxide (8.08 g, 0.202 mole) in 15 ml of water was added to the above mixture over 1 hr period with constant stirring (the internal temperature was maintained at around 5°C). The orange reaction mixture was then heated to 70°C in a water bath for 2 hr and subsequently cooled to above 40°C.

Ethyl chloroformate (10.9 ml, 0.1 mole) was added to the mixture over 1 hr period, and the reaction mixture was then cooled to room temperature. The oily material (methyl isothiocyanate) was placed in a 100 ml three-neck flask with a reflux condenser, a thermometer and 50 ml separating funnel.

Concentrated ammonium hydroxide (9.4 g, 0.15 mole) was then added to the flask over 2 hr period with stirring. The condenser was removed, and the solution was heated on a boiling water bath for 1 hr.

The solution was treated with activated charcoal and filtered. The filtrate was chilled to 4°C overnight. The coloress compact crystals were isolated by filtration. The compound was then recrystallized from water, filtered, and dried. The yield of pure N-[^{14}C-methyl]thiourea is 7.1 g and 792 uCi (79% based on ^{14}C-methylamine hydrochloride) with a m.p. 118-120°C (lit.[10] 119-120.5°C).

N^G-Mono[^{14}C-methyl]-L-arginine monoflavianate

N-[^{14}C-Methyl]thiourea (1.8 g, 20 mmole, 50 uCi) and acetone (20 ml) were placed in a 100 ml flask with a reflux condenser which was surrounded in an ice-water bath, and methyl iodide (1.9 ml, 30 mmole) was then added while stirring. The bath was removed after 2 hr and stirring was continued at room temperature for another 2 hr. Subsequently, the solution was concentrated to dryness under reduced pressure, and the residue was dissolved in 20 ml of water. The solution was treated with activated charcoal, filtered, and the charcoal was washed once with 10 ml of hot water.

The combined solution, which contained N, S-dimethylthiopseudouronium iodide was coupled with L-ornithine whose α-amino group has been masked with cupric acetate (2.0 g, 10 mmole) in 20 ml of 25% ammonium hydroxide[11] and the mixture was stirred for 24 hr at room temperature under a hood.

The preciptate formed was separated by filtration, and washed with 10 ml of 10% ammonium hydroxide. The combined solution was then concentrated to dryness under reduced pressure at around 35°C, and the residue was dissolved in 50 ml of water. The solution was loaded on a Dowex 50 (H^+ form) column (2 cm × 20 cm; 200-400 mesh and 8% cross-linking) in order to remove copper and impurities. The column was washed with 200 ml of water and subsequently eluted with 3 M ammonium hydroxide at a flow rate of 2 ml per minutes. 10 ml fractions were collected and the presence of ornithine and monomethyl arginine was detected by thin-layer chromatography (solvent, ethylacetate : 90% formic acid: water = 7:3:1). The fractions containg ornithine and monomethylarginine were pooled and then concentrated to dryness under reduced pressure at around 35°C. The residue was dissolved in 50 ml of water and loaded on a Dowex 50 (NH_4^+ form) column (2 cm × 15 cm; 200-400 mesh and 8% cross-linking) which was equilibrated with water. The column was washed with 200 ml of water and then eluted by a linear gradient of 0.1-2 M NH_4OH (500 ml) at a flow rate of 2 ml per minute. 10 ml Fractions were collected, and for the detection of ornithine and monomethylarginine, 5 ml of every fraction was spotted on filter paper, dried, and colored with ninhydrin.

The fractions of N^G-mono[^{14}C-methyl]-L-arginine were pooled and evaporated to dryness under reduced pressure at around 35°C, and then redissolved in 20 ml of water. The clear solution was added to a solution of flavianic acid (6.28 g, 20 mmole) which was dissolved in 50 ml of water. The mixture was stored at 4°C overnight. The precipitates were filtered and washed with water, and then recrystallized from water to yield 4.5 g (22 uCi) (44%, based on L-ornithine). m.p. 253-255°C (lit.[5] 252-253°C).

In order to remove flavianic acid from the flavianate, 1 mmole (504 mg, 2.46 uCi) of the flavianate, 20 ml of water, and 5 ml of Dowex 1 (OH^- form) (bed volume) were placed in a 50 ml flask. The mixture was stirred overnight at 4°C. The resin was filtered and washed twice with 5 ml of water. The filtrate was colorless and the yield was 97.5% (2.4 uCi).

RESULT AND DISCUSSION

$$^{14}CH_3NH_2 + CS_2 \longrightarrow {}^{14}CH_3NCS \xrightarrow{NH_3} {}^{14}CH_3NHCSNH_2$$
$$\xrightarrow{CH_3I} {}^{14}CH_3N{=}C(SCH_3)NH_2 \xrightarrow{\text{ornithine}}$$

$^{14}CH_3N = C(NH_2)NHCH_2CH_2CH_2CH(NH_2)COOH$

N^G-mono[^{14}C-methyl]-L-arginine

The crude N, S-dimethylthiopseudouronium iodide which was derived from N-mono[^{14}C-methyl]amine, carbon disulfide, ammonia, and methyl iodide was successfully coupled with L-ornithine whose α-amino group had been blocked by cupric ion in an ammoniacal solution.

N^G-Mono[^{14}C-methyl]-L-arginine formed could be purified by cation-exchange resine liquid chromatography using a linear gradient of aqueous ammonium hydroxide, and further purified by recrystallization from water as a salt of flavianic acid which was insoluble in cold water and soluble in hot water.

The free amino acid which was very hygroscopic and soluble in water could be prepared by mixing the flavianate of the amino acid and strong anion-exchange resine at room temperature as easily.

The purity of N^G-mono[^{14}C-methyl]-L-arginine was tested by thin-layer chromatography and thin-layer electrophoresis, which chromatograms were colored with ninhydrin, and radioactivity was counted by using a scintillation spectrometer.

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REFERENCES

1. Baldwin, G.C. and Carnegie, R.P. (1971) *Science,* **171**, 579-581.
2. Paik, W.K. and Kim, S. (1970) *J. Biol. Chem.* **245**, 88-92.
3. Paik, W.K. and Kim, S. (1980) Protein Methylation, John Wiley and Sons, New York.
4. Kakimoto, Y. and Akazawa, S. (1970) *J. Biol. Chem.* **245**, 5751-5758.
5. Nakajima, T., Matsuoka, Y. and Kakimoto, Y. (1971) *Biochim. Biophys. Acta,* **230**, 212-222.
6. corbin, T.L. and Reporter, M. (1974) *Anal Biochem.* **57**, 310-312.
7. Abou-Gharbia, M., Paik, W.K. and Swern, D. (1981) *J. Labelled Compds. and Radiopharm.* **18**, 777-779.
8. McDermott, J.R. (1976) *Biochem. J.* **154**, 179-184.
9. Cho, Y.B., Furst, G. and Paik, W.K. (1984) *Anal. Biochem.* **139**, 377-382.
10. Cho, Y.B. and Paik, W.K. (1986) *New Med. J.* (korea) **29**, 673-679.
11. Moore, M.L. and Crossley, F.S. (1955) *Org. Syn. Coll. Vol.* **III**, 617-619, John Wiley and Sons, New York.
12. Kurzt, A.C. (1937-1938) *J. Biol. Chem.* **122**, *477.*