Improved Synthesis of 3-¹⁴C-3-Hydroxy-3-methylglutaryl Coenzyme A

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Received April 4, 1975

The reduction of HMG-CoA to mevalonate is catalyzed by HMG-CoA reductase (EC 1.1.1.34). This enzymatic reaction is a rate-limiting step in the biosynthesis of cholesterol in the liver,¹⁾ and participates in the formation of terpenes such as ipomeamarone in sweet potato roots in response to infection with the black rot fungus, *Ceratocystis fimbriata* Ell and Halst.^{2,3)}

To study HMG-CoA reductase, we have to synthesize 3-¹⁴C-HMG-CoA as the substrate of HMG-CoA reductase. The chemical synthesis of HMG-CoA from HMG acid was first described by Hilz *et al.*⁴⁾ and has been employed by many investigators. In recent years, a simple synthesis for HMG anhydride was reported by Goldfarb and Pitot,⁵⁾ which overcame the problem of 3-acetoxy-HMG anhydride contamination during synthesis of HMG anhydride using the method of Hilz *et al.*⁴⁾

Louw *et al.*⁶⁾ and Goldfarb and Pitot⁵⁾ reported that the synthesized HMG-CoA was purified by ascending chromatography on Whatman filter paper No. 3MM with *n*-butanol-acetic acid-water (5:2:3, v/v) for 4 hr at about 24±1°C; HMG-CoA (*R*_f0.35) was separated from CoA (*R*_f0.17) and HMG acid (*R*_f0.76).

We synthesized and purified 3-¹⁴C-HMG-CoA and cold HMG-CoA by the method of Goldfarb and Pitot.⁵⁾ As shown in Fig. 1, however, either labeled or cold HMG-CoA (*R*_f0.31) could be separated from 3-¹⁴C-HMG acid (*R*_f0.89) and CoA disulfide (*R*_f0.18), but not from CoA (*R*_f0.31), which had the same *R*_f value. Thus, the substance (*R*_f0.17) assigned as being CoA by Louw *et al.*⁶⁾ and Goldfarb and Pitot⁵⁾ was probably CoA disulfide because it was detectable under UV radiation and gave a negative nitroprusside test⁷⁾ (Table I). Several attempts to completely separate HMG-

FIG. 1. The Distribution of Radioactivity of 3-¹⁴C-HMG-CoA (upper) and the Schematic Representation of the Patterns of Cold HMG-CoA (A), CoA (B) and CoA Disulfide (C) (under) after Paper Chromatography.

The radiochromatogram (upper) was cut into one-cm sections from origin to front, which were assayed for radioactivity. CoA disulfide was prepared by oxidizing CoA with diluted H₂O₂. CoA sample (B) was separated into two components marked 1 (major, B-1) and 2 (minor, B-2). These substances showed blue fluorescence under UV light. Hydroxamate test¹¹⁾ was carried out by spraying the paper with 14% neutralized hydroxylamine, and then with 15% FeCl₃. HMG-CoA produced brownish yellow color with this test. CoA produced pink color by spraying the paper with nitroprusside reagent.⁷⁾

TABLE I. LOCALIZATION OF SYNTHESIZED HMG-CoA, CoA AND CoA DISULFIDE

The symbols + and - indicated positive and negative reactions, respectively.

	UV radiation	Hydroxamate test	Nitroprusside test	<i>R</i> _f value
A	+	+	-	0.31
B-1	+	-	+	0.31
B-2	+	-	-	0.18
C	+	-	-	0.18

A, B-1, B-2 and C; the same as the legend to Fig. 1.

CoA from CoA by ascending paper chromatography proved unsuccessful.

Hepper and Audley⁸⁾ also reported that HMG-CoA could be separated from CoA disulfide and HMG acid but not from CoA by ascending chromatography on Whatman No. 1 paper with ethanol-acetic acid (1:1, v/v) and HMG-CoA prepared by the method of Hilz

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HMG acid, 3-hydroxy-3-methylglutaric acid; CoA, reduced coenzyme A; CoA disulfide, coenzyme A disulfide.

*et al.*⁴⁾ usually contained a small amount of CoA after ascending chromatography.

When 3-¹⁴C-HMG-CoA is synthesized from 3-¹⁴C-HMG anhydride and CoA with an excess amount of the former, it contains no CoA but the unreacted 3-¹⁴C-HMG anhydride is wasted. Whereas, in the case of the reaction of 3-¹⁴C-HMG anhydride with excess CoA, the resulting 3-¹⁴C-HMG-CoA would contain CoA. Since CoA inhibits HMG-CoA reductase,^{9,10)} the synthesized sample of 3-¹⁴C-HMG-CoA must not be contaminated by CoA. Based on these investigations, we devised a modification to the method of Goldfarb and Pitot.⁵⁾ After the reaction of 3-¹⁴C-HMG anhydride with excess CoA, cold HMG anhydride was further added to the reaction mixture to convert the unreacted CoA into cold HMG-CoA until the nitroprusside test⁷⁾ was negative, indicating the end point of CoA consumption. In this way, we were able to synthesize 3-¹⁴C-HMG-CoA without CoA with relative high specific activity, by using all of the 3-¹⁴C-HMG anhydride added. For example, 3-¹⁴C-HMG anhydride was synthesized from 6.3 μ moles (0.1 mCi) of 3-¹⁴C-HMG acid (15.8 mCi/mole, 10.25 mg/mCi, New England Nuclear) and mixed with 111 μ moles of CoA (purity, 85%; Boehringer Mannheim GmbH), then mixed with excess cold HMG anhydride to yield 99.4 μ moles of 3-¹⁴C-HMG-CoA showing 4100 dpm/nmole of specific activity.

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