CHROM. 4757

Sephadex G-15 column separation of [9,10-^{3}H]stearyl coenzyme A

[9,10-^{3}H]Stearyl coenzyme A, which was synthesized according to the procedure of YOUNG AND LYNEN[1], was isolated by column chromatography using Sephadex G-15 as the stationary phase. Previous methods, by which the acyl-CoA esters were separated by precipitation in acidic solution, were insufficient with respect to a small-scale preparation (10 μmoles), or in cases where only small amounts of the fatty acids had reacted with the coenzyme A. The isolated [9,10-^{3}H]stearyl-CoA was identified by common procedures.

Experimental procedure

An amount of 2.85 mg (10 μmoles) [9,10-^{3}H]stearic acid from Amersham/Searle (spec. act. 2,500 mCi/mmole) was converted into the mixed anhydride (9.5 μmoles), which was then reacted with 17 mg (11 μmoles) coenzyme A from Sigma (in the reduced form) to yield the [9,10-^{3}H]stearyl-CoA[1]. The mixture was then acidified to pH 5 with 1% $HClO_4$. After removal of the tetrahydrofuran from the reaction mixture under reduced pressure and extraction of the unreacted stearic acid with light petroleum ether, the solution (pH 7.5) was applied to a 42 × 1.2 cm column, packed with Sephadex G-15 from Pharmacia. The column chromatographic separation was operated at 4°. The eluting solvent was water containing 0.5% mercaptoethanol to prevent oxidation of the product. Fractions of 2 ml were collected; the elution profile (Fig. 1) was obtained by UV absorption (at 260 mμ, using a Gilford 240 spectrophotometer) and by radioactivity measurements (cocktail: 5 g PPO per 1000 ml dioxane). The fractions 1 to 5 were collected, the solvent removed by freeze drying, and the com-

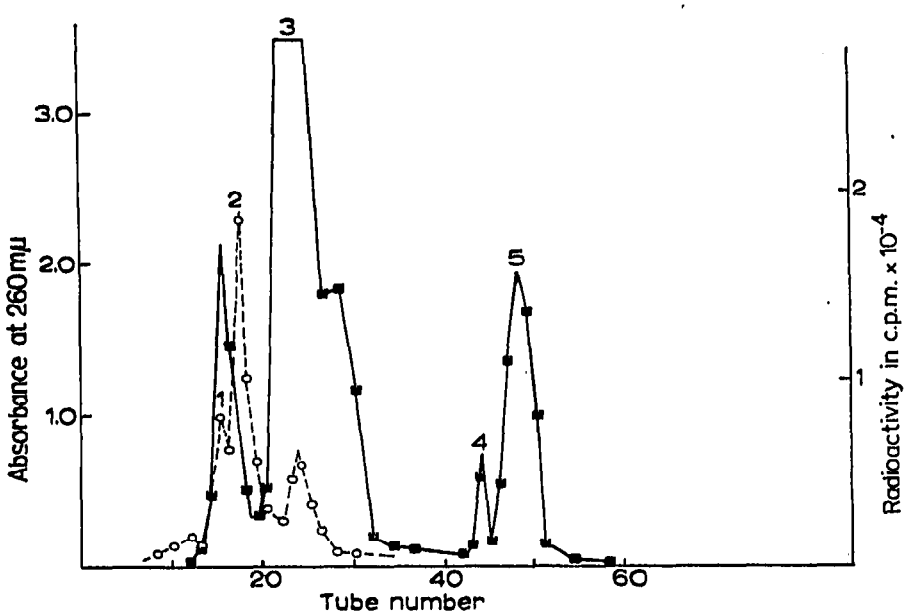

Fig. 1. Separation of the [9,10-^{3}H]stearylCoA on a Sephadex G-15 column. The dimensions of the column were 42 × 1.2 cm. The eluent was water containing 0.5% mercaptoethanol. Fractions of 2 ml were collected. — — — —, Radioactivity curve; ————, UV absorption at 260 mμ. Peaks 1–5 represent fractions 1–5.

pounds were then chromatographed on Whatman No. 3 paper using water–pyridine–isopropanol (1:2:2) as the solvent system[2]. The R_F values were as follows: fract. 1, 0.07; fract. 2, 0.12; fract. 3, 0.31; fract. 4, 0.80; fract. 5, 0.88. The [9,10-^{3}H]stearyl-CoA (fract. 1 and 2) was dissolved in water (pH 7.5) and assayed by absorption at 260 mμ (adenine ring) and by the hydroxamate formation[2]. The yield was 1.6 μmoles (17%).

The occurrence of two peaks (fract. 1 and 2) for the stearyl-CoA, as has also been described for the synthesis of acetyl-CoA[3], cannot be explained at present. The small radioactivity peak under the free coenzyme A peak (fract. 3) may be explained as a result of an incomplete separation of the stearyl-CoA ester from the unreacted CoA. The presence of the two low-molecular weight compounds (fract. 4 and 5) is probably the result of partial alkaline hydrolysis of some of the ester linkages of the coenzyme A molecule[4].

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