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Note

Determination of the thioester purity of fatty acyl-CoA esters*

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Several procedures are currently available for determining the thioester purity of long chain acyl-CoA esters. These include the spectrophotometric measurement of the ferric hydroxamate derivatives of acyl-CoA esters[1–3] spectrophotometric measurement of the ratio of thioester absorption (at 232 nm) to adenine absorption (at 260 nm)[4,5], gas–liquid chromatographic (GLC) analysis of the isocyanate derivatives of acyl hydroxamic acids[6] and GLC analysis of long-chain fatty alcohols after $NaBH_4$ reduction of acyl-CoA esters[7]. Here we report a simple chromatographic and spectrophotometric method for determining thioester purity which does not depend upon the production of thioester derivatives. The apparatus is simple and allows quantitation of several of the CoASH containing compounds expected to be contaminants of long chain acyl CoA thioesters.

MATERIALS AND METHODS

Stock solutions of stearyl-CoA or palmityl-CoA (P-L Biochemicals, Milwaukee, Wisc., U.S.A.) were prepared with 0.07 *M* Tris–HCl buffer (pH 6.0). For each stock solution a sample containing 40–140 nmoles was applied to duplicate channels on Whatman No. 3MM paper. A corresponding amount of buffer was applied to two blank channels. The chromatogram was developed in isobutyric acid–water–conc. NH_4OH (66:33:1)[8], dried in a hood overnight and then inspected under ultraviolet light. Each of the acyl-CoA, free CoA (reduced and oxidized forms together) and corresponding blank regions was then cut out, sliced into strips about 2 mm wide and placed into 10-ml screw-cap centrifuge tubes. A 3-ml volume of 0.07 *M* Tris buffer (pH 7.0) and 150 μl of 5% NaOH were then added to each tube, followed by strong vortexing. The pH of each sample at this point was between 11 and 12. The samples were then left at room temperature overnight to ensure maximal elution. The next day each sample was neutralized with approximately 65 μl of 4.0 *N* HCl. While taking care to leave as much paper fiber behind as possible, about 1.5 ml of each sample was then transferred to a clean centrifuge tube and briefly centrifuged at 300–500 *g*, the supernate removed and re-centrifuged in a clean centrifuge tube.

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Absorbance at 260 nm was then determined for each sample and corrected for any absorbance determined from the blank region. The amount of free CoA present in each sample was calculated using a molar extinction coefficient of 16 mM^{-1} cm^{-1} (ref. 9). The percent thioester purity for each assay was obtained by dividing the amount of free CoA liberated from the thioester region by the total amount of CoA liberated from both regions, then multiplying by 100. The total CoA concentration of each stock solution was determined by measuring the absorbance at 260 nm.

RESULTS AND DISCUSSION

Each chromatogram was withdrawn from the developing tank after 3 h. At this point the solvent front had advanced approximately 14 cm. Long-chain acyl-CoA esters migrated to the region of R_F 0.65–0.80, while oxidized and reduced CoA were located at R_F 0.33–0.47. Free fatty acids (not visible under ultraviolet light) migrate at the solvent front, with trailing back to R_F 0.7–0.8.

TABLE I

PERCENT THIOESTER PURITY AND RECOVERY OF CoA

Sample	*Total CoA concentration (mM)* *	*Thioester purity observed (%)* *	*Recovery of total CoA (%)* **
Palmityl-CoA	2.88	94.2 ± 0.3	88.8 ± 2.1
Stearyl-CoA (lot No. 1)	2.29	75.5 ± 2.2	94.9 ± 3.4
Stearyl-CoA (lot No. 2)	2.03	65.5 ± 0.7	92.8 ± 1.6

* Calculated from the optical density of 260 nm.

** Average of three determinations ± mean deviation

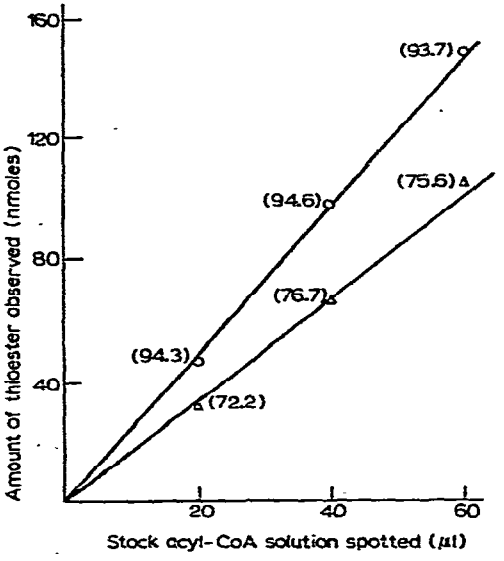

Fig. 1. Determination of thioester concentration in stock solutions of (○) palmityl-CoA and (△) stearyl-CoA. Each number in parentheses is the observed percentage of thioester purity.

Representative data on three acyl-CoA solutions are summarized in Table I. Total recoveries of CoA from chromatographic paper were approximately 90%. As seen in Fig. 1, linear results were obtained upon assaying 30–150 nmoles of thioester, indicating that the method is useful at least over this range.

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