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Short communication

Rapid method for the separation and detection of tissue short-chain coenzyme A esters by reversed-phase high-performance liquid chromatography

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Abstract

A simple and rapid method for the separation and identification of tissue levels of short chain coenzyme A (CoA) esters by a reversed-phase high-performance liquid chromatography with ultraviolet-visible adsorbance detection is described. Samples of liver, heart and kidney tissues were homogenised in 5% sulfosalicylic acid containing 50 μM of dithioerythritol in 1:9 w/v proportion. Following centrifugation, 20 μl of the supernatant were directly injected onto a 3-μm ODS C_{18} column (100 × 4.6 mm I.D.). The separation of acetyl-CoA, malonyl-CoA, methylmalonyl-CoA, succinyl-CoA, propionyl-CoA and free CoASH was achieved in less than 20 min using gradient elution with sodium phosphate, sodium acetate and methanol at a constant flow-rate of 1.5 ml/min. The lowest detection limit was 3 pmol.

1. Introduction

The metabolism of fatty acids in the liver and other organs such as the heart and the kidneys is subjected to complex hormonal and substrate regulation. Short-chain acyl-coenzyme A esters play a crucial role in several steps of lipid metabolism. For example, b-oxidation of newly synthesised fatty acids in the liver is controlled by tissue levels of malonyl-CoA, itself a product of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis [1]. Furthermore, short-chain coenzyme A esters such as acetyl-CoA, propionyl-CoA and methylmalonyl-CoA are intermediates between lipid, carbohydrate and amino acid metabolism [2–4]. Changes in the cellular content of these compounds by either drugs or diseases may influence the harmony of cellular metabolism.

The aim of this paper is to describe a simple and a rapid high-performance liquid chromatographic technique that can be used to identify and quantitative short-chain acyl-CoA levels in different organs. The present work describes a major modification of the method published by Corkey and Deeney [5] and by Bartlett and Causey [6]. Compared with the earlier methods, the total elution time of the different short-chain CoA compounds is reduced by one fourth. Furthermore, the separation of the short-chain coenzymes was achieved at constant flow-rate

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rather than with a changing flow-rate as described earlier [5].

2. Experimental

2.1. Materials

Acetyl-CoA, malonyl-CoA, methylmalonyl-CoA, propionyl-CoA, succinyl-CoA, free coenzyme A (CoASH) and dithioerythritol (DTE) were obtained from Sigma (St. Louis, MO, USA). 5-Sulfosalicylic acid, sodium phosphate, sodium acetate and methanol were purchased from Merck (Darmstadt, Germany). ODS Hypersil (3 μm) was obtained from Shandon Southern (Cheshire, UK). Columns (100 × 4.6 mm I.D.) were packed with ODS Hypersil at 62 MPa using a Shandon column packer.

2.2. Tissue preparation for determination of short-chain acyl-CoA

Pathogen-free male Wistar rats (4 weeks old), weighing between 170 and 180 g, were obtained from Møllegaard Breeding Laboratory (Ejby, Denmark). The animals appeared healthy and were put under light halothane anaesthesia. Their livers, heart and kidney were removed and homogenised immediately in ice-cold 5% sulfosalicylic acid in 50 μM DTE to obtain 10% w/v liver homogenates. Tissue homogenate aliquots of 500 μl were centrifuged at 600 g for 10 min. The resultant supernatants were used to measure the short-chain acyl-CoA esters.

2.3. Chromatography

Aliquots (20 μl) of the supernatants were directly injected onto a column packed with ODS Hypersil (C_{18}), equipped with a guard column packed with Pelliguard LC-18. Absorbance measurements were made at 254 nm. Elution solvent A was 100 mM sodium phosphate and 75 mM sodium acetate adjusted to pH 4.6. Solvent B was 70% solvent A in methanol. The elution was carried out at ambient temperature and the flow-rate was 1.5 ml/min. The profile of the gradient elution was as follows: 0 min, 90% A; 10 min, 60% A; 17.6 min, 10% A. Baseline condition was established again after 8 min of washing with 90% A.

2.4. Instrumentation

A Spectra-Physics SP 8800 solvent delivery system coupled to a Spectra System AS 3000 automated sample processor was used. The chromatographic peaks were identified using a Kratos Spectroflow 773 UV-detector connected by Spectra-Physics 4270 integrator.

2.5. Standard preparation

Individual working standards were prepared by dissolving free CoASH, acetyl-CoA, malonyl-CoA, methylmalonyl-CoA, succinyl-CoA and propionyl-Coa ion 5% sulfosalicylic acid containing 50 μM of DTE. They were then mixed to give a final concentration of 100 μM.

3. Results and discussion

Fig. 1A,B show a typical chromatogram of a standard mixture of the different short-chain CoA esters and a chromatogram obtained from the acid-soluble extract of liver tissue homogenised in 5% sulfosalcyclic acid and 50 μM DTE. In the gradient system adopted, the separation of the different short-chain CoA esters was achieved in less than 20 min in contrast to the 60–80 min run-time required by the earlier method of Corkey and Deeney [5]. Furthermore, the separation of the different CoA compounds was possible at a fixed flow-rate of 1.5 ml/min in the present method in contrast to the continuously changing flow-rate of the old method.

Identification of the peaks from tissue extracts was performed on the basis of similarity in retention times with the chromatographic peaks obtained from the standard mixture containing the various short-chain CoA esters and free CoASH. The typical retention times obtained were: 7.7 min, malonyl-CoA; 9.3 min, free CoASH; 10.9, methylmalonyl-CoA; 12.3 min;

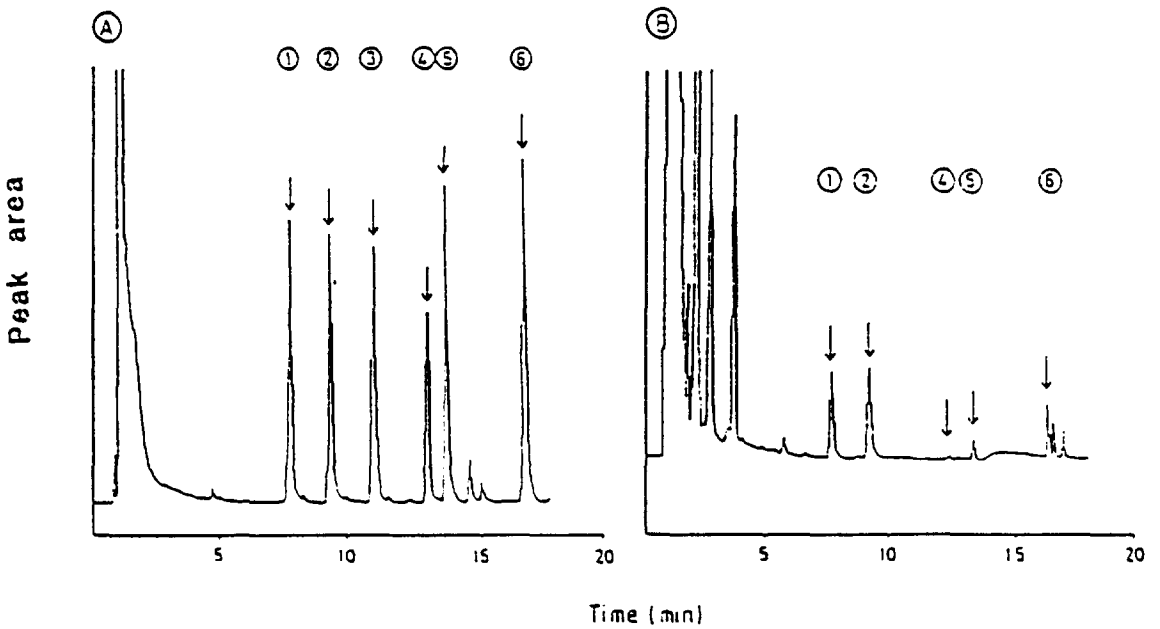

Fig. 1. Chromatogram (A) of mixed standard solution containing 100 μM of free CoASH, acetyl-CoA, malonyl-CoA, methylmalonyl-CoA, propionyl-CoA and succinyl-CoA dissolved in a 5% sulfosalicylic acid containing 50 μM DTE; (B) typical chromatogram of the acyl-CoA esters from acid soluble rat liver extract. Peaks: 1 = malonyl-CoA, 2 = free CoASH, 3 = methylmalonyl-CoA, 4 = succinyl-CoA, 5 = acetyl-CoA and 6 = propionyl-CoA.

succinyl-CoA 13.6 min, acetyl-CoA: and 16.7 min for propionyl-CoA. The recovery obtained by this improved method was approximately 90% for most of the CoA derivatives, except for succinyl-CoA, the recovery of which was about 60%.

In a separate experiment, the CoA ester peaks from the tissue extracts were spiked with increasing concentrations of the different CoA esters of the standard mixture. The results showed a proportional increase in the areas of the peaks from the tissue extract with respect to the concentration of the different CoA esters in the standard mixture (data not shown). The specificity of the method was further demonstrated by the disappearance of all the chromatographic peaks following treatment of the supernatants with 1.5 M potassium hydroxide before injection onto the HPLC system. Moreover, the CoA ester peaks from the tissue extracts acted as substrate for their individual thioesterases.

The reproducibility of the present method was determined by measuring three rat liver samples twice a day, for five days. As shown in Table 1, the variation for the day-to-day as well as for the within-day measurements was low. As little as 10 mg of tissue can be used to prepare samples and the precision of measuring the various CoAs in tissues can be enhanced by performing duplicate analysis.

Standard curves of the different CoA-esters were obtained (data not shown). Table 2 shows

Table 1
Within-run and between-run variation in measurement of short chain acyl-CoA esters from rat liver

Parameters	Concentration (nmol/g liver)	
	Within-run	Between-run
Acetyl-CoA	27.7 ± 7.5	25.1 ± 6.6
Malonyl-CoA	31.9 ± 7.3	40.6 ± 5.3
Methylmalonyl-CoA	1.67 ± 0.21	1.43 ± 0.19
Propionyl-CoA	109.1 ± 11.8	92.9 ± 10.3
Succinyl-CoA	5.36 ± 0.7	4.80 ± 0.34
Free CoASH	76.5 ± 3.5	74.8 ± 5.21

Values are mean ± S.D. from three rats.

Table 2
The linear regression and correlation coefficients (r^2) at 25, 50, 100 and 200 μM concentrations of the various short chain CoA esters in rat liver

Parameters	Linear regression	r^2
Acetyl-CoA	$y = -1.5 \cdot 10^4 + 3985x$	0.998
Malonyl-CoA	$y = -1.6 \cdot 10^4 + 3566x$	0.999
Methylmalonyl-CoA	$y = -1.6 \cdot 10^4 + 3682x$	0.999
Propionyl-CoA	$y = -1.7 \cdot 10^4 + 4367x$	0.999
Succinyl-CoA	$y = 4420 + 117x$	0.872
Free CoASH	$y = -1.6 \cdot 10^4 + 3566x$	0.999

Values are mean ± S.D. from three rats.

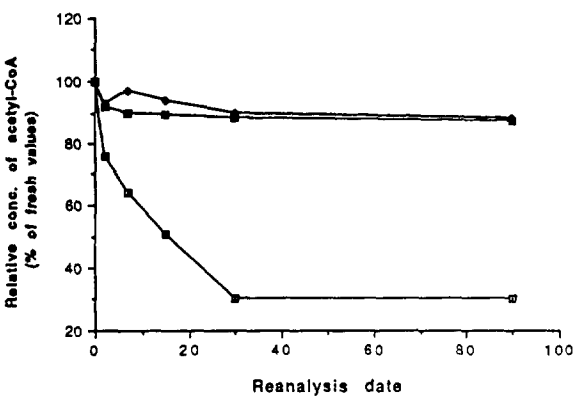

Fig. 2. Effect of storage temperature on the stability of acetyl-CoA levels from acid soluble liver extract. The values show % of a freshly prepared liver extract (day 0, 100%). Storage temperature +4°C (□); −20°C (◇); and −80°C (■) and reanalyzed at day 2, 7, 15, 30 and 90.

the linear regressions and correlation coefficients at 25, 50, 100 and 200 μM concentrations for each compound analysed. The detection limit of the method was as low as 3 pmol for all the compounds at a 20-μl injection volume. However, the volume can be increased to 100 μl to detect smaller quantities of the CoA esters in tissues.

To study the effect of storage temperature on the stability of the CoA esters the acid soluble liver extracts were analysed at different time points. Fig. 2 shows the values obtained for acetyl-CoA measured at day 0 (fresh) and then re-determined at day 2, 7, 15, 30, and 90, respectively. For samples stored at +4°C, the levels decreased by 24, 36, 49 and 70% compared with fresh sample when reanalysed on day 2, 7, 15 and 30, respectively. No further loss was noted at day 90. In contrast, samples stored at −20 and −80°C remained relatively stable when reanalyzed at the time points mentioned above. Except for succinyl-CoA, which is the most unstable, the levels of the other CoA derivatives measured by this method were not greatly affected by freezing and thawing. Fresh samples could be frozen (−20 and −80°C) and thawed up to 5 times without the CoA levels decreasing below 90% that of the original. The maximal loss was 13% of the fresh values even after 3 months of storage. Table 3 shows the levels of the different short-chain CoA esters from healthy rat liver, heart and kidney.

In conclusion, the present paper describes a

Table 3
Levels of short chain CoA esters in rat liver, heart and kidney tissue

Parameters	Concentration (nmol/g tissue)		
	Liver	Heart	Kidney
Acetyl-CoA	27.7 ± 7.5	0.63 ± 0.07	6.9 ± 0.9
Malonyl-CoA	31.9 ± 7.3	10.6 ± 1.3	4.5 ± 0.53
Methylmalonyl-CoA	1.67 ± 0.21	ND[a]	2.6 ± 0.21
Propionyl-CoA	109.1 ± 11.8	52.9 ± 11.3	62.6 ± 10.8
Succinyl-CoA	5.36 ± 0.7	1.20 ± 0.34	5.3 ± 0.83
Free CoASH	76.5 ± 3.5	1.68 ± 0.21	13.7 ± 2.7

[a] Not detectable.
Values represent mean ± S.D. ($n = 3$).

relatively simple and rapid method for the determination of short-chain CoA esters in tissues. As compared to the earlier published method [5], the analytical run-time is considerably shorter for this new method. Furthermore, our method utilises an uncomplicated and fixed-flow-rate elution profile.

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