Effect of propionyl-CoA on enzymic methods for the quantification of acetyl-CoA

CoA plays an important role in intermediary metabolism through the generation of acyl-CoAs as intermediates in a number of metabolic pathways. The measurement of CoA, acetyl-CoA and other acyl-CoAs has provided a useful tool for studying metabolism. The enzymic recycling assay described by Allred & Guy [1] provides excellent sensitivity by utilizing kinetic amplification. In this assay, acetyl-CoA reacts with oxaloacetate to generate citrate, and the oxaloacetate is regenerated from malate with the production of NADH, which is detected by fluorometry. The CoA produced during citrate formation is reconverted to acetyl-CoA from acetyl phosphate, permitting the reactions to recycle and generate multiple molecules of NADH from each molecule of CoA and acetyl-CoA originally in the sample (as described, the assay does not distinguish between CoA and acetyl-CoA).

Propionyl-CoA is generated during the oxidation of several amino acids and odd chain length fatty acids, and accumulates in tissues of patients with propionic acidemia and the methylmalonic acidurias [2]. As enzymic assays for CoA and acetyl-CoA are commonly used, the effect of propionyl-CoA on these assays was examined. Employing the recycling assay of Allred & Guy [1] as described [3], containing approx. 20–50 pmol of CoA or acyl-CoA per incubation, propionyl-CoA standards gave fluorescence values $109 \pm 12\%$ of those with equimolar CoA standards (mean $\pm$ s.d., $n = 3$). The generation of NADH under these conditions presumably reflects metabolism of propionyl-CoA to methylcitrate catalysed by citrate synthase [4]. Kinetically, propionyl-CoA is a poor substrate for citrate synthase as compared with acetyl-CoA, but under the recycling assay conditions the CoA generated from propionyl-CoA subsequently behaves identically to CoA or acetyl-CoA in the original sample. Using the end-point acetyl-CoA assay described by Decker [5], addition of a 10-fold excess of propionyl-CoA (25 nmol) to samples containing acetyl-CoA (2.5 nmol) caused no change in the fluorescence observed in the absence of propionyl-CoA (change in fluorescence

with propionyl-CoA $0.4 \pm 22\%$, mean $\pm$ s.d., $n = 3$). This lack of propionyl-CoA effect probably reflects lower citrate synthase and malate concentrations in the end-point assay, and the choice of a time point just sufficient for reaction completion with acetyl-CoA (in the recycling assay, the time permitted for amplification is much longer than required for a single cycle of acetyl-CoA conversion, and hence propionyl-CoA has time to be metabolized, releasing CoA for normal utilization in the assay).

The importance of the effect of propionyl-CoA in the recycling assay is seen in data from our laboratory, published prior to our appreciating the action of propionyl-CoA in the assay [3]. This study included an analysis of the hepatocyte CoA pool in the presence of propionate. The sum of CoA and acetyl-CoA was measured using the recycling assay of Allred & Guy [1], acetyl-CoA was quantified by the end-point assay of Decker [5] and propionyl-CoA was assayed by an h.p.l.c. method [6]. The free CoA content was determined as the difference between the sum of CoA and acetyl-CoA, and the acetyl-CoA concentration measured directly. However, it is clear that the recycling assay actually measured the sum of CoA, acetyl-CoA and propionyl-CoA. Thus, calculation of the CoA concentration requires subtraction of the acetyl-CoA and propionyl-CoA levels from the amount measured with the recycling assay. The impact of this difference is seen in Table 1. While the qualitative conclusions are not altered, their quantitative significance is enhanced.

In conclusion, propionyl-CoA interferes with CoA/acetyl-CoA analysis by the method of Allred & Guy [1]. Complete and accurate assessments of the cellular CoA pool can be obtained by applying assay methodologies available while recognizing potential sources of interference.

Eric P. BRASS

Division of Clinical Pharmacology (Box C-237), University of Colorado Health Sciences Center, 4200 E. 9th Avenue, Denver, CO 80262, U.S.A.

1. Allred, J. B. & Guy, D. G. (1969) *Anal. Biochem.* **29**, 293–297

Table 1. Effect of propionate and carnitine on the hepatocyte CoA pool

Experiments have been previously reported in detail [3]. The sum of coenzyme A, acetyl-CoA and propionyl-CoA was determined by the method of Allred & Guy [1] and were originally reported as the sum of coenzyme A and acetyl-CoA. 'Reported CoA value' was calculated as the difference between the acetyl-CoA content and the sum of the coenzyme A, acetyl-CoA and propionyl-CoA levels. 'Corrected CoA value' was calculated by the additional subtraction of the propionyl-CoA level measured. Values are nmol/ 10^6 cells and represent the mean $\pm$ s.e.m. with n in parentheses.

Conditions	CoA + acetyl-CoA + propionyl-CoA	Acetyl-CoA	Propionyl-CoA	Reported CoA value	Corrected CoA value
0.8 mM-Butyrate	1.85 ± 0.25 (6)	0.53 ± 0.08 (9)	< 0.02 (7)	1.32	1.32
0.8 mM-Butyrate + 10 mM-propionate	0.86 ± 0.16 (6)	0.25 ± 0.05 (9)	0.28 ± 0.07 (7)	0.61	0.33
0.8 mM-Butyrate + 10 mM-propionate + 10 mM-carnitine	1.07 ± 0.09 (6)	0.31 ± 0.05 (9)	0.14 ± 0.03 (7)	0.76	0.62

2. Chalmers, R. A. & Lawson, A. M. (1982) *Organic Acids in Man: The Analytical Chemistry, Biochemistry and Diagnosis of the Organic Acidurias*, Chapman and Hall, London
 3. Brass, E. P. & Beyerinck, R. A. (1988) *Biochem. J.* **250**, 819–825
 4. Weidman, S. W. & Drysdale, G. R. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 549
 5. Decker, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H., ed.), 2nd edn., pp. 1988–1993, Academic Press, New York
 6. Corkey, B. E., Brandt, M., Williams, R. J. & Williamson, J. R. (1981) *Anal. Biochem.* **118**, 30–41
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Received 21 June 1988