BIOCHEMICAL LETTERS

Does pyruvate carboxylase interfere with the radioactive bicarbonate fixation assay of acetyl-CoA carboxylase?

Davies *et al.* (1982) claim that contamination by pyruvate carboxylase may interfere with the [^{14}C]-bicarbonate fixation assay of acetyl-CoA carboxylase in crude liver preparations. We present evidence here that this is not the case with our assay.

Assay of acetyl-CoA carboxylase involves incubation of the enzyme with [^{14}C]bicarbonate, followed by the addition of HCl and drying overnight at 50°C. Acid-stable radioactivity is then measured. This treatment destroys oxaloacetate in that recovery (Hohorst & Reim, 1965) was <5% when oxaloacetate (up to 4 mM) was subjected to these conditions. Oxaloacetate is destroyed by heating even in neutral solution for 15 min (Bloom & Johnson, 1962) and 3-oxo acids, e.g. acetoacetate, are rapidly decarboxylated by acid (Greenberg & Lester, 1944). Thus, oxaloacetate itself cannot contribute to acid-stable radioactivity.

Oxaloacetate produced by pyruvate carboxylase in crude enzyme preparations may be converted to some other form such as malate or aspartate that is heat stable. Crude preparations (Fig. 1) of liver from rats fed *ad libitum* were assayed for acetyl-CoA carboxylase activity by fixation of [^{14}C]bicarbonate in acid-stable form after 0, 10, 20 and 40 min incubation at 37°C. For comparison, purified acetyl-CoA carboxylase (Allred & Roehrig, 1980) was assayed without preheating. In agreement with previous observations (Allred & Roehrig, 1978), the activity of the 27000g supernatant and the Sephadex G-25 eluate increased 5- and 15-fold, respectively, over the 40 min incubation period (data not shown). Analysis by t.l.c. of the acidified, dried reaction mixtures after crude preparations were heat

without enzyme. The enzymic reaction, in which 0.025 ml of enzyme preparation was incubated with 0.5 ml of reaction mixture (Allred & Roehrig, 1978, except that the specific activity of the bicarbonate was 1200 c.p.m./nmol), was stopped with HCl, dried and the reaction product was resolubilized in ethanol/water (1:1, v/v) and an aliquot was subjected to t.l.c. (Davies *et al.*, 1982) on MN 300 cellulose (Analtech Inc., Newark, DE, U.S.A.). After drying, 1 cm sections of the plate were removed and assayed for radioactivity and enzyme specific activities were calculated on the basis of protein determined by dye binding (Bradford, 1976).

Fig. 1. T.l.c. analyses of reaction products from acetyl-CoA carboxylase assay

Treatments were (a) 27000g supernatant and (b) 105000g supernatant filtered through Sephadex G-25 [both prepared as previously described (Allred & Roehrig, 1978) and both heat activated for 40 min], (c) purified acetyl-CoA carboxylase (Allred & Roehrig, 1980), and (d) [^{13}C]-malonyl-CoA (New England Nuclear) added to reaction mixture

activated for 40 min (Fig. 1) showed that most of the radioactive material comigrated with malonyl-CoA. A second, smaller, peak comigrated with malonic acid. The same pattern was observed for the other time points during heat activation (data not shown). The section of the plate corresponding to the R_F of aspartic or malic acid did not contain measurable radioactivity above background with any enzyme preparation or at any time points. For comparison, authentic [1,3- 14 C]malonyl-CoA was added to reaction mixture without enzyme, acidified, dried and chromatographed along with the enzyme reaction products. The pattern was the same as for the products of the enzymic reaction except that considerably more of the total radioactivity was associated with material comigrating with free malonic acid, which was probably due to the fact that this sample of radioactive malonyl-CoA had been stored frozen for some time. Finally, it was established by radioautography that the radioactivity in the peaks shown in Fig. 1 each represented an individual spot on the t.l.c. plate. It should be noted that the R_F of malonic acid is very near that reported (Myers & Huang, 1969) for oxaloacetate in this solvent system. It is possible that the peak that Davies *et al.* (1982) identified as oxaloacetate may be malonate, produced either by hydrolysis of malonyl-CoA or by removal of the β -carboxyl group of oxaloacetate followed by oxidation of the resultant malonic semialdehyde.

Evidence that a minimal amount, if any, of oxaloacetate was produced in our assay system for acetyl-CoA carboxylase activity was obtained by repeating the above experiment with identical enzyme preparations except that after 1 min, the reaction was stopped by the addition of carrier oxaloacetate followed immediately by 2,4-dinitrophenylhydrazine in HCl and separation of the 2,4-dinitrophenylhydrazones by t.l.c. on cellulose plates (Bloom & Johnson, 1962). Only trace amounts of radioactivity comigrated with the 2,4-

dinitrophenylhydrazone of oxaloacetate when the 27000g supernatant incubated for 0 and 10 min served as the enzyme source. None was detected with any other enzyme reaction product. Since no exogenous pyruvate was added, any pyruvate available would have to have been endogenous. Davies *et al.* (1982) added pyruvate and used large amounts (25% of total volume) in their assay.

Pyruvate carboxylase does not interfere with the assay of acetyl-CoA carboxylase under our experimental conditions because (a) oxaloacetate did not survive the drying process before counting, (b) it was not converted to either aspartate or malate and (c) the production of oxaloacetate even under conditions specifically designed to preserve it was too small to measure.

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- Allred, J. B. & Roehrig, K. L. (1978) *J. Biol. Chem.* **253**, 4826–4829
 Allred, J. B. & Roehrig, K. L. (1980) *J. Lipid Res.* **21**, 488–491
 Bloom, S. J. & Johnson, M. J. (1962) *J. Biol. Chem.* **237**, 2718–2720
 Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
 Davies, D. R., Van Schaftingen, E. & Hers, H.-G. (1982) *Biochem. J.* **202**, 559–560
 Greenberg, L. A. & Lester, D. (1944) *J. Biol. Chem.* **154**, 177–190
 Hohorst, H.-J. & Reim, M. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 335–339, Academic Press, New York
 Myers, W. F. & Huang, K.-Y. (1969) *Methods Enzymol.* **13**, 431–434

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