Biochem. J. (1986) 237, 289–291 (Printed in Great Britain)

Intertissue differences in the hysteretic behaviour of carnitine palmitoyltransferase in the presence of malonyl-CoA

Alison C. LLOYD, Carol A. CARPENTER and E. David SAGGERSON
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

In the presence of malonyl-CoA, the overt form of carnitine palmitoyltransferase (CPT_1) in mitochondria from rat liver, kidney cortex, heart, skeletal muscle and brown adipose tissue shows non-linear time courses, suggesting hysteretic behaviour. The pattern of this hysteresis is similar in heart, skeletal muscle and brown adipose tissue, but the hysteretic behaviour of the enzyme in these three tissues differs markedly from that seen in liver and kidney.

INTRODUCTION

The overt form of carnitine palmitoyltransferase (CPT_1) in mitochondria from various tissues has been shown to be potently inhibited by malonyl-CoA. Under appropriate assay conditions, inhibition of hepatic CPT_1 by malonyl-CoA is slow in onset (Zammit, 1984; Cook, 1984), and it has therefore been suggested (Cook, 1984) that CPT_1 is a 'hysteretic' enzyme (Frieden, 1979). Kinetic arguments have been advanced that malonyl-CoA interacts with a site distinct from the substrate-binding site of CPT_1 (Bird & Saggerson, 1984; Mills *et al.*, 1984; Edwards *et al.*, 1985; Grantham & Zammit, 1986), and this hypothesis has been confirmed by the demonstration that catalytic unit of liver CPT and malonyl-CoA-binding protein(s) are separable chromatographically (Bergseth *et al.*, 1986). Differences between tissues in both of these components appear likely from kinetic studies. Intertissue differences in the malonyl-CoA-binding entity are suggested by the substantially lower values of IC_{50} for inhibition of CPT_1 in heart, skeletal-muscle and brown-adipose-tissue mitochondria compared with those of liver or kidney cortex (Saggerson & Carpenter, 1981, 1982) and by the lower values of K_D for binding of malonyl-CoA to high-affinity sites in heart and skeletal muscle compared with those in liver (Mills *et al.*, 1983, 1984; Bird & Saggerson, 1984). Intertissue differences in the catalytic entity of the enzyme are suggested from comparisons of sensitivity to inhibition by 2-bromopalmitoyl-CoA. Whereas CPT_1 in mitochondria from liver and kidney is very sensitive to this inhibitor, CPT_1 from skeletal muscle, heart or brown adipose tissue shows substantially less sensitivity (Saggerson & Carpenter, 1986). In view of the possibility that the interacting components of CPT_1 might differ between tissues, we have investigated hysteretic behaviour of CPT_1 in mitochondria from five rat tissues.

MATERIALS AND METHODS

Animals

These were male Sprague–Dawley rats (160–180 g) bred at University College London and maintained as described by Saggerson & Carpenter (1986).

Chemicals

These were obtained and treated as described by Saggerson *et al.* (1982).

Isolation of mitochondria

Mitochondria were obtained from heart and liver as described by Saggerson (1982), from interscapular brown adipose tissue as described by Saggerson & Carpenter (1982) and from kidney cortex and skeletal muscle as described by Saggerson & Carpenter (1981). In all cases mitochondria were finally suspended in ice-cold 0.3 M-sucrose medium containing 10 mM-Tris/HCl buffer (pH 7.4), 1 mM-EGTA and 1 mM-dithiothreitol. Mitochondrial protein was measured by the method of Lowry *et al.* (1951), with bovine albumin as standard.

Assay of CPT_1

This was measured at 25 °C as the incorporation of [^{3}H]carnitine into palmitoylcarnitine (Saggerson *et al.*, 1982). All assays contained the following final concentrations: 150 mM-sucrose, 25 mM-Hepes buffer (pH 7.0), 60 mM-KCl, 1 mM-EGTA, 1 mM-dithiothreitol, fatty acid-poor albumin (3.9 mg/ml), 70 μM-palmitoyl-CoA, 0.4 mM-L-carnitine, 0.5 μCi of L-[^{3}H]carnitine/ml and mitochondrial protein at approx. 200 μg/ml. Malonyl-CoA was also present as indicated.

Three types of assay procedure were followed; the common feature of all three was that mitochondria were preincubated at 25 °C for 7 min with the sucrose/Hepes/KCl/EGTA/dithiothreitol/albumin before addition of carnitine at zero time. In general the incubations were 15 ml.

In control assays, palmitoyl-CoA was added at −2 min.

In malonyl-CoA type A assays, palmitoyl-CoA was added at −2 min and malonyl-CoA at zero time.

In malonyl-CoA type B assays, malonyl-CoA was added at −2 min and palmitoyl-CoA at zero time.

Samples (1.0 ml) of assay mixtures were removed at various times and added to 1.0 ml of ice-cold 1.2 M-HCl. Further treatment and measurement of the reaction product was as described by Saggerson *et al.* (1982).

Abbreviations used: CPT_1, the overt form of carnitine palmitoyltransferase (EC 2.3.1.21); IC_{50}, the concentration of an inhibitor causing 50% inhibition.

RESULTS AND DISCUSSION

The assay conditions were chosen such that control assays from which malonyl-CoA was absent and in which mitochondria were preincubated with palmitoyl-CoA for 2 min before addition of carnitine were linear for at least 8 min (see Figs. 1 and 2; $r \geqslant 0.999$ in all five tissues). Malonyl-CoA assay type A shown in Fig. 1(*a*), in which liver mitochondria were preincubated with palmitoyl-CoA before addition of malonyl-CoA (and carnitine), demonstrates an effect essentially the same as that observed by Zammit (1984) and Cook (1984), i.e. malonyl-CoA inhibition of the enzyme was slow in onset. A steady rate of reaction was only obtained after approx. 4 min, and was 1.2 ± 0.2 nmol/min per mg of protein, representing a decrease of $54 \pm 7\%$ compared with the control rate of 2.7 ± 0.1 nmol/min per mg of protein. When the order of additions was reversed in the type B assay, such that mitochondria were preincubated for 2 min with malonyl-CoA before addition of palmitoyl-CoA (and carnitine), non-linearity of the time course was again observed (Fig. 1*a*), with establishment of a steady rate after approx. 2 min. This rate was 1.0 ± 0.1 nmol/min per mg of protein, which is a decrease of $63 \pm 2\%$ relative to the control. The decreases relative to the control in the steady-state rates obtained in malonyl-CoA assays type A and B were not significantly different from each other, suggesting that order of addition of malonyl-CoA and palmitoyl-CoA has little effect on the final steady state of the enzyme. There was therefore no evidence for the

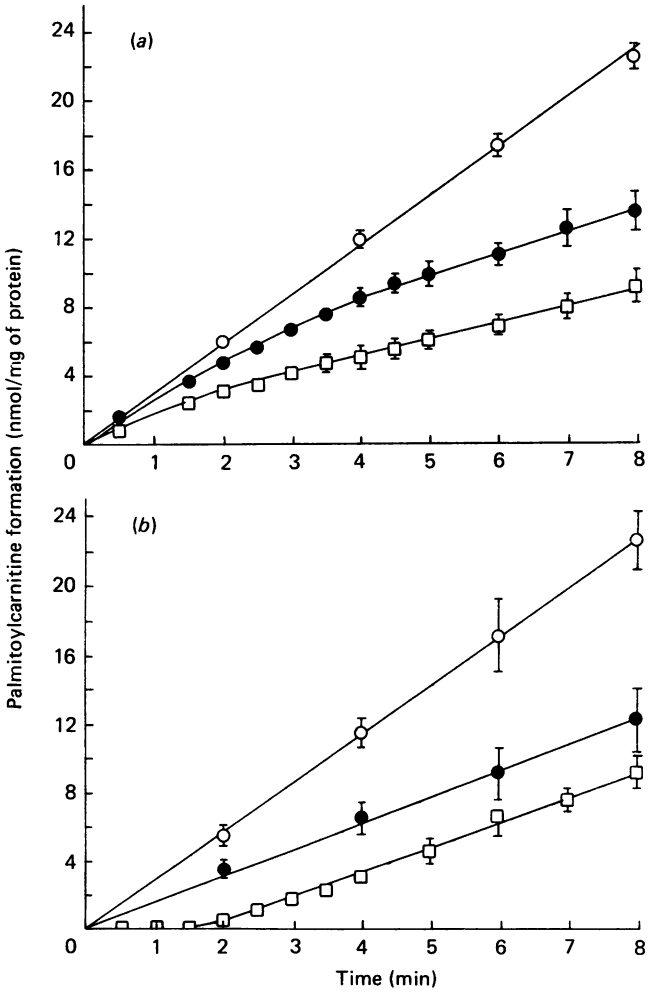

Fig. 1. Time courses of CPT_1 assays with liver (*a*) and heart (*b*) mitochondria

○, Control assays; ●, malonyl-CoA type A assays; □, malonyl-CoA type B assays. Where appropriate, malonyl-CoA was present at 25 μM and 50 nM with liver and heart assays respectively. The bars indicate S.E.M. ($n = 4$).

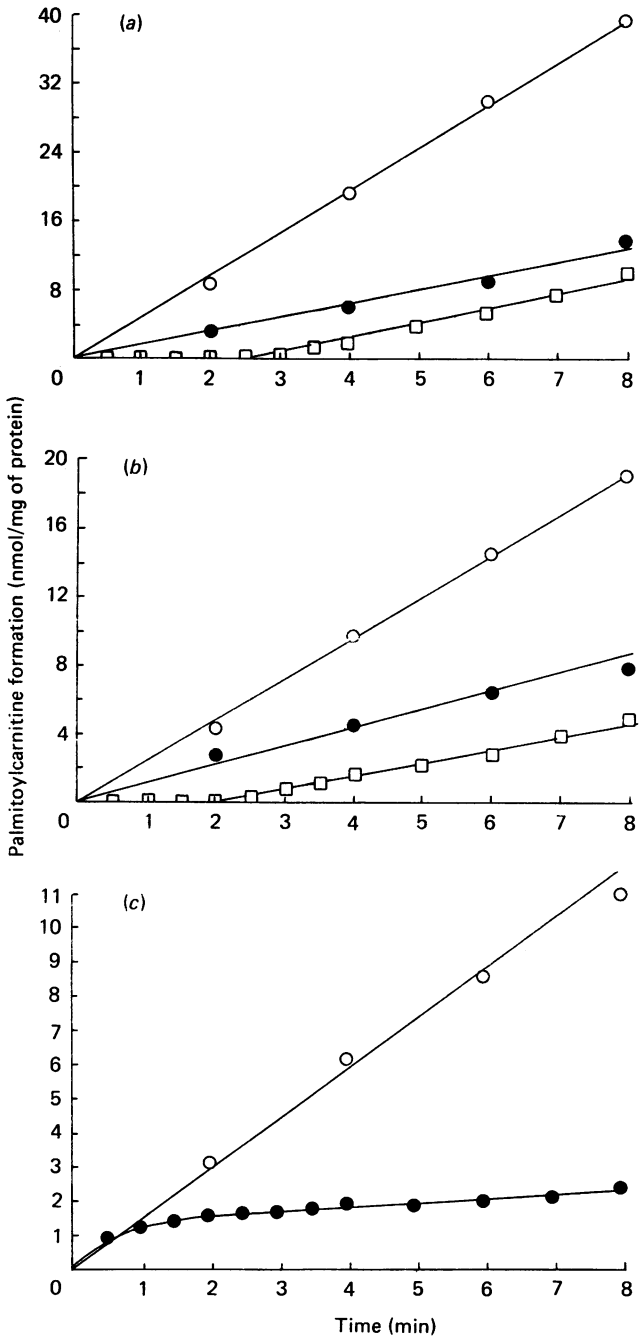

Fig. 2. Time courses of CPT_1 assays with brown-adipose-tissue (*a*), skeletal-muscle (*b*) and kidney-cortex (*c*) mitochondria

Symbols are as for Fig. 1; $n = 2$. Where appropriate, malonyl-CoA was present at 50 nM, 20 nM and 10 μM with brown-adipose-tissue, skeletal-muscle and kidney-cortex assays respectively.

'sensitization' phenomenon reported by Zammit (1983, 1984) on the basis of short-term non-steady-state assays of the liver enzyme. Fig. 1(*b*) summarizes a similar experiment using heart mitochondria with malonyl-CoA again at a concentration that approximately half-inhibits the enzyme. The modes whereby inhibited steady-state rates were achieved were totally different from those seen with liver CPT_1. There was no perceptible delay in onset of inhibition in the type A assay. In malonyl-CoA type B assays, where there was a 2 min preincubation with malonyl-CoA before addition of palmitoyl-CoA (and carnitine), the enzyme appeared to commence the time course in a totally inactive state and took approx. 2 min to achieve a steady-state rate. Again, the order of addition of malonyl-CoA and palmitoyl-CoA did not influence the final rates, in that the control rate was 2.9 ± 0.3 nmol/min per mg of protein and steady-state rates in malonyl-CoA assays A and B were 1.5 ± 0.3 and 1.5 ± 0.2 nmol/min per mg of protein respectively, representing decreases of $48 \pm 5\%$ and $48 \pm 1\%$ relative to the control.

Sensitivity of CPT_1 in liver and heart mitochondria to inhibition by malonyl-CoA is increased with decreasing pH (Stephens *et al.*, 1983; Mills *et al.*, 1984). Further studies (results not shown) were therefore performed to see whether these hysteretic effects were pH-dependent. Type B malonyl-CoA assays were performed with liver and heart mitochondria at different pH values under conditions otherwise identical with those shown in Figs. 1(*a*) and 1(*b*). Relative steady-state rates obtained for liver CPT_1 ($n = 3$) were pH 7.4 = 100, pH 7.0 = 54, pH 6.6 = 20, and for heart CPT_1 (n = 3) were pH 7.4 = 100, pH 7.0 = 53, pH 6.6 = 22. Although pH influenced these final steady-state rates, it had little effect upon the hysteresis, since in all six cases there were delays of 2–3 min after the addition of carnitine and palmitoyl-CoA before these steady rates were obtained.

Figs. 2(*a*) and 2(*b*) show that the brown-adipose-tissue and skeletal-muscle enzyme was similar to CPT_1 in heart mitochondria in that there was no delay in onset in inhibition in malonyl-CoA type A assays, but that after prior incubation with malonyl-CoA (type B assay) the enzymes were inactive for 2–3 min after addition of the substrates. Again, both type A and B assays yielded essentially the same final steady-state rates, with no evidence of a 'sensitization' phenomenon. It was necessary to lower the malonyl-CoA concentration to 20 nM when assaying the skeletal-muscle enzyme, since 50 nM-malonyl-CoA (as used for heart or brown-adipose-tissue CPT_1) almost totally inhibited the enzyme. Fig. 2(*c*) shows, by contrast, that kidney cortex CPT_1 in a type A assay had a hysteretic pattern similar to that seen with the liver enzyme but dissimilar to that of the other extra hepatic enzymes. Previous work has suggested that rat liver and kidney-cortex CPT_1 activities have similar sensitivity to malonyl-CoA (Saggerson & Carpenter, 1981). In the present study these two tissues showed some differences, by virtue of the liver enzyme being somewhat less sensitive and the kidney enzyme being rather more sensitive than previously reported. The reasons for these discrepancies are not clear, but do not alter the conclusion that liver and kidney CPT_1 show a different pattern of hysteretic behaviour from that shown by the three other tissues. It is proposed that these differences must reflect tissue differences in rate constants for transformations between different activity states and conformations of the components of this complex enzyme.

E.D.S. is grateful to the Medical Research Council for a project grant.

REFERENCES

Bergseth, S., Lund, H. & Bremer, J. (1986) Biochem. Soc. Trans. **14**, 671–672
Bird, M. I. & Saggerson, E. D. (1984) Biochem. J. **222**, 639–647
Cook, G. A. (1984) Biochem. J. **224**, 1015–1018
Edwards, M. R., Bird, M. I. & Saggerson, E. D. (1985) Biochem. J. **230**, 169–179
Frieden, C. (1979) Annu. Rev. Biochem. **48**, 471–489
Grantham, B. D. & Zammit, V. A. (1986) Biochem. J. **233**, 589–593
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Mills, S. E., Foster, D. W. & McGarry, J. D. (1983) Biochem. J. **214**, 83–91
Mills, S. E., Foster, D. W. & McGarry, J. D. (1984) Biochem. J. **219**, 601–608
Saggerson, E. D. (1982) Biochem. J. **202**, 397–405
Saggerson, E. D. & Carpenter, C. A. (1981) FEBS Lett. **129**, 229–232
Saggerson, E. .D. & Carpenter, C. A. (1982) Biochem. J. **204**, 373–375
Saggerson, E. D. & Carpenter, C. A. (1986) Biochem. J. **236**, 137–141
Saggerson, E. D., Tselentis, B. S. & Carpenter, C. A. (1982) Biochem. J. **208**, 667–672
Stephens, T. W., Cook, G. A. & Harris, R. A. (1983) Biochem. J. **212**, 521–524
Zammit, V. A. (1983) Biochem. J. **210**, 953–956
Zammit, V. A. (1984) Biochem. J. **218**, 379–386

Received 12 March 1986/21 April 1986; accepted 23 April 1986