THE LOCATION OF GLYCEROL PHOSPHATE ACYLTRANSFERASE AND FATTY ACYL CoA SYNTHASE ON THE INNER SURFACE OF THE MITOCHONDRIAL OUTER MEMBRANE

H. G. NIMMO
Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

Received 19 March 1979

1. Introduction

In rat liver the enzymes long-chain fatty acyl CoA synthetase (fatty acid CoA ligase (AMP), EC 6.2.1.3) and glycerol phosphate acyltransferase (acyl CoA-*sn*-glycerol 3-phosphate 1-acyltransferase, EC 2.3.1.15) are found in both the outer membranes of mitochondria and the microsomal fraction [1–4]. It has been shown that the mitochondrial and microsomal glycerol phosphate acyltransferases are distinct isoenzymes [5] but it is not yet clear whether they carry out different metabolic roles. The partitioning of fatty acids between esterification and oxidation is controlled by diet and by hormones [6,7]. β-Oxidation is primarily a mitochondrial process (although it may also occur in peroxisomes [8]). The properties of the mitochondrial fatty acyl CoA synthetase and glycerol phosphate acyltransferase may be of considerable importance in the control of fatty acid metabolism in liver. In this report I present evidence indicating that both fatty acyl CoA synthetase and glycerol phosphate acyltransferase lie on the inner surface of the outer mitochondrial membrane.

2. Materials and methods

2.1. *Materials*

Trypsin (TPCK-treated) was obtained from Worthington Biochemical Corp., Freehold, NJ 07728. Soyabean trypsin inhibitor and palmitic acid were obtained from Sigma (London) Chemical Co., Poole, Dorset. ADP, NADP, hexokinase and glucose 6-phosphate dehydrogenase were obtained from the Boehringer Corp. (London) Ltd, Lewes, Sussex. [9,10-^{3}H]Palmitic acid was obtained from the Radiochemical Centre, Amersham, Bucks. The sources of other reagents were as described in [5,9].

2.2. *Assay methods*

Glycerol phosphate acyltransferase was assayed as in [5]. Fatty acyl CoA synthetase was assayed using [^{3}H]palmitic acid [10]. Rotenone-insensitive NADH-cytochrome *c* reductase [11] and adenylate kinase [12] were assayed by standard methods. Protein was estimated using the method in [13].

2.3. *Preparation of mitochondria and incubations with trypsin*

Male Wistar rats (~200 g) deprived of food overnight were used in all experiments. Liver mitochondria were prepared and washed as in [14]. A particle-free supernatant was prepared by centrifugation of the post-mitochondrial supernatant at 100 000 × *g* for 60 min. Mitochondria were resuspended in either isotonic medium (0.25 M sucrose, 5 mM potassium phosphate, 0.1 mM EDTA, 1 mM dithiothreitol (pH 7.5)) or hypotonic medium (0.025 M sucrose, 5 mM potassium phosphate, 0.1 mM EDTA, 1 mM dithiothreitol, (pH 7.5)) as in [11]. Samples of resuspended mitochondria were incubated at 30°C for 5–10 min and then trypsin (5 mg/ml in 1 mM HCl) was added to give the final concentrations indicated in the text. After various times aliquots were removed and an excess of soyabean trypsin inhibitor (inhibitor:trypsin 3:1, w/w) was added. The samples were stored at 0°C and assayed for enzyme activity within 30 min.

 Elsevier/North-Holland Biomedical Press

3. Results and discussion

Studies on the accessibility of mitochondrial enzymes to trypsin in isotonic and hypotonic conditions have been used to indicate whether they are located on the outer surface of or within the outer mitochondrial membrane [11,15]. Rotenone-insensitive NADH-cytochrome *c* reductase is located on the outer surface of the outer membrane whereas adenylate kinase is located in the inter-membrane space [11,14]. Figures 1a,d show the results of control experiments in which the sensitivities of adenylate kinase and rotenone-insensitive NADH-cytochrome *c* reductase, respectively, to inactivation by trypsin were examined. The adenylate kinase was completely resistant to trypsin in mitochondria incubated under isotonic conditions. However, when the mitochondria were incubated under hypotonic conditions, ~60–65% of the total adenylate kinase activity was rapidly destroyed by trypsin. Clearly the protease cannot cross the outer membranes of mitochondria incubated in isotonic conditions. The rotenone-insensitive NADH-cytochrome *c* reductase activity was destroyed rapidly by trypsin in both isotonic and hypotonic media, as would be expected for an enzyme located on the outer surface of the outer membrane.

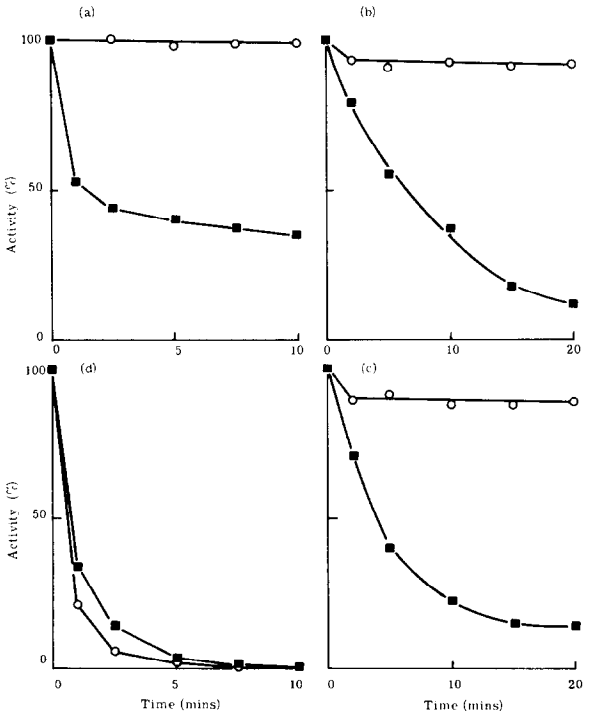

Fig.1. The effect of trypsin on enzyme activities in mitochondria. Mitochondria were prepared and incubated with trypsin as in section 2. In (a)–(c) the final concentration of trypsin was 0.04 mg/ml and in (d) it was 0.01 mg/ml. In each case the concentration of mitochondrial protein was 4 mg/ml. The mitochondria were suspended in either isotonic medium (○) or hypotonic medium (■). The enzymes assayed were: (a) adenylate kinase; (b) glycerol phosphate acyltransferase; (c) fatty acyl CoA synthase; (d) rotenone-insensitive NADH-cytochrome *c* reductase. Control experiments showed that in each case < 5% of the total activity was lost if trypsin was omitted from the incubation.

Figures 1b,c show the effects of trypsin on glycerol phosphate acyltransferase and fatty acyl CoA synthase, respectively. When the mitochondria were incubated in isotonic conditions some 10–15% of each activity was destroyed by trypsin. However under hypotonic conditions both enzymes were rapidly and extensively (> 80%) destroyed by trypsin. A semilog plot for the inactivation of glycerol phosphate acyltransferase was linear to > 90% inactivation, whereas the corresponding plot for the inactivation of fatty acyl CoA synthase was linear only over the first 30% of the inactivation.

These results indicate that only ~10% of the glycerol phosphate acyltransferase and fatty acyl CoA synthase activities were accessible to trypsin in mitochondria incubated in isotonic medium. To assess the degree to which the outer mitochondrial membranes were damaged during isolation, the proportion of the total adenylate kinase activity that was recovered in the 100 000 × *g* supernatant fraction was measured. Over a series of 4 experiments this proportion was 9 ± 4% (mean ± SD). The proportions of glycerol phosphate acyltransferase and fatty acyl CoA synthase that were susceptible to trypsin were estimated to be 12 ± 5% and 10 ± 5%, respectively. I interpret these results as indicating that ~90% of the mitochondria were isolated with their outer membranes intact. The glycerol phosphate acyltransferase and fatty acyl CoA synthase in these mitochondria are inaccessible to trypsin. The remaining 10% of the mitochondria had sustained damage to their outer membranes and had lost all their adenylate kinase. The acyltransferase and synthase in these damaged mitochondria were accessible to trypsin.

It is evident from fig.1a that even in mitochondria

incubated under hypotonic conditions ~35% of the adenylate kinase activity was resistant to inactivation by trypsin. This is surprising in view of the fact that > 80% of the glycerol phosphate acyltransferase and fatty acyl CoA synthase activities were susceptible to trypsin. It may indicate that under these conditions some of the adenylate kinase is membrane-associated and resistant to trypsin. After incubation in 2 mM EDTA, > 90% of the adenylate kinase in rat brain mitochondria was found [15] to be inactivated by trypsin. I have confirmed these results using rat liver mitochondria (not illustrated) but I found that incubation in 2 mM EDTA was not suitable for a study of glycerol phosphate acyltransferase, because in these conditions the enzyme lost activity quite rapidly even in the absence of trypsin.

The results presented above indicate that the glycerol phosphate acyltransferase and fatty acyl CoA synthase activities of the outer mitochondrial membrane lie on the inner surface of this membrane. This may have important implications for the control of hepatic fatty acid metabolism. The branch-point between esterification and β-oxidation is at the level of the enzymes glycerol phosphate acyltransferase and 'outer' carnitine palmitoyltransferase. Fatty acid oxidation has been suggested to be regulated by inhibition of the latter enzyme by malonyl CoA [16]. This enzyme is believed to be loosely attached to the outer surface of the inner mitochondrial membrane [16–18]. Since both fatty acyl CoA synthase and glycerol phosphate acyltransferase seem to be located on the inner surface of the outer membrane, it appears that the fate of hepatic fatty acids may be decided in the inter-membrane space. This implies that the mitochondrial glycerol phosphate acyltransferase is more important than the microsomal isoenzyme in the control of lipid synthesis. This view is supported by the observation that the activity of the mitochondrial enzyme but not the microsomal enzyme is affected by insulin and in diabetes [19,20].

Acknowledgement

This work was supported by a grant from the Medical Research Council.

References

[1] De Jong, J. W. and Hülsmann, W. C. (1970) Biochim. Biophys. Acta 197, 127–135.
[2] Aas, M. (1971) Biochim. Biophys. Acta 231, 32–47.
[3] Daae, L. N. W. and Bremer, J. (1970) Biochim. Biophys. Acta 210, 92–104.
[4] Monroy, G., Rola, F. H. and Pullman, M. E. (1972) J. Biol. Chem. 247, 6884–6894.
[5] Nimmo, H. G. (1979) Biochem. J. 177, 283–288.
[6] Mayes, P. A. (1970) in: Adipose Tissue (Jeanrenaud, B. and Hepp, D. eds) pp. 186–195, Academic Press, London.
[7] Geelen, M. J. H., Groener, J. E. M., De Haas, C. G. M., Wisserhof, T. A. and Van Golde, L. M. H. (1978) FEBS Lett. 90, 57–60.
[8] Lazarow, P. B. (1978) J. Biol. Chem. 253, 1522–1528.
[9] Nimmo, H. G. and Houston, B. (1978) Biochem. J. 176, 607–610.
[10] Jason, C. J., Polokoff, M. A. and Bell, R. M. (1976) J. Biol. Chem. 251, 1488–1492.
[11] Kuylenstierna, B., Nicholls, D. G., Hoomoller, S. and Ernster, L. (1970) Eur. J. Biochem. 12, 419–426.
[12] Haslam, R. J. and Mills, D. C. B. (1967) Biochem. J. 103, 773–784.
[13] Bradford, M. M. (1976) Anal. Biochem. 72, 248–254.
[14] Sottocasa, G. L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) Methods Enzymol. 10, 447–463.
[15] Mayer, R. J. and Hubscher, G. (1971) Biochem. J. 124, 491–500.
[16] McGarry, J. D., Leatherman, G. F. and Foster, D. W. (1978) J. Biol. Chem. 253, 4128–4136.
[17] Chase, J. F. A. and Tubbs, P. K. (1972) Biochem. J. 129, 55–65.
[18] Hoppel, C. L. and Tomec, R. J. (1972) J. Biol. Chem. 247, 832–841.
[19] Bates, E. J., Topping, D. L., Sooranna, S. R., Saggerson, D. and Moyes, P. A. (1977) FEBS Lett. 84, 225–228.
[20] Bates, E. J. and Saggerson, D. (1977) FEBS Lett. 84, 229–232.