IMMUNOLOGICAL ASSAY OF THE MITOCHONDRIAL ACETYL-CoA ACETYLTRANSFERASE IN CRUDE LIVER HOMOGENATE

Ralf MENKE and Walter HUTH
with the technical assistance of Ulrike MÖLLER
Physiologisch-Chemisches Institut der Universität Göttingen, Humboldtallee 7, 3400 Göttingen, FRG

Received 28 July 1980

1. Introduction

The hepatic cell contains a cytosolic acetyl-CoA acetyltransferase, a mitochondrial acetyl-CoA acyltransferase (EC 2.3.1.16) and acetyl-CoA acetyltransferase as well as a peroxisomal acetyl-CoA acyltransferase [1–3]. The mitochondrial acetyl-CoA acetyltransferase (mAAT) (EC 2.3.1.9), which catalyzes the first step in the biosynthesis of ketone bodies, has been shown to exist in two forms, transferases A and B [2]. Its transferase activity increases in metabolic situations with an elevated ketonaemia [4], and evidence has been reported that the enzyme is involved in the regulation of ketogenesis [2,5–7]. The aim of the study was to quantitate the amount of mAAT present in crude liver homogenates from rats under various metabolic conditions. In view of the numerous transferase activities present in a crude liver homogenate we used an immunochemical assay with antibodies raised against the purified enzyme. Our results demonstrate that the amount of mAAT remains unchanged under conditions in which the rate of ketogenesis differs considerably. This indicates that, in vivo, the activity rather than the amount of enzyme protein is modulated.

2. Materials and methods

Acute diabetes was induced in rats by alloxan injections [5]. Liver homogenates were prepared by conventional homogenization and centrifugations and the mitochondria were solubilized in a medium containing 0.05 M sodium phosphate (pH 6.6), 1 mM EDTA, 0.5 mM dithioerythritol, 20% (w/v) glycerine and 0.5% (w/v) Triton X-100. Then the homogenates were centrifuged for 60 min at 105 000 × *g*. DNA was determined as in [8]. Acetyl-CoA acetyltransferase activity was assayed in the presence of 80 mM potassium [2]. Antibodies against mAAT were raised in rabbits [2] and their ability to immunoprecipitate mAAT in liver homogenate was tested [2]. Rocket immunoelectrophoresis was carried out in 1% (w/v) agarose A [9] using 30.7 $\mu g/cm^2$ gel of IgC anti-mAAT for 3–4 h at a field-strength of 10 V/cm in a LKB Multiphor chamber. Transferases A and B were isolated from 1 g rat liver by chromatography on phosphocellulose (column 80 × 6 mm), buffer 0.1 M sodium phosphate (pH 6.8), containing 0.14 M NaCl, 0.5 mM dithioerythritol and 20% (w/v) glycerine with a gradient from 0.28–0.63 M Na^+ (15 ml each) fractionated in 0.5 ml. Fused rocket immunoelectrophoresis was performed subsequently [10]. Samples of 5 μl from the chromatographic fractions were put into the wells and were allowed to diffuse radially for 4 h followed by electrophoresis during 10 h at 2 V/cm.

Abbreviation: mAAT, mitochondrial acetyl-CoA acetyltransferase

3. Results and discussions

The mAAT was proved to be immunologically distinct from the mitochondrial acetyl-CoA acyltransferase, from the cytosolic acetyl-CoA acetyltransferase [2] and from the peroxisomal acetyl-CoA acyltransferase [3]. Therefore rocket immunoelectrophoresis can be employed to quantitate the amount of mAAT directly in liver homogenates with purified mAAT as a standard. Both forms A and B of this enzyme are measured simultanously in this way due to immuno-

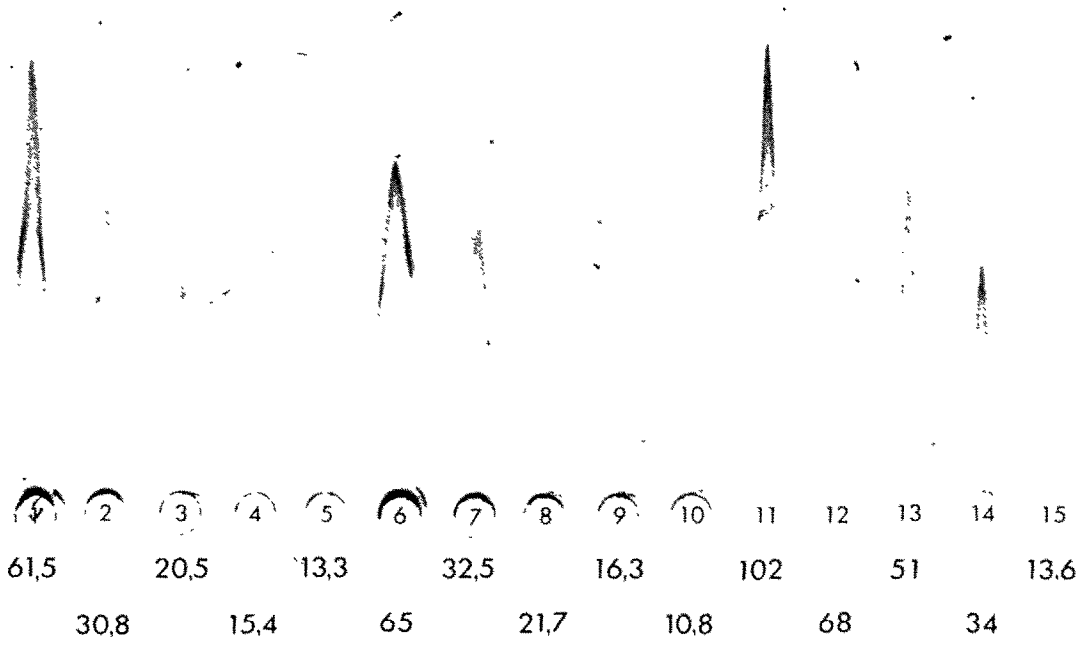

Fig.1. Assay of mitochondrial acetyl-CoA acetyltransferase by rocket immunoelectrophoresis of rat liver homogenates. The amounts of liver homogenate total protein (μg) and of antigen protein (ng) added to each well are indicated. The wells contain: (1–5) liver homogenate I; (6–10) liver homogenate II; (11–15) mAAT antigen.

logical cross-reactivity [2]. Each run was calibrated by a standard of the purified antigen protein (fig.1). The standard curve obtained (fig.2) was used to calculate the immunoreactive protein in the liver homogenates by linear regression.

Ketone body concentrations in blood are elevated in starved and diabetic animals reflecting increased rates of hepatic ketogenesis in these metabolic states (table 1). The mAAT content in livers from starved and alloxan diabetic rats did not significantly differ from the content in livers from fed rats when based on 100 g body wt and/or on the DNA content. With liver wet wt as reference, however, the amount of immunoreactive protein was significantly elevated in starved rats. Thus the amount of mAAT protein appears not to correlate with the observed states of ketonaemia.

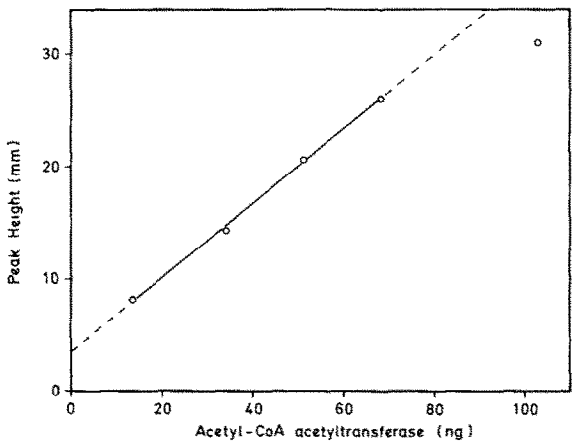

Fig.2. Standard curve for purified acetyl-CoA acetyltransferase by rocket immunoelectrophoresis.

The maximal acetoacetyl-CoA synthesizing capacity of mAAT of 18.8 μmol . min^{-1} . 100 g body wt^{-1} was calculated from the immunoreactive protein content (table 1) and the specific activity of the purified mAAT (22.0 μmol . min^{-1} . mg $protein^{-1}$) [2]. This can account for the highest ketogenic rates observed in alloxan diabetic rats (10.9 μmol . min^{-1} . 100 g body wt^{-1}) [12]. Obviously only a part of the maximal synthesizing activity can be expressed in vivo due to the inhibitory action of CoASH and acetoacetyl-CoA on the mAAT [6,7,13]. CoASH may be of physiological importance to the ketogenic rates, for the ketone body production only correlates with the

Table 1
Liver mitochondrial acetyl-CoA acetyltransferase content in rats of different metabolic states

Metabolic state of rats	Total ketone bodies in blood (μmol/ml)	Immunoreactive mAAT (μg/g liver wet wt)	(μg/100 g body wt)	(μg/mg DNA)
Fed (12)	0.21 ± 0.05	206.5 ± 38.7	852.1 ± 153.8	103.3 ± 19.4
Starved (12)	1.01 ± 0.19[a]	281.8 ± 45.9[a]	783.7 ± 131.9[c]	96.1 ± 15.7[c]
Alloxan-diabetic (10)	9.67 ± 6.8[b]	196.1 ± 40.5[c]	697.5 ± 165.2[c]	–[d] –[d]

[a] $p < 0.001$; [b] $p < 0.01$; [c] not significant; [d] not determined

Ketone bodies were determined as in [11]. Values are means ±SD; no. expt. are in parenthesis

acetyl-CoA/CoASH ratio [14,15].

The two forms of the mAAT, A and B, differ in isoelectric points and in specific activities [2]. Variation of the relative amounts may thus be important for the actual rate of ketogenesis. These amounts of mAAT A and B were measured by a sequence of chromatography on phosphocellulose and fused rocket immunoelectrophoresis. A typical result of an immunochemical elution profile is shown in fig.3. From the area of the two peaks of immunoprecipitates a mean ratio A/B of 0.94 ± 0.13 was calculated for the transferases, which remained unchanged when blood ketone bodies increased. The relative amounts of A and B forms of mAAT can be concluded not to change during starvation or alloxan-induced diabetes.

The amount of the immunoreactive mAAT does not correlate with the flux through ketogenic pathway in vivo. However, these results do not exclude a possible regulatory role of mAAT in this pathway as a consequence of modulation of enzyme activity expressed under various metabolic conditions.

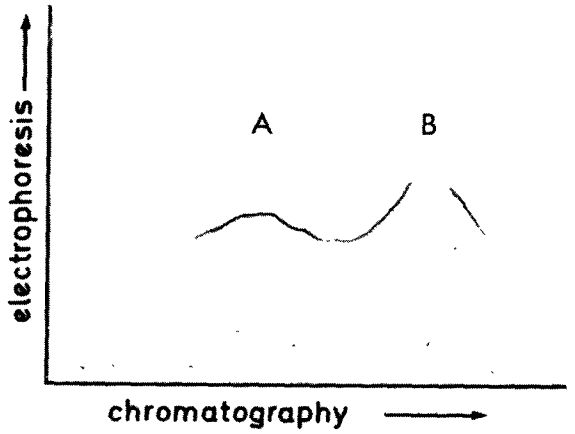

Fig.3. Fused rocket immunoelectrophoresis. Immunochemical elution profile from chromatography on phosphocellulose. The relative amount of the two forms of mAAT A and B, were calculated from the areas of immunoprecipitates by graphical integration. Number of experiments: fed $n = 13$; starved $n = 9$; alloxan diabetic $n = 5$.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft Hu 260/3. We wish to thank Matthijs Lopes-Cardozo for critically reading the manuscript.

References

[1] Middleton, B. (1973) Biochem. J. 139, 109–121.
[2] Schwabe, D. and Huth, W. (1979) Biochim. Biophys. Acta 575, 112–120.
[3] Miyazawa, S., Osumi, T. and Hashimoto, T. (1980) Eur. J. Biochem. 103, 589–596.
[4] Reed, W. D., Ozand, P. T., Tildon, J. T. and Cornblath, M. (1977) Biochem. J. 164, 27–32.
[5] Huth, W., Dierich, Ch., Von Oeynhausen, V. and Seubert, W. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 635–649.
[6] Huth, W., Jonas, R., Wunderlich, I. and Seubert, W. (1975) Eur. J. Biochem. 59, 475–489.
[7] Huth, W., Stermann, R., Holze, G. and Seubert, W. (1978) in: Biochemical and clinical aspects of ketone body metabolism (Soling, H. D. and Seufert, C. D. eds) pp. 11–19, Thieme, Stuttgart.
[8] Burton, K. (1956) Biochem. J. 62, 315–323.
[9] Weeke, B. (1973) in: Quantitative immunoelectrophoresis (Axelsen, N. H. et al. eds) pp. 37–46, Universitetsforlaget, Oslo.

[10] Axelsen, N. H. (1973) in: Quantitative immunoelectrophoresis (Axelsen, N. H. et al. eds) pp. 71–77, Universitetsforlaget, Oslo.
[11] Williamson, D. H., Mellanby, J. and Krebs, H. A. (1962) Biochem. J. 82, 90–98.
[12] Bates, M. W., Krebs, H. A. and Williamson, D. H. (1968) Biochem. J. 110, 655–661.
[13] Huth, W. and Menke, R. (1980) Hoppe Seyler's Z. Physiol. Chem. 361, 613.
[14] Lee, L. P. K. and Fritz, I. B. (1972) Canad. J. Biochem. 50, 120–127.
[15] Siess, E. A., Brocks, D. G. and Wieland, O. H. (1976) FEBS Lett. 69, 265–271.