CARNITINE OCTANOYLTRANSFERASE. EVIDENCE FOR A NEW ENZYME IN MITOCHONDRIA

H.E.SOLBERG

Institute of Clinical Biochemistry, University of Oslo, Rikshospitalet, Oslo 1, Norway

Received 19 November 1970

1. Introduction

Two enzymes have been shown to catalyze the reversible transfer of acyl groups from acyl-CoA to L-carnitine: the carnitine acetyltransferase (acetyl-CoA:carnitine *O*-acetyltransferase, EC 2.3.1.7) [1, 2] and the carnitine hexadecanoyltransferase (hexadecanoyl-CoA:carnitine *O*-hexadecanoyltransferase, EC 2.3.1.-) [3–6]. They are localized in the membranous part of the mitochondria [7]. Preparations of both enzymes transfer also medium chain acyl groups, but at relatively low rates [2, 8, 9]. In this report evidence is presented for the existence of a third carnitine acyltransferase showing maximum activity when medium chain acyl groups (C_6–C_9) are transferred. This enzyme, carnitine octanoyltransferase (octanoyl-CoA: carnitine *O*-octanoyltransferase) is present in the mitochondria of different rat tissues and as a contamination in a commercial preparation of carnitine acetyltransferase.

2. Materials and methods

Carnitine acetyltransferase (specific activity approx. 80 units/mg) was obtained from Boehringer and Soehne GmbH, Mannheim, Germany. L-Acylcarnitines [10], L-Me-^{3}H-carnitine [11], and acyl-CoA derivatives [12] were synthesized. Other chemicals were those previously used [10].

Mitochondria were obtained from the tissues of a male Wistar/MøIl rat (approx. 200 g) [13]. The particles were suspended in 0.15 M KCl without washing, and disrupted by sonication (Branson Model S 75 sonifier, 10 sec at 2.4 A, medium tip). The carnitine acyltransferase activity was assayed spectrophotometrically at 232 nm [14] (substrates: L-acylcarnitines/CoASH) or at 412 nm with 5,5′-dithiobis-(2-dinitrobenzoate) as reagent [2] (substrates: acyl-CoA derivatives/L-carnitine). A Hitachi Perkin-Elmer (Model 124) double-beam spectrophotometer equipped with a scale expanding recorder was used. The isotope exchange assay was performed according to Norum [9] when long chain acylcarnitines (C_{12}–C_{16}) were substrates. Otherwise the previously described [10] modification of the exchange assay was used. Protein was determined according to Lowry et al. [15].

3. Results

The existence of a third carnitine acyltransferase was first suspected when the previously reported investigation [10] of the commercial carnitine acetyltransferase was extended to include medium chain L-acylcarnitines as substrates. Fig. 1 shows that the transfer rates of the medium chain acyl groups (C_6–C_{10}) were greater than expected from the known specificity of carnitine acetyltransferase [2, 8]. Similar results were obtained in experiments with other batches of the commercial enzyme. The enzyme preparation did not contain carnitine hexadecanoyltransferase activity. When the enzyme activity was assayed by the isotope exchange method or by measuring the reaction in the reverse direction, i.e. with acyl-CoAs with varying chain length of the acyl groups, the same high reactivity of the medium chain acyl derivatives was observed.

In order to exclude that our results with medium

North-Holland Publishing Company – Amsterdam

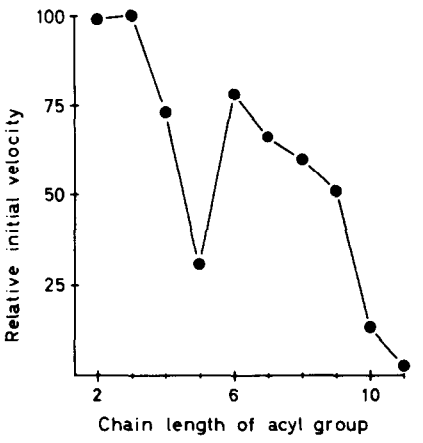

Fig. 1. Acyl group specificity of a commercial preparation of carnitine acetyltransferase. The assays were performed at 30° by measuring the initial velocity at 232 nm (see Methods). The reaction mixtures contained in a final volume of 3 ml: enzyme, 0.75 μg protein; L-acylcarnitine, 500 μM; CoASH, 100 μM; tris-HCl buffer (pH 7.8), 100 mM. The reactions were started by the addition of enzyme.

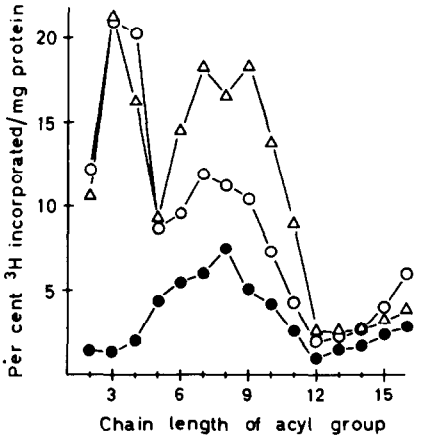

Fig. 2. Carnitine acyltransferase activity of disrupted mitochondria of rat tissues incubated with different acylcarnitines. The isotope exchange assay was used. The incubation mixtures contained in a final volume of 1 ml: liver mitochondria (●——●), 2.0 mg protein; heart mitochondria (△——△), 1.1 mg protein; or testis mitochondria (○——○), 0.8 mg protein; L-acylcarnitine, 500 μM; ^{3}H-carnitine (approx. 1.2 × 10^6 cpm), 500 μM; CoASH, 100 μM; GSH, 500 μM; KCN, 100 μM; tris-HCl buffer pH 7.8, 100 mM. The reactions were started by the addition of labelled carnitine and run for 4 min at 30°. The specific activities are expressed as percent incorporation of ^{3}H into acylcarnitines per mg mitochondrial protein. The values shown for liver mitochondria are means of duplicates.

chain substrates were due to some unkown kinetic behaviour of the carnitine acetyltransferase in our assay systems, we chose to compare the transferase activities in mitochondria from tissues with low and high activities of the carnitine acetyltransferase. This activity is high in rat heart and testis and low in rat liver [16]. Fig. 2 shows the results obtained with the isotope exchange assay on disrupted mitochondria from these tissues. The liver mitochondria showed low transferase activity when acetyl- or propanoylcarnitine were substrates, in striking contrast to the high activity of the acetyltransferase in heart or testis mitochondria. Propanoylcarnitine was the best substrate for the acetyltransferase in this assay system [10]. The mitochondria contained carnitine hexadecanoyltransferase activity with an acyl group specificity for C_{12}–C_{16} acylcarnitines similar to that found by Norum [9]. In addition all three kinds of mitochondria transferred medium chain acyl groups at high rates (maximum activity C_7–C_9).

4. Discussion

The reported results show that the high transfer rates of medium chain acyl groups are independent of both carnitine acetyltransferase and hexadecanoyltransferase. Only the existence of a third, hitherto unknown, carnitine acyltransferase with maximum activity in the middle region seems to explain this observation.

The role of carnitine medium chain acyltransferase is probably analogous to that of the acetyltransferase and the hexadecanoyltransferase [3–5, 17]: to catalyze the carnitine dependent transfer of fatty acyl-CoA derivatives through the inner mitochondrial membrane. If the short and the long chain carnitine acyltransferases were the only two transferases present in the mitochondria, medium chain acyl groups would probably be transferred at rather low rates. Octanoyl-

carnitine is however oxidized by mitochondria of different tissues at rates comparable to those observed with acetylcarnitine and hexadecanoylcarnitine [3].

The medium chain acyltransferase present as a contamination in the commercial acetyltransferase can explain why this enzyme preparation was better than hexadecanoyltransferase as an auxiliary enzyme in the assay of fatty acid activating enzymes [18] for acids up to C_8.

Chase [8] did not observe high activities with medium chain acyl-CoAs when assaying carnitine acetyltransferase obtained from the same source as the commercial preparation (pigeon breast muscle). His enzyme was, however, purified by recrystallizations to constant specific activity of approx. 120 units/mg [19], while the commercial enzyme had only 3/4 of this activity. Norum's specificity studies [9] were also performed on a relatively pure enzyme preparation. The reason why the carnitine octanoyltransferase has remained undiscovered until now, seems to be that no specificity studies have been done on pure enzyme preparations or on mitochondria.

Acknowledgements

The helpful suggestions by Dr. Jon Bremer and the skilled technical assistance of Miss W.Thorbjørnsen are gratefully appreciated.

References

[1] S.Friedman and G.Fraenkel, Arch. Biochem. Biophys. 59 (1955) 491.
[2] I.B.Fritz, S.K.Schultz and P.A.Srere, J. Biol. Chem. 238 (1963) 2509.
[3] J.Bremer, J. Biol. Chem. 237 (1962) 3628.
[4] J.Bremer, J. Biol. Chem. 238 (1963) 2774.
[5] I.B.Fritz and K.T.N.Yue, J. Lipid Res. 4 (1963) 279.
[6] K.R.Norum, Acta Chem. Scand. 17 (1963) 1487.
[7] K.R.Norum and J.Bremer, J. Biol. Chem. 242 (1967) 407.
[8] J.F.A.Chase, Biochem. J. 104 (1967) 510.
[9] K.R.Norum, Biochim. Biophys. Acta 89 (1964) 95.
[10] H.E.Solberg and J.Bremer, Biochim. Biophys. Acta 22(1970) 372.
[11] J.Bremer and K.R.Norum, J. Biol. Chem. 242 (1967) 1744.
[12] E.R.Stadtman, in: Methods in Enzymology, Vol. 3, eds. S.P.Colowick and N.O.Kaplan (Academic Press, New York, 1957) p. 931.
[13] D.K.Myers and E.C.Slater, Biochem. J. 67 (1957) 558.
[14] J.F.A.Chase and P.K.Tubbs, Biochem. J. 99 (1966) 32.
[15] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[16] N.R.Marquis and I.B.Fritz, J. Biol. Chem. 240 (1965) 2193.
[17] J.Bremer, J. Biol. Chem. 237 (1962) 2228.
[18] M.Aas, Biochim. Biophys. Acta, in press.
[19] J.F.A.Chase, D.J.Pearson and P.K.Tubbs, Biochim. Biophys. Acta 96 (1965) 162.