Vol. 88, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 13, 1979 Pages 1140-1146

Hydroxymethylglutaryl coenzyme A reductase activity in hamster adrenal mitochondria purified on a sucrose gradient

Benjamin Preiss and Jean-Guy Lehoux†

Départements de Biochimie
et de Gynécologie-Obstétrique
Faculté de Médecine
Université de Sherbrooke
Sherbrooke, Québec, Canada, J1H 5N4

Received April 20, 1979

SUMMARY

Hamster adrenal homogenates were fractionated by differential centrifugation to obtain crude mitochondrial and microsomal pellets. The mitochondria were further purified on a linear sucrose density gradient. The crude mitochondrial fraction was separated into three bands on the gradient. One of the bands (band 3, $D_{20}^{20} = 1.165$) contained all the measurable cytochrome C oxidase activity. Band 3 also contained the highest specific activity of HMG-CoA reductase corresponding to a 1.9 fold enrichment compared to the crude mitochondrial pellet. The evidence presented supports the possibility that a part of the HMG-CoA reductase activity in hamster adrenals is associated with mitochondria.

INTRODUCTION

The rate of cholesterol biosynthesis in a variety of mammalian cells appears to be determined by the activity of the enzyme hydroxymethylglutaryl coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34) (1). In the adrenal gland cholesterol is the precursor of the corticosteroids, in addition to its general role in the maintenance of the tissue. HMG-CoA reductase activities

† Scholar of the Medical Research Council of Canada.

0006-291X/79/111140-07$01.00/0

were first determined in adrenal preparations from different species (2,3). In hamster adrenals comparatively high HMG-CoA reductase activities were found in crude microsomal and mitochondrial fractions from normal animals (3). It appeared of interest to investigate further the possibility that adrenal HMG-CoA reductase activity may be associated with mitochondria, at least in some animal species. We found that beef adrenal cortex mitochondria were enriched in HMG-CoA reductase activity after purification on a linear sucrose gradient, although their specific activity remained very low compared to that of the microsomal fraction (4). In this communication we present results showing that a part of the HMG-CoA reductase activity in hamster adrenals is associated with the mitochondrial fraction. After separation of washed mitochondria on a linear sucrose gradient, the band containing practically all the cytochrome C oxidase activity is also enriched in HMG-CoA reductase activity.

MATERIALS AND METHODS

R, S[3-^{14}C]HMG-CoA and R, S[5-^{3}H]mevalonic acid (dibenzylethylenediamine salt) were obtained from New England Nuclear. HMG-CoA was from P-L biochemicals. Mevalonolactone, $NADP^{+}$, DTT, cytochrome C, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were from Sigma. Unisil was obtained from the Clarkson Chemical Co., Williamsport, PA. Syrian hamsters were obtained from Robidoux Inc. St. Constant, Quebec, and were kept for 3 weeks in an isolated room with an alternating light-dark cycle (light 06.00 to 18.00h, dark 18.00 to 06.00h). Animals were sacrificed by decapitation between 09.00 and 10.00h. Whole adrenal glands from 20 male or female hamsters were homogenized in 0.05M tris, pH 7.4, 0.25M sucrose, 0.01M $MgCl_2$, 0.001M Mg-EDTA (homogenization buffer) and fractionated under the conditions of Frühling et al. (5) to obtain mitochondrial, "intermediate" and microsomal fractions. The "intermediate" fractions were discarded. The mitochondrial fraction, after the third wash (6), was suspended in homogenization buffer (crude mitochondrial fraction) and added to a 24% to 56% W/V linear sucrose gradient containing

0.05M tris-HCl pH 7.4, 0.01M $MgCl_2$ and 0.001M Mg-EDTA, using a modification of the procedure of Shimizu et al. (7). Gradients were centrifuged at 63,000xg for 1h in a Beckman SW 50.1 rotor (7). The gradient fractions, collected with a pasteur pipette, were diluted in homogenization buffer and centrifuged at 10,000xg for 10 minutes. The pellets were suspended in the appropriate buffer for the HMG-CoA reductase or cytochrome C oxidase assay. The microsomal fraction (crude microsomal fraction) and part of the crude mitochondrial fraction were kept on ice and assayed together with the gradient fractions after suspension of the final pellets in the appropriate buffer for each assay. For the HMG-CoA reductase assay subcellular fractions were suspended in a medium containing 0.30M sucrose, 0.03M nicotinamide, 0.01M potassium phosphate pH 7.0 and 0.001M dithiothreitol (freshly added). Fractions were made up to 100µl in the same medium and incubated in a total volume of 200µl containing final concentrations of 5mM potassium phosphate, pH 7.0, 150mM sucrose, 15mM nicotinamide, 2mM sodium EDTA, 4.3mM dithiothreitol, 1 unit/ml glucose-6-phosphate dehydrogenase, 20mM glucose-6-phosphate, 3mM $NADP^+$, 0.5mM and 0.05µCi [3-^{14}C]R, S-HMG-CoA. Samples were incubated for 30 minutes at 37°C and assayed as described before (4).

For the cytochrome C oxidase assay,pellets were suspended in 50mM potassium phosphate buffer, pH 7.5 and assayed by the modification of Hodges and Leonard (8) of the method of Wharton and Tzagoloff (9). Protein was determined by the method of Lowry et al. (10).

RESULTS AND DISCUSSION

The crude mitochondrial fraction was separated on a 24% to 56% W/V linear sucrose gradient as described in Materials and Methods. The gradient separated the crude mitochondrial fraction into three bands, band 1, a rather diffuse colorless band (D_{20}^{20} = 1.2-1.22), band 2, a sharp colorless band (D_{20}^{20} = 1.18-1.185) and band 3, a sharp brownish band (D_{20}^{20} = 1.165). The

Table I

Cytochrome C oxidase activity

Fraction	Cytochrome C oxidase activity (in μmoles/mg protein/min)	Number of determinations
Crude mitochondrial fraction	0.242 ± 0.030*	4
Crude microsomal fraction	0.07	2
Gradient		
Band 1 (176μg protein)	0	4
Band 2 (260μg protein)	0	4
Band 3 (1115μg protein)	0.353 ± 0.014*	4

* ± S.D.

distribution of cytochrome C oxidase activity and total protein among the gradient fractions is shown in Table I. Band 3 contains all the measurable cytochrome C oxidase activity.

In preliminary experiments the linearity of the HMG-CoA reductase assay was established between 6 and 36 micrograms of crude or purified (band 3) mitochondrial protein per 0.2ml incubation mixture. Subsequent determinations were done in that range of protein concentrations. Table II shows the specific activities of HMG-CoA reductase in the three gradient fractions and in the crude mitochondrial and microsomal fractions. Band 3 has the highest specific activity of HMG-CoA reductase among the gradient fractions, representing a 1.9 fold purification of the crude mitochondrial fraction. The evidence presented here strongly supports the conclusion that HMG-CoA reductase activity is associated with hamster adrenal mitochondria.

Table II

HMG-CoA reductase activity

Fraction	HMG-CoA reductase activity (in nmoles/mg protein/30 min)	Number of determinations
Crude mitochondrial fraction	4.3 ± 0.39*	4
Crude microsomal fraction	12.56	2
Gradient		
Band 1	4.6	2
Band 2	2.6	2
Band 3	8.2 ± 0.97*	4

* ± S.D.

In the yeast Saccaromyces cerevisiae HMG-CoA reductase is localized in the mitochondria (7,11). In the crypt cells of rat intestinal mucosa, HMG-CoA reductase activity was reported to be both in the mitochondrial and in the microsomal fractions obtained by differential centrifugation (12). We have previously reported the presence of relatively high specific activities of HMG-CoA reductase in crude mitochondrial fractions from hamster and chick adrenals, homogenized in a 0.1M phosphate buffer medium (3). In the present study the conditions of Frühling et al. (5) were followed in order to minimize the possibility of contamination of the mitochondria with the microsomal fraction.

Adrenal cortex mitochondria contain the enzymes necessary for the biosynthesis of pregnenolone from cholesterol (13). The hamster is a vertebrate species in which the rate of cholesterol synthesis in the adrenals is normally very high compared to the rat (14). In contrast with rat adrenals, the cho-

lesterol ester content in hamster adrenals is very low (15,16). In the rat, adrenal HMG-CoA reductase activity is normally very low (3,17). Reductase activity in rat adrenal microsomes was enhanced after depletion of the adrenal cholesterol esters following treatment with 4-aminopyrazolopyrimidine (18).

It would be of interest to compare the properties of the mitochondrial and microsomal HMG-CoA reductase activities and their regulation under different physiological conditions. Future work could also establish whether HMG-CoA reductase is present in mitochondria from other steroid hormone producing tissues of hamster and other species.

ACKNOWLEDGEMENT

We thank miss Hélène Beaudoin for expert technical assistance. This research was supported by the Banting Research Foundation and the Medical Research Council of Canada.

REFERENCES

1. Rodwell, V.W., Nordstrom, J.L. and Mitschelen, J.J. (1976) Adv. Lipid Res, 14, 2-74.

2. Lehoux, J.-G., Tan, L. and Preiss, B. (1975). Endocrinology 96 (Suppl. A449), 275.

3. Lehoux, J.-G., Tan, L. and Preiss, B. (1977). Gen. Comp. Endocrinol. 33, 133-138.

4. Preiss, B., Tan, L. and Lehoux, J.-G. (1978). Can. J. Biochem. 56, 958-962.

5. Frühling, J., Pecheux, F. and Penasse, W. (1974). Histochemistry, 42, 145-162.

6. Sand, G., Frühling, J., Penasse, W. and Claude, A. (1972) J. Microscopie 15, 41-66.

7. Shimizu, I., Nagai, J., Hatanaka, H., Saito, E. and Katsuki, H. (1971). J. Biochem. 70, 175-177.

8. Hodges, T.K. and Leonard, R.T. (1975) in Methods in Enzymology,(Fleischer, S. and Packer, L. eds.)Vol. 32B, pp. 392-406. Academic Press New York.

9. Wharton, D.C. and Tzagoloff, A. (1967) in Methods in Enzymology,(Estabrook, R.W. and Pullman, M.E. eds.)Vol. 10, pp. 245-250.

10. Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

11. Shimizu, I., Nagai, J., Hatanaka, H. and Katsuki, H. (1972) Biochim. Biophys. Acta 296, 310-320.

12. Shefer, S., Hauser, S., Lapar, V. and Mosbach, E.H. (1972) J. Lipid Res. 13, 402-412.

13. Halkerston, I.D.K., Eichhorn, J. and Hechter, O. (1959) Arch. Biochem. Biophys. 85, 287-289.

14. Lloyd, B.J. (1972) Gen. Comp. Endocrinol. 19, 428-431.

15. Miroff, M.B.C. (1954) Doctoral dissertation University of Michigan, Publ. no. 8382.

16. J.-G. Lehoux and B. Preiss, unpublished results.

17. Balasubramaniam, S., Goldstein, J.L., and Brown, M.S. (1977) Proc. Natl. Acad. Sci. USA 74, 1421-1425.

18. Balasubramaniam, S., Goldstein, J.L., Faust, J.R., Brunschede, G.Y., and Brown, M.S. (1977) J. Biol. Chem. 252, 1771-1779.