Properties of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Solubilized from Rat Liver and Hepatoma*

(Received for publication, March 26, 1974)

MICHAEL S. BROWN, SUZANNA E. DANA, AND MARVIN D. SIPERSTEIN†

From the Divisions of Gastroenterology-Liver and Metabolism, Department of Internal Medicine, University of Texas, Southwestern Medical School, Dallas, Texas 75235

SUMMARY

In hepatomas, the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-controlling enzyme in cholesterol biosynthesis, is not normally suppressed by cholesterol. To examine the biochemical mechanism of this loss of feedback control of cholesterol synthesis, a comparison was made of the properties of 3-hydroxy-3-methylglutaryl coenzyme A reductase after solubilization and partial purification from microsomes of normal rat liver and of a minimal deviation rat hepatoma. The solubilized enzyme from the two sources behaved identically with respect to substrate kinetics, heat inactivation, cold inactivation, and gel filtration.

By the use of a new method that permits assay of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity after polyacrylamide gel electrophoresis, it was shown that the solubilized hepatoma enzyme and the normal liver enzyme had similar electrophoretic mobility. It is concluded that the failure of hepatomas to suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase activity is not due to the production of an altered form of the enzyme.

When an animal consumes cholesterol in the diet, a compensatory reduction in hepatic cholesterol synthesis occurs (1-4). It is now well established that this feedback control mechanism acts specifically at the site of synthesis of mevalonic acid (5-8), a reaction catalyzed by the enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase. When liver cells become malignant they lose their ability to lower HMG-CoA reductase activity in response to cholesterol feeding (9, 10). As a result, in all primary and transplantable hepatomas of animals and man that have been studied, feedback regulation of cholesterol synthesis is consistently absent (11-14).

Since hepatomas characteristically produce isozymic forms of enzymes that are low or absent in normal adult liver tissue (15, 16), it has been postulated that the deletion of feedback control of HMG-CoA reductase activity in these tumors might be due to the production of an abnormal form of HMG-CoA reductase that is insensitive to inhibition by cholesterol (17). Experiments to evaluate this hypothesis are lacking, however, because HMG-CoA reductase has been resistant to solubilization from liver microsomes, and no comparison of the properties of the purified enzyme from normal liver and hepatomas has therefore been conducted. Recently, we have developed a method for the solubilization and partial purification of microsomal HMG-CoA reductase from rat liver (18). The enzyme from normal liver was shown to have several distinctive properties such as cold inactivation, which could be prevented by salt only at concentrations approaching 4 M, and heat stability in the presence of 4 M salt. In the current studies, we have compared these properties of HMG-CoA reductase solubilized from normal rat liver and hepatoma. In addition, we have devised a method of polyacrylamide gel electrophoresis that preserves the activity of the enzyme and permits direct measurement of its electrophoretic mobility. The results indicate that HMG-CoA reductase solubilized from the hepatoma is indistinguishable from the enzyme solubilized from normal liver.

EXPERIMENTAL PROCEDURE

Materials—-d-glucose 6-phosphate (monosodium salt) and TPN were obtained from Sigma Chemical Co. Coenzyme A and yeast glucose 6-phosphate dehydrogenase (350 units per mg) were purchased from Boehringer Mannheim. Dithiothreitol (A grade) was purchased from Calbiochem. All inorganic reagents used throughout were of analytical reagent grade and were obtained from J. T. Baker Chemical Co. Plastic-backed thin layer chromatography sheets coated with Silica Gel G (without gumgum) were from Brinkmann Instruments. Agarose A-0.5w, 200 to 400 mesh, was from Bio-Rad Laboratories. Materials for polyacrylamide gel electrophoresis were from Eastman. Sodium barbital was from Merck.

Unlabeled dl-mevalonic acid lactone was purchased from Sigma Chemical Co. dl-3-Hydroxy-3-methyl[3-3H]glutaric acid (specific activity 3.26 mCi per mmole) and dl-[5-3H]mevalonic acid (dibenzyloxyhemiamelaine salt) (specific activity 6.7 G i per mmole) were from New England Nuclear. dl-[2-3H]Mevalonic acid lac-
Hepatoma and Liver Tissue—The minimal deviation Morris hepatoma 9121 was grown by implantation into the thigh muscles of rats of inbred ACI/f Mai strain (Microbiological Associates). This tumor has been shown previously to lack feedback regulation of cholesterol synthesis (16) and HMG-CoA reductase activity (9). Livers were obtained from tumor-free rats of the same strain. Rats were housed in gang cages, kept on a diet of rat pellets, and exposed to continuous lighting. Animals were killed at 9 a.m.

Assay for HMG-CoA Reductase Activity—The method used has been reported previously in detail (18). The enzyme was added to 0.2 ml of a solution containing 0.1 mM KPO4, 0.02 mM glucose-6-phosphate, 2.5 mM TPN, 0.7 unit of glucose-6-phosphate dehydrogenase, and 5 mM dithiothreitol at pH 7.5. The assays were started by the addition of [3H]HMG-CoA (1.76 mCi per mmole, specific activity 5 mCi per mmole) was from Amersham-Searle Corp.

Hepatoma 9121 was grown by implantation into the thigh muscles of rats of the inbred ACI/f Mai strain (Microbiological Associates). This tumor has been shown previously to lack feedback regulation of cholesterol synthesis (16) and HMG-CoA reductase activity (9). Livers were obtained from tumor-free rats of the same strain. Rats were housed in gang cages, kept on a diet of rat pellets, and exposed to continuous lighting. Animals were killed at 9 a.m.

Assay for HMG-CoA Reductase Activity—The method used has been reported previously in detail (18). The enzyme was added to 0.2 ml of a solution containing 0.1 mM KPO4, 0.02 mM glucose-6-phosphate, 2.5 mM TPN, 0.7 unit of glucose-6-phosphate dehydrogenase, and 5 mM dithiothreitol at pH 7.5. The assays were started by the addition of [3H]HMG-CoA (1.76 mCi per mmole, specific activity 5 mCi per mmole) was from Amersham-Searle Corp.

Hepatoma 9121 was grown by implantation into the thigh muscles of rats of the inbred ACI/f Mai strain (Microbiological Associates). This tumor has been shown previously to lack feedback regulation of cholesterol synthesis (16) and HMG-CoA reductase activity (9). Livers were obtained from tumor-free rats of the same strain. Rats were housed in gang cages, kept on a diet of rat pellets, and exposed to continuous lighting. Animals were killed at 9 a.m.

Assay for HMG-CoA Reductase Activity—The method used has been reported previously in detail (18). The enzyme was added to 0.2 ml of a solution containing 0.1 mM KPO4, 0.02 mM glucose-6-phosphate, 2.5 mM TPN, 0.7 unit of glucose-6-phosphate dehydrogenase, and 5 mM dithiothreitol at pH 7.5. The assays were started by the addition of [3H]HMG-CoA (1.76 mCi per mmole, specific activity 5 mCi per mmole) was from Amersham-Searle Corp.

Hepatoma 9121 was grown by implantation into the thigh muscles of rats of the inbred ACI/f Mai strain (Microbiological Associates). This tumor has been shown previously to lack feedback regulation of cholesterol synthesis (16) and HMG-CoA reductase activity (9). Livers were obtained from tumor-free rats of the same strain. Rats were housed in gang cages, kept on a diet of rat pellets, and exposed to continuous lighting. Animals were killed at 9 a.m.
0.005 and 0.1 mM.

rapid inactivation at 4°C, with protection by salt only at concentrations approaching 4 M. Moreover, the first order decay rate of enzyme activity at 4°C was identical for the two enzymes. Fourth, the enzymes have similar size as measured by agarose gel filtration. Fifth, the enzymes have similar charge as measured by polyacrylamide gel electrophoresis. Sixth, the substrate saturation kinetics for the two enzymes are similar.

Thus, the data provide no support for the hypothesis that the hepatoma produces an isoenzyme of HMG-CoA reductase that differs from the one produced by normal liver. By the nature of our approach, however, using the solubilized enzyme, we cannot exclude the possibility that when the enzyme is located in the microsomal membrane it behaves differently in the hepatoma than in the normal liver. For example, it is possible that in our solubilization procedure we have separated HMG-CoA reductase from another protein, produced in normal liver, that shares a unique property of liver HMG-CoA reductase, namely, rapid inactivation at 4°C, with protection by salt only at concentrations approaching 4 M.

Kₘ values for dL-HMG-CoA (2.5 × 10⁻⁴ M) and TPNH (0.11 × 10⁻⁴ M) were similar for both enzymes (Fig. 1).

Studies of susceptibility to heat inactivation revealed similar patterns for HMG-CoA reductase solubilized from liver and hepatoma when heated in the presence of 4 M KCl (Fig. 2). Both enzymes were stable to moderate heating and showed inactivation of approximately 65% and 70%.

Solubilized hepatoma HMG-CoA reductase showed cold lability that was identical with that of the liver enzyme (Fig. 3). When incubated at 4°C in a solution containing 1 M KCl both enzymes were inactivated with first order kinetics and a half-life of approximately 6 min. Both enzymes were protected from cold inactivation when the concentration of KCl was increased to 4 M.

When solubilized liver and hepatoma HMG-CoA reductase were subjected to gel filtration on Agarose A-0.5m both enzymes showed a similar elution profile, corresponding to an apparent molecular weight of 200,000 (18) (Fig. 4).

The similarity of solubilized liver and hepatoma HMG-CoA reductase was indicated further by their migration when subjected to electrophoresis in polyacrylamide gels at pH 8.0 (Fig. 5). By incorporating 30% glycerol into the gel and conducting the electrophoresis at 25°C, it was possible to recover about 80% of the applied activity in a single band that coincided with a band of protein stained with Coomassie blue. When measured by this technique, the enzymes from both the hepatoma and liver had similar Rₚ values.

DISCUSSION

The data presented in this paper show that HMG-CoA reductase from a rat hepatoma manifests several distinctive properties identical with those of the enzyme from normal liver. First, the two enzymes can be solubilized from microsomes in similar yield and with a similar degree of purification by a technique that releases only 20 to 25% of the total protein from the membrane (18). Second, both enzymes show similar heat stability in the presence of 4 M KCl. Third, the hepatoma enzyme shares a unique property of liver HMG-CoA reductase, namely, rapid inactivation at 4°C, with protection by salt only at concentrations approaching 4 M.
forms a complex with the reductase on the microsomes and regula-
s its activity. The absence of this hypothetical regulatory
protein in the hepatoma might result in the observed deletion of
feedback suppression of HMG-CoA reductase activity. Against
this hypothesis is the observation that direct addition of choles-
terol to normal liver microsomes in vitro does not inhibit HMG-
CoA reductase activity (7, 18).

The evidence that the lack of suppression of HMG-CoA redu-
ductase in hepatomas is not due to the production of an altered
form of HMG-CoA reductase is consistent with previous data
that indicate that suppression of reductase activity by cholesterol
is achieved by decreasing the synthesis of new enzyme mole-
cules, both in liver (21, 22) and cultured fibroblasts (23). The defec-
tive regulation of HMG-CoA reductase in the hepatoma, then,
does not appear to be due to an alteration in the enzyme itself,
but rather in one of the processes that is necessary for cholesterol-
induced suppression of enzyme synthesis. This defect may for
example involve an altered binding of cholesterol by hepatoma
tissue (24).

Other investigators have apparently not observed cold lability
when rat liver HMG-CoA reductase was solubilized by other
methods, such as bile acid treatment (25), lyophilization of mi-
crosomes (26), or gradual freeze-thawing (27). As previously
reported (18), in our hands HMG-CoA reductase shows cold labi-
ility after solubilization by three methods, all of which yield large
amounts of activity per g of liver: 4 mM KCl treatment of mi-
crosomes, phospholipase A digestion, and freezing followed by gly-
cerol extraction. Although in the current study only the latter
method was used, in other experiments we have observed that
the hepatoma enzyme also showed cold lability when solubilized
with 4 mM KCl. When attempts were made in our laboratory to
solubilize HMG-CoA reductase from rat liver microsomes by bile
acid treatment or by lyophilization, the yields were less than 10%
of those achieved with the current methods, and it is therefore
possible that the failure of some investigators to demonstrate the
cold lability of HMG-CoA reductase may be due to loss during
preparation of the major, cold-sensitive portion of the enzyme.

REFERENCES
Biol. Chem. 201, 137–141
Biol. Chem. 206, 465–469
81
241, 602–609
7. LIPNITZ, T. C. (1957) J. Biol. Chem. 224, 999–998
1882
11. SIPERSTEIN, M. D., and FAGAN, V. M. (1964) Cancer Res. 24,
1108–1115
Cancer Res. 26, 7–11
Proc. 32, 2168–2173
27, 797–800
16. KNOX, W. E. (1972) Enzyme Alterations in Fetal, Adult and
Neoplastic Rat Tissues, Karger, Basel
17. SIPERSTEIN, M. D. (1965) Developmental and Metabolic Control

Fig. 4. Agarose gel filtration of HMG-CoA reductase from liver
and hepatoma. Nine-tenths milliliter of normal liver HMG-CoA
reductase from Step 4 of purification (Table I) containing 14.9
units of HMG-CoA reductase activity and 1.1 mg of protein was
applied to a column (1.0 × 46 cm) containing Agarose A-0.5m
equilibrated with Buffer A containing 3 mM KCl. The column was
developed at room temperature with the same buffer at a flow rate
of 0.25 ml per min. Fractions (1.5 ml) were collected and aliquots
were assayed for HMG-CoA reductase activity. The following
day 0.9 ml of hepatoma HMG-CoA reductase from Step 4 (Table I)
containing 68 units of HMG-CoA reductase activity and 4.3 mg of
protein was applied to the same column equilibrated with the same
buffer, and fractions were collected in an identical manner.

Fig. 5. Polysacrylamide gel electrophoresis of HMG-CoA redu-
ductase from normal liver and hepatoma. The fractions contain-
ing enzyme activity from the agarose column runs (Fig. 4) were
pooled, concentrated to 1.3 ml, and dialyzed overnight against
three changes of 100 ml of 0.05 M Tris-phosphate buffer, pH 6,
containing 50% (w/v) glycerol and 5 mM dithiothreitol. Aliquotes
(25 μl) of the dialyzed material were applied to polysacrylamide
gel electrophoresis tubes prepared as described under "Experi-
mental Procedure" and run for 2 hours, as described. The gels
were removed immediately from the tubes, sliced and slices repre-
senting 5 mm of gel were placed in tubes containing 220 μl of the
standard assay mixture and incubated for 30 min at 37 °C in a shak-
ing Duboff incubator. The reactions were stopped and the mela-
ionate isolated as described under "Experimental Procedure." The
initial aliquots of the liver enzyme contained 0.015 unit of
activity and the aliquot of the hepatoma enzyme contained 0.095
unit. The tracking dye progressed 4.0 cm in the normal enzyme
electrophoresis as opposed to 5.0 cm in the case of the hepatoma
enzyme, and the data are thus expressed as the Rf (distance of the
slice from the origin divided by the distance traveled by the track-
ing dye).
Mechanisms in Neoplasia, pp. 427–451, Williams & Wilkins, Baltimore


Properties of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Solubilized from Rat Liver and Hepatoma
Michael S. Brown, Suzanna E. Dana and Marvin D. Siperstein


Access the most updated version of this article at http://www.jbc.org/content/249/20/6585

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/20/6585.full.html#ref-list-1