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 transplanted 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—dl-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. Protein-backed thin layer chromatography sheets coated with silica gel G (without gypsum) were from Brinkmann Instruments. Agarose A-0.5m, 200 to 400 mesh, was from Bio-Rad Laboratories. Materials for polyacrylamide gel electrophoresis were from Eastman. Sodium boribital was from Merek.

Unlabeled dl-mevalonic acid lactone was purchased from Sigma Chemical Co. dl-Hydroxy-3-methyl-[3-14C]glutaric acid (specific activity 5.46 mCi per mmole) and dl-[5-3H]mevalonic acid (specific activity 6.7 G per mmole) were from New England Nuclear. dl-[2-14C]Mevalonic acid lac-
tone (specific activity 5 mCi per mmole) was from Amersham-Searle Corp.

Hepatoma and Liver Tissue—The minimal deviation Morris hepatoma 9212 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 (9) 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 M K2HPO4, 0.02 M 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 [3-14C]HMG-CoA (1.76 mCi per mmole, prepared as described (9)) to a final concentration of 8.7 × 10^{-6} M. After a period ranging from 15 to 60 min at 37°C (adjusted to remain within the linear range of the assay), the reaction was stopped by the addition of 0.02 ml of 0.1 N HCl. After addition of 3 μmoles of [5-3H]mevalonolactone (12,500 cpm per pmole), the mevalonolactone was extracted with ether, isolated by thin layer chromatography, and the radioactivity was determined in a liquid scintillation counter. One unit of HMG-CoA reductase activity is that activity which will convert 1 nmole of HMG-CoA to mevalonate in 1 min at 37°C.

Polyacrylamide Gel Electrophoresis—The electrophoresis system was based on the Gel System Number 6 of Mauger (20) with the following modifications: (a) all solutions contained 30% (w/v) glycerol and 1 mM dithiothreitol; (b) the concentration of acrylamide in the lower gel was reduced to 5%; (c) the spacer gel buffer contained 25 mM Tris-phosphate (pH 6.0). Electrophoresis was conducted at room temperature for 2 hours, at 2 mA per tube in siliconized glass tubes (5 × 65 mm). Under these conditions the enzyme stacked at pH 7.0 and ran at pH 8.0.

RESULTS

Solubilisation of Liver and Hepatoma HMG CoA Reductase—Five rats with transplanted hepatomas (9212) and six normal rats were killed with ether and the hepatomas were removed from the former and the livers were removed from the control animals. The hepatomas (totaling 125 g) and livers (totaling 35 g) were placed in 2:1 (v/w) ice-cold homogenization medium consisting of 0.3 M sucrose and 10 mm 2-mercaptoethanol and the cells were disrupted in a Dounce homogenizer with 15 strokes of a loose-fitting pestle. The homogenates were centrifuged twice for 10 min at 12,000 × g in a refrigerated centrifuge and the supernatant fraction was then centrifuged for 60 min at 100,000 × g at 4°C.

The microsomal pellets were then drawn up repeatedly through a hypodermic needle in one-half the original volume of solution containing 0.8 M sucrose, 5 mM dithiothreitol, and 0.05 mM EDTA (pH 7.5), and an aliquot was removed for assay for HMG-CoA reductase activity and protein content (Step 1, Table I). The mixture was centrifuged immediately at 100,000 × g for 60 min and the pellets (each consisting of the microsomal membranes from 0 g of tissue) were frozen in liquid nitrogen for 30 s and thawed slowly at room temperature. Each pellet was then suspended by drawing through a hypodermic needle in 1.0 ml of solution containing 50% glycerol, 50 mM K2HPO4, 5 mM dithiothreitol, and 1 mM EDTA (pH 7.5). The mixtures were incubated at room temperature for 30 min, then diluted 1:5 with buffer containing 50 mM K2HPO4, 5 mM dithiothreitol, 1 mM EDTA, and 1.25 mM KCl (pH 7.5), and centrifuged at 100,000 × g for 60 min at 25°C. All further steps were conducted at room temperature. The supernatants were adjusted to 4 M KCl and dialyzed for 16 hours against two changes of 50 volumes of buffer containing 50 mM K2HPO4, 5 mM dithiothreitol, 1 mM EDTA, and 1.25 mM KCl (pH 6.9) (Buffer A), plus 4 M KCl. Aliquots of this dialyzed supernatant were assayed for HMG-CoA reductase activity and protein content (Step 3, Table I). The pellets from the centrifugation were resuspended in Buffer A and aliquots were assayed for HMG-CoA reductase activity. For both the liver and hepatoma enzymes all of the original activity that was not present in the supernatant could be accounted for by the activity remaining in the pellet.

The dialyzed supernatants were heated at 65°C and the resultant precipitate was removed by centrifugation at 100,000 × g for 30 min at 25°C. The supernatants were concentrated 10-fold by ultrafiltration using an Amicon UM-10 membrane, and aliquots of the concentrated material were assayed for HMG-CoA reductase activity and protein content (Step 4, Table I). The solubilized enzymes were then divided into aliquots, quick-frozen by immersion in liquid nitrogen, and stored at -195°C. This procedure resulted in yields of 54 and 44% of the initial activities of the liver and hepatoma enzymes, respectively, with purifications of 73- and 47-fold (Table I).

Properties of Solubilized HMG-CoA Reductase from Liver and Hepatoma—The substrate saturation curves for TPNH and HMG-CoA for the solubilized liver and hepatoma enzymes were similar and double-reciprocal plots indicated that the apparent

| Table I | Simultaneous purification of HMG-CoA reductase from liver and hepatoma | Details for See "Experimental Procedure" |
**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 KC1. Third, the hepatoma enzyme shares a unique property of liver HMG-CoA reductase, namely, rapid inactivation at 4°, with protection by salt only at concentrations approaching 4 M. Moreover, the first order decay rate of enzyme activity at 4° 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 situ on 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°, with protection by salt only at concentrations approaching 4 M. Moreover, the first order decay rate of enzyme activity at 4° 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 situ on 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

![Graph](https://example.com/graph.png)
forms a complex with the reductase on the microsomes and regulates 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 cholesterol to normal liver microsomes in vitro does not inhibit HMG-CoA reductase activity.

The evidence that the lack of suppression of HMG-CoA reductase 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 molecules, both in liver (21, 22) and cultured fibroblasts (23). The defective regulation of HMG-CoA reductase in the hepatoma, then, does not appear to be due to an alteration in the enzyme itself, but 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 microsomes (26), or gradual freeze-thawing (27). As previously reported (18), in our hands HMG-CoA reductase shows cold lability after solubilization by three methods, all of which yield large amounts of activity per g of liver: 4 m KCl treatment of microsomes, phospholipase A digestion, and freezing followed by glyceral extraction. Although in the present 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 m 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
17. Siperstein, M. D. (1965) Developmental and Metabolic Control
Mechanisms in Neoplasia, pp. 427-451, Williams & Wilkins, Baltimore

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