3-Hydroxy-3-methylglutaryl coenzyme A reductase in human liver microsomes: active and inactive forms and cross-reactivity with antibody against rat liver enzyme[1]

Bo Angelin, Kurt Einarsson, Lars Liljeqvist, Klas Nilsell, and Renu A. Heller

Departments of Medicine and Surgery, Karolinska Institutet at Huddinge University Hospital, Stockholm, Sweden, and Department of Biological Sciences, Stanford University, Stanford, CA

Abstract 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme catalyzing the rate-limiting step in cholesterol biosynthesis, exists in one active (dephosphorylated) and one inactive (phosphorylated) form in liver microsomes obtained from several animal species. The present study was undertaken in order to determine *a*) whether the human enzyme also exists in active and inactive readily interconvertible forms; *b*) whether the large inter-individual variation in HMG-CoA reductase activity observed in normal man can be explained by variations in the activation state of the enzyme; and *c*) to characterize the reactivity of antibodies raised against rat liver HMG-CoA reductase with the intact human microsomal enzyme. HMG-CoA reductase activity, assayed in microsomes prepared in the presence of 50 mM NaF, was only 17 ± 3% of the activity observed in microsomes prepared from the same liver in the absence of fluoride. Preincubation of microsomes prepared in NaF with alkaline phosphatase resulted in a tenfold increase of enzyme activity, while the activity of microsomes prepared without fluoride was increased also (by about 45%) with this treatment. On the other hand, the activated enzyme could be inactivated by incubation of microsomes with Mg-ATP. In eleven normal weight, normolipidemic gallstone patients, the HMG-CoA reductase activity determined in microsomes prepared without NaF ("standard procedure") reflected well both the "expressed" activity (in microsomes prepared with NaF) and the "total" (fully activated) enzyme activity; correlation coefficients were +0.80 and +0.84, respectively. Preincubation of human liver microsomes with rabbit antiserum against partially purified HMG-CoA reductase from rat liver resulted in a 72 ± 6% inhibition of enzyme activity. The fraction of enzyme activity maximally inhibited was relatively constant over a wide range of individual reductase activities. Immunotitration studies suggested that the affinity of the antiserum used may be higher for the active than for the inactive form of the enzyme. In studies of three additional patients, the inclusion of protease inhibitors in the homogenizing media did not influence the properties of the enzyme with regard to activation-inactivation or to immunoreactivity. It is concluded *a*) that the human microsomal HMG-CoA reductase exists in interconvertible active and inactive forms; *b*) that the inter-individual variation of enzyme activity present in untreated normal subjects is mostly the consequence of varying amounts of enzyme protein; and *c*) that antibodies raised against rat liver HMG-CoA reductase cross-react with the microsomal human enzyme.—**Angelin, B., K. Einarsson, L. Liljeqvist, K. Nilsell, and R. A. Heller.** 3-Hydroxy-3-methylglutaryl coenzyme A reductase in human liver microsomes: active and inactive forms and cross-reactivity with antibody against rat liver enzyme. *J. Lipid Res.* 1984. **25:** 1159–1166.

Supplementary key words cholesterol synthesis • enzyme phosphorylation-dephosphorylation • liver biopsy

The microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (mevalonate: NADP oxidoreductase, E.C. 1.1.1.34) catalyzes the rate-limiting step in cholesterol biosynthesis. The activity of this enzyme is subject to an intricate feed-back regulation, still incompletely known in its details (2, 3). Previous studies in several animal species have indicated that hepatic HMG-CoA reductase exists in active and inactive forms (4–8). The inactive form of the enzyme appears to be converted into the active one by dephosphorylation of the enzyme (9–11). Due to the effect of phosphatases of the cytosol, activation of HMG-CoA reductase during the preparation of liver microsomes has been described (5, 7, 8). In all previous studies of the human hepatic HMG-CoA reductase, the activity has been assayed using microsomes prepared in the absence of fluoride (12–22).

The present study was undertaken to determine whether human hepatic microsomal HMG-CoA reductase exists in active and inactive forms, and whether variation

Abbreviations: EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; HMG, 3-hydroxy-3-methylglutaryl; HMG-CoA reductase, mevalonate:NADP oxidoreductase, EC 1.1.1.34; PMSF, phenylmethanesulfonyl fluoride.

[1] Presented in part at the 54th Scientific Session of the American Heart Association, Dallas, Texas, November 16–19, 1981; published in abstract form (1).

Downloaded from www.jlr.org by guest, on June 18, 2012

in enzyme activation might explain the relatively wide inter-individual variation of enzyme activity that has been reported in presumably healthy subjects (12). Furthermore, we wanted to determine whether antibodies prepared against the rat liver enzyme cross-react with human HMG-CoA reductase.

METHODS

Subjects

Fourteen patients, four males and ten females, with uncomplicated cholesterol gallstone disease were studied. Their ages ranged from 39 to 68 (mean 48) years and their relative body weights[2] from 87 to 131 (mean 106)%. None of the patients had clinical or laboratory evidence of hyperlipoproteinemia, overconsumption of alcohol, or diseases affecting liver, kidney, or thyroid function (cf. 12).

Experimental procedure

The patients were all admitted to the Department of Surgery for elective cholecystectomy at least 3 days prior to surgery. A detailed clinical and laboratory examination was performed in all cases. The patients were fed the regular hospital diet, which contained 35% fat, 45% carbohydrate, and 20% protein based on caloric intake. The dietary intake of cholesterol was about 0.5 mmol/day.

All operations were performed between 9 and 10 AM after a 12-hr fast. Standardized anesthesia was given with thiopentothal induction and continuous treatment with nitrous oxide, diazepam, and fentanyl. Immediately after opening the abdomen a liver biopsy was obtained from the left lobe of the liver and immediately rinsed in ice-cold 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose, 10 mM EDTA, and 10 mM dithiothreitol. The biopsy was then divided into two (in some cases four) equal pieces, and microsomes were prepared using NaCl- or NaF-containing buffer (see below). A specimen of the biopsy was sent for histological examination. Liver morphology was normal as judged by light microscopy in all cases.

Informed consent was obtained from each patient before operation. The ethical aspects of the study were approved by the Ethical Committee of Karolinska Institutet.

Materials

[3-^{14}C]HMG-CoA (sp act 19 mCi/mmol) was obtained from New England Nuclear Corp., Boston, MA, and diluted with unlabeled material (from P-L Biochemicals, Inc., Milwaukee, WI) to yield a specific radioactivity of 5.5 mCi/mmol. DL-[2-^{3}H]mevalonic acid lactone (sp act 125 mCi/mmol) was obtained from Radiochemical Center, Amersham, England. Unlabeled mevalonic acid lactone, EDTA, EGTA, NADP, ATP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, dithiothreitol, PMSF, and leupeptin were purchased from Sigma Chemical Co., St. Louis, MO. *E. coli* alkaline phosphatase, suspended in 2.6 M ammonium sulfate (30–60 units/mg protein) was obtained from Sigma Chemical Co., St. Louis, MO. Just prior to use, the enzyme suspension was centrifuged at 12,000 *g* for 45 min at 4°C. The supernatant was discarded and the pellet was suspended in 20 mM imidazole buffer, pH 7.4, containing 5 mM dithiothreitol. Rabbit antiserum to partially purified HMG-CoA reductase from rat liver was prepared exactly as described (23–25). The antiserum was frozen in aliquots at −20°C until used.

Preparation of liver microsomes

Two aliquots of the liver biopsy (approximately 0.5–1 g) were weighed and placed into 9:1 (v/w) cold 50 mM Tris-HCl buffer, pH 7.4, containing 0.3 M sucrose, 10 mM EDTA, 10 mM dithiothreitol, and 50 mM NaCl or 50 mM NaF, respectively. In some experiments, the liver tissue was divided into four pieces, and homogenates were prepared in both NaCl- and NaF-containing buffers in the presence and absence of protease inhibitors (EGTA, 5.0 mM; PMSF, 1.0 mM; and leupeptin, 50 μM). The liver specimens were homogenized with a Polytron 10S and the homogenate was filtered through two layers of gauze to remove connective tissue. The filtrate was rehomogenized in a Potter-Elvehjem homogenizer equipped with a loose-fitting Teflon pestle. The homogenate was centrifuged at 20,000 *g* for 15 min. The supernatant fluid was centrifuged at 100,000 *g* for 60 min. The microsomal fraction obtained was suspended in the homogenizing medium and recentrifuged at 100,000 *g* for 60 min. The washed microsomal fraction was then suspended in 20 mM imidazole buffer, pH 7.4, containing 5 mM dithiothreitol, to a volume corresponding to that of the 20,000 *g* supernatant fluid.

The protein concentrations of the microsomal fractions were determined by the method of Lowry et al. (26).

Assay of HMG-CoA reductase activity

In the standard assay for HMG-CoA reductase activity, the microsomal fraction (30–100 μg of protein) was preincubated for 15 min at 37°C in a total volume of 0.2 ml containing 100 mM phosphate buffer, pH 7.4, 10 mM imidazole buffer, pH 7.4, 5 mM dithiothreitol, 10 mM EDTA, 3 mM NADP, 12 mM glucose-6-phos-

[2] Calculated as body weight (kg)/(height (cm) − 100) × 100%.

Downloaded from www.jlr.org by guest, on June 18, 2012

phate, and 1 unit of glucose-6-phosphate dehydrogenase. The HMG-CoA reductase assay was then initiated with the addition of 90 nmol of [3-^{14}C]HMG-CoA, 0.5 μCi, dissolved in 25 μl of distilled water, giving a total substrate concentration of 0.4 mM. The incubation was run for 15 min and was stopped by the addition of 25 μl of 6 M HCl. Tritium-labeled mevalonic acid, 0.01 μCi, was added together with 3 mg of unlabeled mevalonic acid lactone and the incubation mixture was allowed to stand overnight at room temperature for complete lactonization. In all experiments, enzyme assays were carried out in duplicate.

In some experiments, microsomes were preincubated with or without 2 mM ATP, and 4 mM $MgCl_2$ for 15 min. In other experiments the microsomes were preincubated in the presence or absence of *E. coli* alkaline phosphatate, 10 units, for 60 min at 37°C before the addition of cofactors and [3-^{14}C]HMG-CoA. In the experiments with antiserum, the microsomes were preincubated with 40 μl of undiluted or diluted rabbit antiserum or pre-immune rabbit serum for 15 min prior to incubation (25).

After lactonization, the incubation mixture was subjected to thin-layer chromatography with benzene–acetone 1:1 (v/v) as the developing solvent. The mevalonic acid lactone zone was located and scraped off into a counting vial. A Packard liquid scintillation spectrometer, Model 3003, was used for determining the radioactivity using Instagel as scintillator liquid. Corrections for losses were made by the internal standard.

RESULTS

The first objective of the present investigation was to determine whether active and inactive forms of HMG-CoA reductase exist in the human liver, and whether activation of enzymatic activity might occur during the preparation of microsomes. When microsomes prepared in the presence of NaF were assayed for HMG-CoA reductase activity, their mean enzymatic activity was only 17 ± 3 (SEM)% of microsomes prepared in the absence of NaF (**Table 1**). Since the actions of both specific and nonspecific phosphatases and kinases are inhibited by NaF-containing buffer (5–11), this implied that a considerable activation of HMG-CoA reductase takes place during the standard microsomal preparation.

That this activation was reversible and probably the consequence of dephosphorylation of the enzyme was demonstrated by the incubation of microsomes (prepared without fluoride) with Mg-ATP. This treatment, which activates kinases phosphorylating HMG-CoA reductase (5–11), resulted in inactivation of HMG-CoA reductase by 67 ± 17% (Table 1). On the other hand, the preincubation of microsomes (prepared in the presence of fluoride) with alkaline phosphatase, which results in dephosphorylation of the enzyme (7), gave a tenfold activation of reductase activity (Table 1). Some activation was also seen when microsomes prepared in the absence of fluoride were treated with alkaline phosphatase (Table 1).

TABLE 1. Relative HMG-CoA reductase activity in human liver microsomes

Source of Enzyme[a]	Relative Enzyme Activity[b]
	%
Microsomes prepared without fluoride (n = 11)	100[c]
Microsomes prepared with fluoride (n = 11)	17 ± 3
Microsomes prepared without fluoride, preincubated with alkaline phosphatase (n = 6)	143 ± 25
Microsomes prepared with fluoride, preincubated with alkaline phosphatase (n = 4)	166 ± 42
Microsomes prepared without fluoride, preincubated with Mg-ATP (n = 4)	33 ± 17

[a] Number of subjects studied within parentheses.
[b] Expressed as percentage of corresponding control incubation of microsomes prepared without fluoride.
[c] Mean absolute activity, 79 ± 15 pmol/min per mg protein.

These in vitro studies are consistent with the concept that microsomal HMG-CoA reductase of human liver, like that of other vertebrates (5–8), exists in an active (probably dephosphorylated) and an inactive (probably phosphorylated) form. These two forms are fully interconvertible in vitro. The activity of microsomes preincubated with alkaline phosphatase would represent the total amount of enzyme present, whereas that observed using microsomes prepared with NaF would represent the "expressed" amount of enzyme. The state of activation can thus be calculated as the ratio between the activity of NaF-prepared microsomes (expressed) and that of phosphatase-treated microsomes (total). This ratio averaged 12 ± 3% in six subjects (Table 1), indicating that, similar to what has been demonstrated in several animal species (4–8), a considerable proportion of HMG-CoA reductase is inactive in the basal situation.

In all previous studies of the human hepatic HMG-CoA reductase, the activity has been assayed using microsomes prepared in the absence of fluoride (12–22). It was, therefore, of major interest to determine whether this standard assay adequately reflects expressed and/or total microsomal HMG-CoA reductase activity. As seen in **Fig. 1**, the activities of microsomes prepared in the absence of fluoride correlated well both with the activities of microsomes prepared with fluoride ($r = +0.80$, n = 11, $P < 0.005$) and with the activities of

JOURNAL OF LIPID RESEARCH ASBMB

Downloaded from www.jlr.org by guest, on June 18, 2012

JOURNAL OF LIPID RESEARCH

ASBMB

Downloaded from www.jlr.org by guest, on June 18, 2012

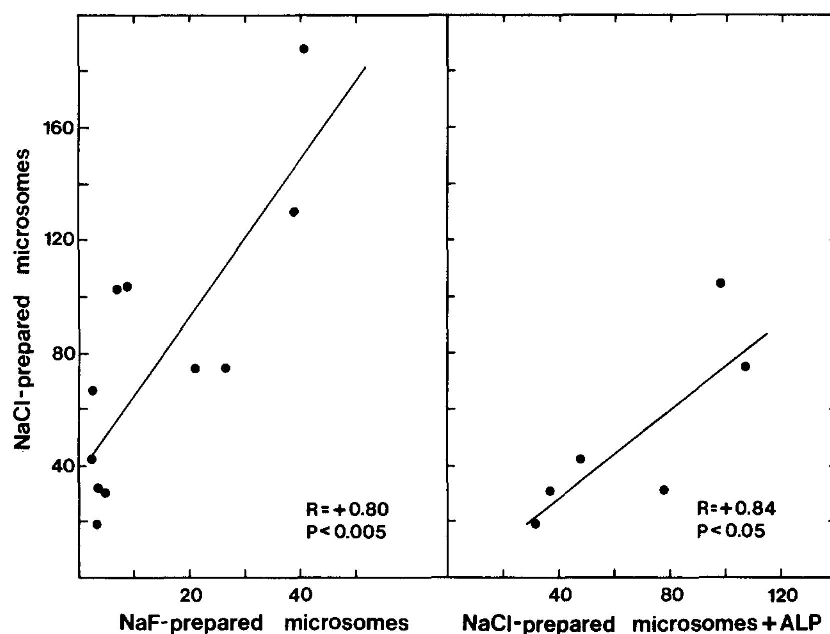

Fig. 1. Relationships between the absolute activity of HMG-CoA reductase (pmol/min per mg protein) in microsomes prepared in the absence (NaCl-prepared) and presence (NaF-prepared) of fluoride, and after preincubation with alkaline phosphatase (NaCl-prepared + ALP). See Methods for experimental details. Data from experiments in 11 and 6 patients, respectively.

the fully activated enzyme ($r = +0.84$, $n = 6$, $P < 0.05$). This also indicates that the activation state of the enzyme is relatively constant in the basal situation, suggesting that the large inter-individual variation of enzymatic activity seen is predominantly the consequence of variation of the amount of enzyme protein.

If variation of enzyme concentration is a major determinant of HMG-CoA reductase activity in human liver microsomes, it would be of great importance for clinical research to develop alternative methods for determining enzyme concentration. Assays based on immunological techniques would be of particular interest. We therefore determined whether antibodies raised against HMG-CoA reductase from rat liver reacted with the human enzyme. As seen in **Table 2,** using rabbit antiserum against partially purified hepatic HMG-CoA reductase from the rat (23), which was highly inhibitory of rat liver microsomal enzyme, we were able to demonstrate a substantial inhibition (72 ± 6%) of the human microsomal enzyme also. No inhibition of activity was seen using pre-immune rabbit serum (Table 2). Maximum inhibition of activity was proportionally constant over a wide range of enzyme activity (**Fig. 2**). Furthermore, quantitative immunotitration was possible using serial dilutions of antiserum; a representative example is shown in **Fig. 3.**

The fact that the polyvalent antiserum used in the present study exhibited a profound inhibition of enzymatic activity indicates that its binding to the microsomal enzyme strongly affects, directly or indirectly, the active site of the HMG-CoA reductase molecule. It is reasonable to assume that the change in enzymatic activity brought about by the reversible in vitro conversion of the enzyme is the consequence of conformational changes affecting the active site. This reasoning prompted us to examine whether the amount of antiserum required to inhibit a certain fraction of the enzyme molecules was different when a high proportion of the enzyme was inactive. As seen in **Fig. 4,** the immunotitration curves were somewhat different for microsomal HMG-CoA reductase prepared in the presence and in the absence of fluoride. With decreasing concentrations of antiserum, proportionally more of the activity was inhibited (at similar

TABLE 2. Inhibition of HMG-CoA reductase activity in human liver microsomes by preincubation with antiserum against partially purified rat liver enzyme

Source of Enzyme	Preincubation	Relative Enzyme Activity
		%
Rat liver microsomes, prepared without fluoride	Buffer	100
	Antiserum	9
Human liver microsomes, prepared without fluoride	Buffer	100
	Antiserum	28 ± 6[a]
	Pre-immune serum	104 ± 4

[a] Mean ± SEM of seven patients.

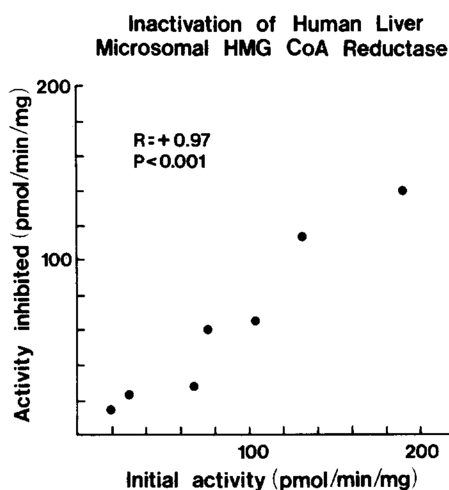

Fig. 2. Relationship between the amount of HMG-CoA reductase activity (pmol/min per mg protein) present and the amount of enzyme inhibited by preincubation with 40 μl of antiserum prior to assay. See Methods for experimental details. Data from experiments in seven patients.

concentrations of enzyme protein) by a specific concentration of antiserum when the state of activation of the enzyme was low. Although not conclusive, this may indicate that the active and inactive forms of the enzyme are antigenically different, and that the antiserum used in this work thus may be somewhat more active against the active form.

Recent studies have demonstrated that the HMG-CoA reductase molecule is very sensitive to cleavage by

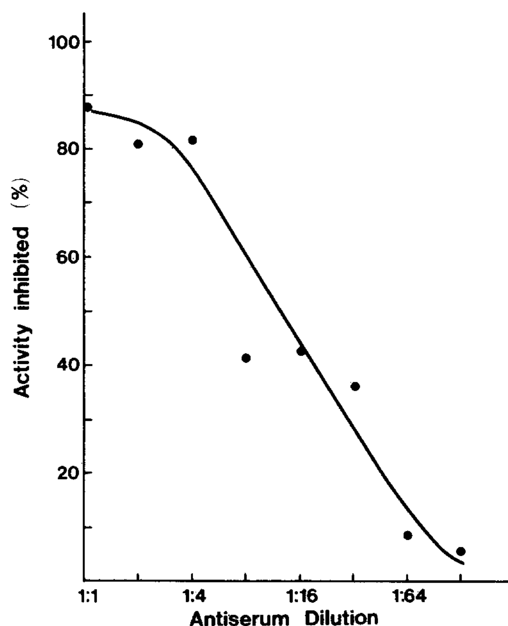

Fig. 3. Immunotitration curve for microsomal HMG-CoA reductase. Microsomes, prepared in the absence of fluoride, were preincubated for 15 min with 40 μl of antiserum, diluted as marked. Uninhibited activity was 130 pmol/min per mg protein.

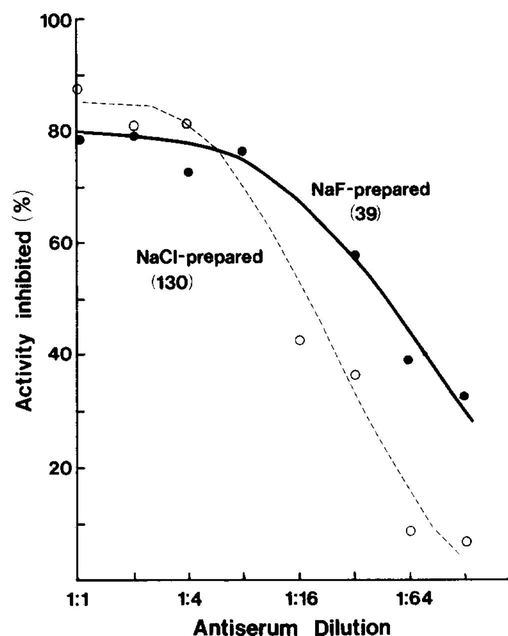

Fig. 4. Immunotitration curves for microsomal HMG-CoA reductase. Equal amounts of microsomes (100 μg/assay), prepared in the absence (NaCl-prepared) or presence (NaF-prepared) of fluoride, were preincubated for 15 min with 40 μl of antiserum, diluted as marked. Uninhibited activity was 130 pmol/min per mg protein for NaCl-prepared, and 39 pmol/min per mg protein for NaF-prepared microsomes.

lysosomal proteases (27–29). In our studies of the first eleven patients, protease inhibitors were not included in the homogenization or preparation media, nor were such inhibitors present during the partial purification of rat liver reductase that yielded the enzyme preparation used for immunization (23). Although the subunits that result from such proteolytic cleavage of the rat enzyme are known to retain enzymatic activity, it was considered of major importance to determine whether the observed effects of activation-inactivation and immunological cross-reactivity are also demonstrable with HMG-CoA reductase of human microsomes prepared in the presence of protease inhibitors. We therefore performed additional studies in three patients, where EGTA, PMSF, and leupeptin were included in the buffers from the excision of the biopsy and onwards. As seen in **Table 3,** the difference between enzymatic activities of microsomes prepared with and without NaF was not influenced by the presence of protease inhibitors. Furthermore, the activation by alkaline phosphatase and the inhibition by antibody to the rat liver enzyme were still present when lysosomal proteases were efficiently inhibited (Table 3).

DISCUSSION

The present study has clearly demonstrated that the human liver contains both active and inactive forms of

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

TABLE 3. Lack of influence of protease inhibitors on properties of HMG-CoA reductase in human liver microsomes[a]

Source of Enzyme	Relative Enzyme Activity	
	Without Protease Inhibitors	With Protease Inhibitors
	%	
Microsomes prepared without fluoride	100 (137)[b]	100 (142)[b]
Microsomes prepared with fluoride	30	25
Microsomes preincubated with alkaline phosphatase	146	153
Microsomes prepared without fluoride, preincubated with preimmune serum[c]	109	108
Microsomes prepared without fluoride, preincubated with antiserum[c]	57	65

[a] Microsomes were prepared with 50 mM NaF or NaCl in the presence or absence of protease inhibitors (EGTA, 5.0 mM; PMSF, 1.0 mM; leupeptin, 50 μM). See Methods for details.

[b] Absolute activity (pmol/min per mg protein) given in parentheses. Data represent means of three experiments (three patients).

[c] Pre-immune rabbit serum and antireductase antiserum were diluted 1:4 in these experiments.

microsomal HMG-CoA reductase in vivo. The in vitro experiments with alkaline phosphatase and Mg-ATP strongly indicate that, similar to what has been found in other species (4–9), the active form of the enzyme is dephosphorylated and the inactive phosphorylated. The (artifactual) activation of the enzyme that occurs during the preparation of the microsomal fraction in the absence of fluoride may give rise to some concern when considering the results of clinical studies on the regulation of HMG-CoA reductase in man. It is therefore of major importance that we were able to demonstrate that the activity of microsomes prepared using the "standard" procedure, i.e., without fluoride, closely reflected both the total and the initially expressed activity. It should be pointed out, however, that this refers to measurements under highly standardized conditions; it is, therefore, still possible that some of the variations in absolute enzymatic activity seen between different investigations may be related to factors such as delayed onset of microsomal preparation, etc.

Even under standardized conditions, a wide range of absolute enzymatic activities is seen in normal subjects (12). The present study has demonstrated that this inter-individual variation is not due to variation of the state of activation of microsomal HMG-CoA reductase. Instead, differences between individuals with regard to the absolute amount of microsomal enzyme protein appear to occur. This is in agreement with studies in the rat, where most if not all of the variation of the enzymatic activity under different physiological conditions appears to be the consequence of variation in the amount of enzyme protein (7, 25, 30–33). It remains to be studied whether the changes in hepatic HMG-CoA reductase activity that have been observed to occur in humans with hypertriglyceridemia (15) or morbid obesity (19) and during treatment with chenodeoxycholic acid (12, 14, 16, 17) or phenobarbital (14) are the consequence of changes in enzymatic activation and/or concentration.

It is reasonable to assume that the region of HMG-CoA reductase containing the active site is relatively similar in different species. In agreement with this concept, antiserum to rat liver reductase exerted considerable inhibitory effect on the human microsomal enzyme also. The finding of cross-reactivity is of major practical importance, because this means that subsequent studies on the regulation of HMG-CoA reductase activity in human liver under different physiological perturbations will be possible with the help of such antisera. The finding of a possible difference in antigenicity of active and inactive forms of HMG-CoA reductase may indicate that modulation of activity is exerted by conformational changes of the enzyme molecule. Conformational changes of rat liver reductase leading to alterations in antigenicity have recently been demonstrated in response to specific inhibitors (34); these authors did not observe any major difference in enzyme antigenicity between active and inactive forms, however. The presence of such a phenomenon with other antisera, as seen in the present study, may explain some of the discrepancies reported with regard to the regulation of HMG-CoA reductase activity (25, 30–34).

Cross-reactivity between human and rat liver HMG-CoA reductase has been reported in a few patients previously (20, 21); the present study represents the first detailed study. The phenomenon of altered antigenicity due to activation/inactivation of HMG-CoA reductase may make physiological measurements of the enzyme less valid; the emergence of monoclonal antibodies against HMG-CoA reductase (35, 36) could provide a solution to this problem. Clearly, further studies of the human enzyme will be of great interest.

The expert technical assistance of Ms. Gunvor Alvelius and Ms. Lisbet Benthin, and the skillful preparation of the manuscript by Ms. Lena Ericsson are gratefully acknowledged. This study was supported by grants from the Swedish Medical Research Council (03X-04793), the Wiberg Foundation, and the Harald and Greta Jeansson Foundation, Dr. Angelin was supported in part by fellowships from the Ernst Klenk Foundation and from the Karolinska Institute.

Manuscript received 19 September 1983.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB
JOURNAL OF LIPID RESEARCH
JLR

REFERENCES

1. Angelin, B., K. Einarsson, L. Liljeqvist, K. Nilsell, and R. Heller. 1981. HMG-CoA reductase in human liver: active and inactive forms and cross-reactivity with antibody against rat liver enzyme. *Circulation,* **64:** IV–270 (abstract).
2. Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen. 1976. Regulation of HMG-CoA reductase. *Adv. Lipid Res.* **14:** 1–74.
3. Brown, M. S., and J. L. Goldstein. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21:** 505–517.
4. Beg, Z. H., D. W. Allman, and D. M. Gibson. 1973. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. *Biochem. Biophys. Res. Commun.* **54:** 1362–1369.
5. Nordstrom, J. L., V. W. Rodwell, and J. J. Mitschelen. 1977. Interconversion of active and inactive forms of rat liver hydroxymethylglutaryl-CoA reductase. *J. Biol. Chem.* **252:** 8924–8934.
6. Gibson, D. M., and T. S. Ingebritsen. 1978. Reversible modulation of liver hydroxymethylglutaryl CoA reductase. *Life Sci.* **23:** 2649–2664.
7. Brown, M. S., J. L. Goldstein, and J. M. Dietschy. 1979. Active and inactive forms of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the liver of the rat. Comparison with the rate of cholesterol synthesis in different physiological states. *J. Biol. Chem.* **254:** 5144–5149.
8. Hunter, C. F., and V. W. Rodwell. 1980. Regulation of vertebrate liver HMG-CoA reductase via reversible modulation of its catalytic activity. *J. Lipid Res.* **21:** 399–405.
9. Beg, Z. H., J. A. Stonik, and H. B. Brewer, Jr. 1978. 3-Hydroxy-3-methylglutaryl coenzyme A reductase: regulation of enzymatic activity by phosphorylation and dephosphorylation. *Proc. Natl. Acad. Sci. USA.* **75:** 3678–3682.
10. Keith, M. L., V. W. Rodwell, D. H. Rogers, and H. Rudney. 1979. In vitro phosphorylation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Analysis of ^{32}P-labeled inactivated enzyme. *Biochem. Biophys. Res. Commun.* **90:** 969–975.
11. Gil, G., M. Sitges, and F. G. Hegardt. 1981. Preparation of highly radioactive homogenous ^{32}P-labeled hydroxymethylglutaryl coenzyme A reductase from rat liver. *Arch. Biochem. Biophys.* **210:** 224–229.
12. Ahlberg, J., B Angelin, and K. Einarsson. 1981. Hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and biliary lipid composition in man: relation to cholesterol gallstone disease and effects of cholic acid and chenodeoxycholic acid treatment. *J. Lipid Res.* **22:** 410–422.
13. Salen, G., G. Nicolau, S. Shefer, and E. H. Mosbach. 1975. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology.* **69:** 676–684.
14. Coyne, M. J., G. G. Bonorris, L. I. Goldstein, and L. J. Schoenfield. 1976. Effects of chenodeoxycholic acid and phenobarbital on the rate-limiting enzymes of hepatic cholesterol and bile acid synthesis in patients with gallstones. *J. Lab. Clin. Med.* **87:** 281–291.
15. Ahlberg, J., B. Angelin, I., Björkhem, K., Einarsson and B. Leijd. 1979. Hepatic cholesterol metabolism in normo- and hyperlipidemic patients with cholesterol gallstones. *J. Lipid Res.* **20:** 107–115.
16. Carulli, N., M. Ponz de Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria. 1980. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short term feeding of chenodeoxycholic and ursodeoxycholic acid. *J. Lipid Res.* **21:** 35–43.
17. Maton, P. N., H. J. Ellis, M. J. P. Higgins, and R. H. Dowling. 1980. Hepatic HMG CoA reductase in human cholelithiasis: effects of chenodeoxycholic and ursodeoxycholic acids. *Eur. J. Clin. Invest.* **10:** 325–332.
18. Balasubramaniam, S., K. A. Mitropoulos, S. Venkatesan, N. B. Myant, T. J. Peters, A. Postiglione, and M. Mancini. 1981. Analytical fractionation of human liver microsomal fractions: localization of cholesterol and of the enzymes relevant to its metabolism. *Clin. Sci.* **60:** 435–439.
19. Angelin, B., L. Backman, K. Einarsson, L. Eriksson, and S. Ewerth. 1982. Hepatic cholesterol metabolism in obesity: activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Lipid Res.* **23:** 770–773.
20. Tanaka, R. D., P. A. Edwards, S-F. H. Lan, E. M. Knöppel, and A. M. Fogelman. 1982. Purification of 3-hydroxy-3-methylglutaryl coenzyme A reductase from human liver. *J. Lipid Res.* **23:** 523–530.
21. Beg, Z. H., J. A. Stonik, and H. B. Brewer, Jr. 1982. Isolation and immunological characterization of 3-hydroxy-3-methylglutaryl coenzyme A reductase from human liver. *Biochem. Biophys. Res. Commun.* **107:** 1013–1020.
22. Angelin, B., S. Ewerth, and K. Einarsson, 1983. Ursodeoxycholic acid treatment in cholesterol gallstone disease: effects on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, biliary lipid composition, and plasma lipid levels. *J. Lipid Res.* **24:** 461–468.
23. Heller, R. A., and M. A. Shrewsbury. 1976. 3-Hydroxy-3-methylglutaryl coenzyme A reductase from rat liver. Its purification, properties and immunochemical studies. *J. Biol. Chem.* **251:** 3815–3822.
24. Beirne, O. R., R. Heller, and J. A. Watson, 1977. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in minimal deviation hepatoma 7288 C. Immunological measurements in hepatoma tissue culture cells. *J. Biol. Chem.* **252:** 950–954.
25. Hardgrave, J. E., R. A. Heller, M. G. Herrera, and T. J. Scallen. 1979. Immunotitration of 3-hydroxy-3-methylglutaryl coenzyme A reductase in various physiological states. *Proc. Natl. Acad. Sci. USA.* **76:** 3834–3838.
26. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
27. Ness, G. C., S. C. Way, and P. S. Wickham. 1981. Proteinase involvement in the solubilization of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Biochem. Biophys. Res. Commun.* **102:** 81–85.
28. Chin, D. J., K. L. Luskey, J. R. Faust, R. J. MacDonald, M. S. Brown, and J. L. Goldstein. 1982. Molecular cloning of 3-hydroxy-3-methylglutaryl coenzyme A reductase and evidence for regulation of its mRNA. *Proc. Natl. Acad. Sci. USA.* **79:** 7704–7708.
29. Kennelly, P. J., K. G. Brandt, and V. W. Rodwell. 1983. 3-Hydroxy-3-methylglutaryl-CoA reductase: solubilization in the presence of proteolytic inhibitors, partial purification, and reversible phosphorylation-dephosphorylation. *Biochemistry.* **22:** 2784–2788.
30. Edwards, P. A., D. Lemongello, J. Kane, I. Shechter, and A. M. Fogelman. 1980. Properties of purified rat hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and

Downloaded from www.jlr.org by guest, on June 18, 2012

regulation of enzyme activity. *J. Biol. Chem.* **255:** 3715–3725.
31. Kleinsek, D. A., A. M. Jabalquinto, and J. W. Porter. 1980. In vivo and in vitro mechanisms regulating rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase activity. *J. Biol. Chem.* **255:** 3918–3923.
32. Jenke, H. S., M. Löwel, and J. Berndt. 1981. In vivo effect of cholesterol feeding on the short term regulation of hepatic hydroxymethylglutaryl coenzyme A reductase during the diurnal cycle. *J. Biol. Chem.* **256:** 9622–9625.
33. Dugan, R. E., T. A. Baker, and J. W. Porter, 1982. Regulation of short-term changes in hepatic β-hydroxy-β-methylglutaryl-CoA reductase activity. *Eur. J. Biochem.* **125:** 497–503.
34. Rogers, D. H., and H. Rudney. 1982. Modification of 3-hydroxy-3-methylglutaryl coenzyme A reductase immunoinhibition curves by substrates and inhibitors. Evidence for conformational changes leading to alterations in antigenicity. *J. Biol. Chem.* **257:** 10650–10658.
35. Heller, R. A., P. Hoy, and P. P. Jones. 1982. Development of monoclonal antibodies to 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Biochem. Biophys. Res. Commun.* **106:** 412–421.
36. Clark, R. E., G. G. Martin, M. C. Barton, and D. J. Shapiro. 1982. Production and characterization of monoclonal antibodies to rat liver microsomal 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Proc. Natl. Acad. Sci. USA.* **79:** 3734–3738.

ASBMB JOURNAL OF LIPID RESEARCH JLR

Downloaded from www.jlr.org by guest, on June 18, 2012