*Proc. Natl. Acad. Sci. USA*
Vol. 80, pp. 1516–1520, March 1983
Biochemistry

# Overproduction of a $M_r$ 92,000 protomer of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in compactin-resistant C100 cells

(undegraded protomer/regulation of cholesterol synthesis)

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*Communicated by Robert T. Schimke, December 6, 1982*

**ABSTRACT** **We describe a cell line, designated C100, that displays a 100-fold increase in the major regulatory enzyme of the cholesterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA; mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34]. Immunoprecipitation of [$^{35}$S]methionine-labeled enzyme from C100 microsomal membranes prepared in the presence of the protease inhibitors phenylmethylsulfonyl fluoride and leupeptin revealed two up regulated proteins: a major band of $M_r$ 92,000 and a minor band of $M_r$ 63,000. We conclude that the $M_r$ 92,000 protein is probably the intact form of HMG-CoA reductase protomer based on the following criteria. (*i*) It is a highly up regulated microsomal membrane protein that coincides with the increase in HMG-CoA reductase specific activity in this cell line. (*ii*) It is recognized by a specific HMG-CoA reductase antiserum under a variety of stringencies. (*iii*) Isolation and solubilization of [$^{35}$S]methionine-labeled C100 microsomal membranes in the absence of protease inhibitors resulted in the disappearance of the $M_r$ 92,000 protein and the appearance of two proteins of $M_r$ 52,000 and 38,000. (*iv*) Analysis of cells labeled for 30 min with [$^{35}$S]methionine, well under the half-life of HMG-CoA reductase, revealed only the $M_r$ 92,000 protein to be present in total cell extract. (*v*) The previously reported single immunoprecipitation polypeptide for HMG-CoA reductase of $M_r$ 62,000 [Chin, D. J., Luskey, K. L., Anderson, R. G. W., Faust, J. R., Goldstein, J. L. & Brown, M. S. (1982) *Proc. Natl. Acad. Sci. USA* 79, 1185–1189] can be isolated and appears to be the result of both proteolysis and sample preparation for NaDodSO$_4$ gel electrophoresis. Analysis of C100 cells labeled with [$^{35}$S]methionine for 24 hr indicates that the predominant steady-state form of the enzyme is the $M_r$ 92,000, rather than the $M_r$ 63,000, protein, further suggesting that the two proteins do not have a classical precursor–product relationship.**

3-Hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA; mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] has been a focus of interest because it is the key regulatory enzyme in the cholesterol biosynthetic pathway and the branch pathway that results in nonsterol products such as dolichol, ubiquinone, and isopentenyladenosine (1–7). Located in the endoplasmic reticulum, HMG-CoA reductase usually constitutes <0.01% of total cellular protein (8). However, the enzymatic level within the cell is highly dependent on the presence or absence of various regulatory agents in the growth medium, including plasma low density lipoprotein, oxygenated sterols, free cholesterol, hormones, and pathway intermediates (1, 7, 9–11). Although the precise mechanisms are not well defined, a number of studies indicate that regulation occurs at the level of enzyme modification via phosphorylation–dephosphorylation (7, 11–17) and through changes in the rates of synthesis or degradation (1, 7, 18–23).

The availability of the fungal metabolite compactin, a potent competitive inhibitor of the enzyme (24, 25), has enabled us to select cell lines that overproduce HMG-CoA reductase (26). In cell lines reported so far, the modes of regulation and the biochemical properties of the enzyme appear to remain intact after selection for compactin resistance (26, 27). These cell lines should prove valuable in defining how the various regulatory agents control the level of HMG-CoA reductase within the cell.

Recently, the enzyme has been shown to be susceptible to proteolysis when solubilized in the absence of protease inhibitors (27, 28). To accurately monitor the regulation of the enzyme, it is necessary to be able to distinguish the intact enzyme from its proteolysis products. Further, it is critical to discriminate between the cleavage products that result from manipulation of the sample and those that result from *in vivo* processing. Clearly, the task of defining various forms of the enzyme would be facilitated with an overproducing cell line.

In this paper, we describe a cell line, designated C100, that overproduces HMG-CoA reductase 100-fold and displays the stringent regulation via serum cholesterol characteristic of the parental cell line. In the process of characterizing the C100 cells, we developed a straightforward and highly reproducible protocol for solubilization of the microsomal enzyme. Most importantly, this cell line has enabled us to identify a $M_r$ 92,000 protomer of HMG-CoA reductase. In addition, we suggest that the previously reported $M_r$ values of the enzyme [62,000 (28) and 53,000 (22)] are artifacts of sample preparation for NaDodSO$_4$ gel electrophoresis and proteolysis during enzyme isolation.

## MATERIALS AND METHODS

**Materials.** L-[$^{35}$S]Methionine (1,200 Ci/mmol; 1 Ci = 37 GBq) and DL-3-hydroxy-3-methyl[3-$^{14}$C]glutaryl-coenzyme A (26.2 mCi/mmol) were obtained from New England Nuclear. DL-3-Hydroxy-3-methylglutaryl-coenzyme A was purchased from P-L Biochemicals. All other chemicals were of highest quality from commercial sources. Compactin was provided by Akiro Endo.

**Cell Maintenance and Selection for Compactin Resistance.** C13/SV28 cells (a simian virus 40-transformed baby hamster kidney cell line) were provided by George Stark (Department of Biochemistry, Stanford University School of Medicine). C13/SV28 cells were maintained as monolayers in minimal essential medium (ME medium; GIBCO) supplemented with nonessen-

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Abbreviations: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; PhMeSO$_2$F, phenylmethylsulfonyl fluoride; ME medium, minimal essential medium; MVA, mevalonic acid.

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tial amino acids and 5% delipidated fetal calf serum (29). Inoculation conditions were as follows: $4 \times 10^5$ cells per 75-cm$^2$ flask (4 ml), $10^6$ cells per 75-cm$^2$ flask (10 ml), $2 \times 10^6$ cells per 150-cm$^2$ dish (20 ml), $12 \times 10^6$ cells per 850-cm$^2$ roller bottle (100 ml). Fresh medium was added every 2 days.

Initially, $10^6$ cells were exposed to 2 $\mu$M compactin in the growth medium. Those cells surviving grew to confluency and were trypsinized and seeded into 75-cm$^2$ flasks. This procedure was repeated with 5, 11, 22, 56, 113, and 225 $\mu$M compactin. The last selection step resulted in three colonies, one of which was designated C100. This cell line has been maintained in the presence of 225 $\mu$M compactin since March 1981.

**Extract Preparation and Assay of HMG-CoA Reductase.** Cell extracts for enzyme assays were prepared as described by Ryan *et al.* (26), protein concentrations were determined by a modification (30) of the procedure of Lowry *et al.* (31). HMG-CoA reductase assays were conducted by the method of Shapiro *et al.* (32).

**Microsome Preparation for Solubilization of HMG-CoA Reductase.** Cell monolayers in ten 850-cm$^2$ roller bottles ($\approx 7 \times 10^8$ cells) were first washed and then harvested by scraping in ice-cold Dulbecco's phosphate-buffered saline (without $Ca^{2+}$ or $Mg^{2+}$). All subsequent manipulations were at 0–4°C and all solutions contained 1 mM phenylmethylsulfonyl fluoride (PhMeSO$_2$F)/100 $\mu$M leupeptin. Extract preparation and subcellular fractionation were carried out by the procedure of Fries and Rothman (33). The postnuclear supernatant was centrifuged at 100,000 × *g* for 60 min. The resulting microsomal membrane pellet was suspended in 5 ml of buffer A (40 mM potassium phosphate, pH 7.0/100 mM sucrose/50 mM KCl/4 mM EDTA/10 mM dithiothreitol/1 mM PhMeSO$_2$F/100 $\mu$M leupeptin) using a Potter–Elvejham homogenizer with motor-driven pestle. Five milliliters of buffer B (buffer A/1 M KCl) was added slowly with homogenization and the extract was sonicated with two 5-sec bursts with a probe sonicator. The sonicate was centrifuged at 100,000 × *g* for 60 min, the pellet was resuspended, and the procedure was repeated with buffer C [buffer A/buffer B (1:1)/1% Triton X-100]. The extract was centrifuged at 100,000 × *g* for 3 hr, and the supernatant was frozen and stored in liquid $N_2$.

**[$^{35}$S]Methionine Labeling and Extract Preparation.** Cells were inoculated in 150-cm$^2$ tissue culture dishes or 850-cm$^2$ roller bottles. Inoculation densities were adjusted as follows so that each cell type would be ≈75% confluent after 6 days: cells grown in the presence of 225 $\mu$M compactin [C100(+) cells], $2 \times 10^6$/dish; cells grown in the absence of compactin [C100(−) cells], $1.2 \times 10^6$/dish. Cells were given fresh medium 24 hr prior to labeling. Labeling commenced when monolayers were switched to growth medium containing [$^{35}$S]methionine at 25 $\mu$Ci/ml (5 ml per 150-cm$^2$ dish, 25 ml per 850-cm$^2$ roller bottle) and continued as indicated. Incorporation was stopped by washing the monolayers with ice-cold phosphate-buffered saline/2 mM unlabeled methionine. Subsequent steps were performed at 0–4°C. Total cell extracts were prepared by lysing the monolayers with 1 ml of buffer C, harvesting the lysate, and sonicating with two 5-sec bursts. The extract was centrifuged at 12,500 × *g* for 5 min, and the supernatant was frozen in liquid $N_2$. Microsomal membranes were prepared and solubilized as described above. Also, we have lysed the cells and solubilized HMG-CoA reductase in hypotonic buffer (10 mM sodium phosphate, pH 7.0/1 mM EDTA/10 mM dithiothreitol/0.1 mM leupeptin/1 mM PhMeSO$_2$F) and observed the same results.

**Immunoprecipitations.** Comparisons of C100(+) and C100(−) extracts were made by immunoprecipitating equivalent amounts of protein. Samples were diluted to a final volume of 100 $\mu$l with buffer D (10 mM sodium phosphate, pH 7.0/0.5 M NaCl/0.5% Nonidet P-40/2 mM methionine/10 mM dithiothreitol/1 mM PhMeSO$_2$F/100 $\mu$M leupeptin) containing ovalbumin at 1 mg/ml, treated with 10 $\mu$l of a 1:10 dilution of antiserum or nonimmune serum in buffer D, and incubated at 24°C for 30 min. This was followed by the addition of 50 $\mu$l of 10% (wt/vol) IgGsorb (Enzyme Center, Boston) in buffer C and further incubation at 24°C for 15 min. The immunoprecipitates were centrifuged at 12,500 × *g* for 5 min, and the pellets were washed three times with buffer D/0.1% NaDodSO$_4$ as described by Jones (34). The final pellets were suspended in 30 $\mu$l of electrophoresis sample buffer [62.5 mM Tris·HCl, pH 6.8/2.3% NaDodSO$_4$/5.0% 2-mercaptoethanol/10% (wt/vol) glycerol] with sonication. Samples were incubated at 37°C for 5 min prior to gel electrophoresis unless otherwise indicated.

Immunoprecipitation parameters were varied extensively as suggested by Jones (34); the parameters included pH, salt concentration, time, temperature, and detergent type and concentration. All of these variations gave the same results.

**NaDodSO$_4$/polyacrylamide Gel Electrophoresis.** Acrylamide (10%) gels were run by the procedure of Jones (35) and fluorographed by the method of Laskey (36).

## RESULTS

**Levels of HMG-CoA Reductase in the C100 Cell Line.** We have derived a cell line, C100, that is resistant to the presence of 225 $\mu$M compactin in the growth medium. The cell line has expressed a stable phenotype of a 100-fold increase in HMG-CoA reductase activity (Table 1) for 18 months when maintained in medium containing delipidated serum and 225 $\mu$M compactin. Under these conditions, C100 cells have a doubling time of 29 hr compared with the parental cell value of 18 hr. In addition, C100 cells have been grown in the absence of compactin for as long as 6 months and, during this time, they expressed the phenotype typical of the parental cell line in regard to HMG-CoA

Table 1. HMG-CoA reductase activity in C13/SV28 and C100 cells grown in various media

| Cell line | Addition to culture medium | Incubation medium | HMG-CoA reductase activity |
|---|---|---|---|
| C13/SV28 | | ME medium/delipidated fetal bovine serum | 0.15 ± 0.05 (10) |
| | | ME medium/delipidated fetal bovine serum/22.5 $\mu$M compactin* | 1.4 ± 0.13 (5) |
| | | ME medium/fetal bovine serum | 0.025 ± 0.01 (5) |
| C100 | | ME medium/delipidated fetal bovine serum | 0.20 ± 0.11 (10) |
| | 225 $\mu$M compactin | ME medium/delipidated fetal bovine serum/225 $\mu$M compactin | 17.0 ± 5.0 (10) |
| | | ME medium/fetal bovine serum | 0.04 ± 0.01 (5) |
| | 225 $\mu$M compactin | ME medium/fetal bovine serum/225 $\mu$M compactin | 2.3 ± 1.3 (5) |

Cells were seeded into 25-cm$^2$ flasks in ME medium/5% delipidated fetal bovine serum (culture medium) with or without 225 $\mu$M compactin. On day 5, culture medium was changed as indicated and, 24 hr later, cells were harvested. Reductase activity is expressed as (nmol of mevalonic acid formed/min)/mg; results are mean ± SD. Numbers in parentheses are numbers of experiments.
*Maximum concentration in which C13/SV28 cells could be maintained for 24 hr and remain viable.

Table 2. Solubilization of HMG-CoA reductase activity from C100 membranes

| Step | % total protein | | % original activity | | Specific activity | |
|---|---|---|---|---|---|---|
| | Sup. | Pellet | Sup. | Pellet | Sup. | Pellet |
| 100,000 × *g* | 60 | 40 | 0 | 100 | 0 ± 0.01 | 54 ± 15 |
| 0.5 M KCl wash | 20 | 21 | 3 | 98 | 2 ± 1.5 | 110 ± 36 |
| 0.5 M KCl/1% Triton X-100 | 13 | 9 | 89 | 12 | 220 ± 75 | 14 ± 7 |

Specific activity is expressed as (nmol of MVA formed/min)/mg; results are mean ± SD for an average of six determinations. HMG-CoA reductase specific activity of whole cell extracts was 17 (nmol of MVA formed/min)/mg. C100 cells were seeded into ten 850-$cm^2$ roller bottles at a density of 12 × $10^6$ cells per bottle in 100 ml of ME medium/5% delipidated bovine serum/225 μM compactin and harvested on day 6. Sup., supernatant.

reductase activity and doubling time. However, when 225 μM compactin was reintroduced in the growth medium, HMG-CoA reductase activity increased to its former 100-fold elevated level within 5 days. There is a 12-fold difference between the specific activity of the enzyme in C100(+) cells and the maximum specific activity induced in parental, C13/SV28, cells after incubation in compactin-containing medium (Table 1). In all three cell types, C100(+), C100(−), and C13/SV28, the half-life of the enzyme is 2–4 hr, as determined by cycloheximide arrest of protein synthesis (data not shown).

**Regulation of HMG-CoA Reductase Activity.** In Table 1, we show that HMG-CoA reductase in the C100 variant cell line is subject to regulation by whole serum, both in the presence and absence of 225 μM compactin. The parental cells exhibit an ≈80% decrease in enzymatic activity when fetal bovine serum is substituted for delipidated fetal bovine serum in the growth medium. A similar decrease is seen when C100 cells are grown in delipidated fetal bovine serum without compactin for 5 days and then switched to medium containing fetal bovine serum. C100 cells maintained in delipidated fetal bovine serum/compactin and then grown for 24 hr in fetal bovine serum/compactin display an 85% decrease in HMG-CoA reductase specific activity. We have not determined whether the enzymatic activity returns to the parental level with prolonged growth in fetal bovine serum/225 μM compactin or whether a higher basal level is maintained in the C100 cell line.

**Solubilization of HMG-CoA Reductase.** To examine HMG-CoA reductase further in the C100 cells, we attempted to solubilize the hamster microsomal enzyme by the freeze-thaw technique used routinely to solubilize the rat liver enzyme (37). However, we found that the enzymatic activity consistently remained membrane bound (data not shown). Therefore, we developed a detergent-based solubilization scheme for HMG-CoA reductase that is straightforward and reproducible for both C100 and hamster liver microsomes (Table 2). Proteolysis is minimized by carrying out each step at 0–4°C and including 1 mM $PhMeSO_2F$ and 100 μM leupeptin in the microsomal preparation and solubilization buffers. Routinely, we obtained an ≈13-fold increase in specific activity between whole cell extracts and solubilized microsomes and a 4-fold increase between microsomes and soluble enzyme. Use of Triton X-100 alone, without 0.5 M KCl, results in solubilization of only 50–60% of the enzymatic activity. The enzyme is not solubilized by sonication in the presence of 0.5 M KCl, which suggests that HMG-CoA reductase is not a peripheral membrane protein.

**Immunoprecipitation of HMG-CoA Reductase.** To examine the relative amounts of HMG-CoA reductase in the various cell types we used an antiserum raised against the purified $M_r$ 52,000 fragment of the rat liver enzyme previously described (21). Although several groups have reported that antiserum to the rat liver enzyme recognizes the hamster enzyme, we wanted to determine the degree of crossreactivity with enzyme solubilized by our procedure. Therefore, we titrated solubilized C100 HMG-CoA reductase with the rabbit antiserum. As shown in Fig. 1, with a starting specific activity of 200 [nmol of mevalonic acid (MVA)/min]/mg, ≈50% of the activity was removed by a 1:100 dilution of antiserum. Ten percent of the original activity remained when undiluted antiserum was used. We concluded that the crossreactivity between the antiserum raised against the rat liver enzyme was sufficiently stringent to further analyze the soluble C100 enzyme.

We wanted to determine whether the amount of HMG-CoA reductase protein in C100(+) and C100(−) cells corresponded to the 100-fold difference in specific activity. We labeled both types of cells for 1 hr with [$^{35}$S]methionine and immunoprecipitated the soluble enzyme. Analysis of the precipitated radiolabeled protein showed that 20–100 times more protein was immunoprecipitated from C100(+) cells than from C100(−) cells, thus demonstrating that the increase in activity is indeed due to an increased amount of enzyme (data not shown).

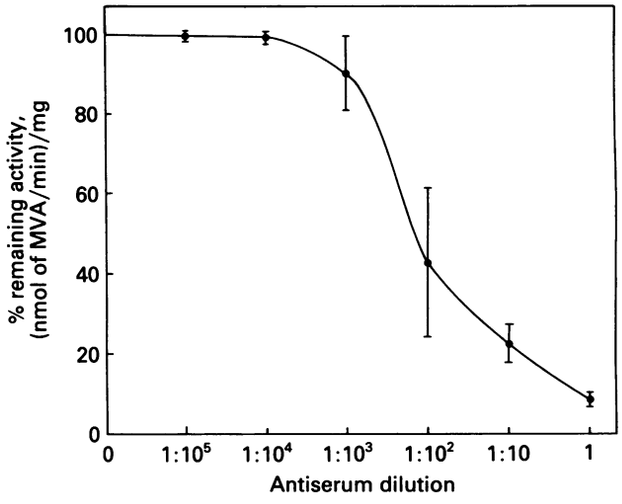


FIG. 1. Crossreactivity of rat HMG-CoA reductase antiserum with enzyme from C100 cells. C100(+) cells were harvested from ten 850-$cm^2$ roller bottles after 6 days of growth and subjected to subcellular fractionation, and the enzyme was solubilized. The starting specific activity of the enzyme was 200 (nmol of MVA/min)/mg. An aliquot of soluble enzyme (≈40 μg/ml) was diluted 1:4 with buffer C and rabbit antiserum (21) was diluted as indicated with buffer C. Immunoprecipitations were carried out as follows. Twenty-five microliters of antiserum was added to 100 μl of diluted soluble enzyme and the mixture was incubated at 24°C for 30 min. Then, 50 μl of 10% (wt/vol) IgGsorb in buffer C was added and incubation was continued at 24°C for 15 min. The IgGsorb was pelleted at 12,500 × *g* for 10 min at 4°C. Twenty-five microliters of supernatant was assayed for HMG-CoA reductase activity. Two control immunoprecipitations showed that neither incubation with IgGsorb nor treatment at 24°C inhibited HMG-CoA reductase activity.

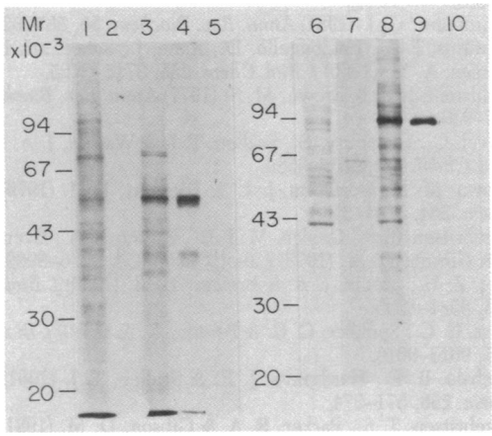


FIG. 2. $NaDodSO_4$ gel electrophoresis analysis of C100 microsomes prepared with and without $PhMeSO_2F$ and leupeptin. C100(+) and C100(−) cells in 850-$cm^2$ roller bottles were maintained in ME medium/5% delipidated fetal bovine serum with or without 225 μM compactin. After 6 days of growth, [$^{35}$S]methionine (25 μCi/ml) was added to each bottle in 25 ml of growth medium. One hour later, the labeled cells were harvested and processed with (lanes 6–10) or without (lanes 1–5) $PhMeSO_2F$ and leupeptin in the microsomal isolation and solubilization buffers. Lanes: 1 and 6, total C100(−) cell extract; 2 and 7, C100(−) extract together with HMG-CoA reductase antiserum; 3 and 8, total C100(+) cell extract; 4 and 9, C100(+) extract together with HMG-CoA reductase antiserum; 5 and 10, C100(+) extract together with nonimmune rabbit serum. Gels were fluorographed for 2 days at −70°C except for lane 7, which was exposed for 8 days. $^{35}$S radioactivity applied: lanes 2, 5, 7, and 10, not detectable; lane 4, 6,050 dpm; lanes 9 and 5, 300 dpm.

Initially, we isolated, solubilized, and immunoprecipitated the enzyme in the absence of protease inhibitors. When we analyzed these products by gel analysis (Fig. 2, lane 4), we found proteins of $M_r$ 52,000 and 38,000 that were recognized specifically by the antiserum and highly upregulated in the C100(+) cells compared with the C100(−) cells. However, the observation of Ness *et al.* (27) that rat liver HMG-CoA reductase is susceptible to proteolysis prompted us to include $PhMeSO_2F$ and leupeptin in the microsomal isolation, solubilization, and immunoprecipitation buffers. Immunoprecipitation of enzyme prepared under these conditions revealed a major protein of $M_r$ 92,000 and a minor protein of $M_r$ 63,000 present in C100(+) cells and in very reduced amounts in C100(−) cells (Fig. 2, lanes 9 and 7, respectively). Clearly, most of this radiolabeled protein is accounted for in the C100(+) cells by the $M_r$ 92,000 protein. On the other hand, the $M_r$ 63,000 protein appears the more prominent of the two in C100(−) cells. The significance of this latter observation is unclear.

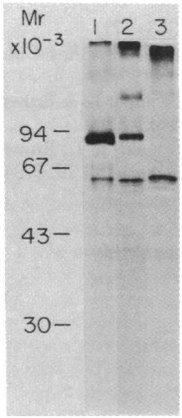


FIG. 3. Effect of heating the $M_r$ 92,000 protomer of HMG-CoA reductase at 90°C. [$^{35}$S] Methionine-labeled C100(+) microsomes were prepared, solubilized, and immunoprecipitated. Lanes: 1, C100(+) extract together with HMG-CoA antiserum was heated at 37°C for 5 min in $NaDodSO_4$ gel sample buffer; 2, C100(+) extract together with HMG-CoA reductase antiserum was heated at 90°C for 1.5 min; 3, C100(+) extract together with HMG-CoA reductase antiserum was heated at 90°C for 3 min. The gel was fluorographed for 8 days at −70°C. $^{35}$S radioactivity applied: lane 1, 5,280 dpm; lane 2, 5,778 dpm; lane 3, 7,813 dpm.

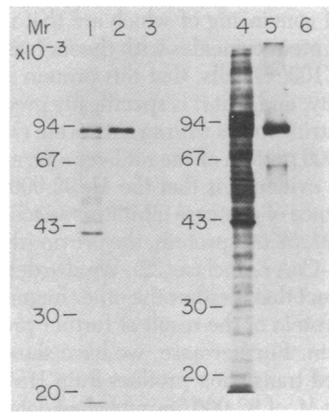


FIG. 4. $NaDodSO_4$ gel electrophoresis of total cell extracts and immunoprecipitates from C100 cells labeled for 30 min or 24 hr with [$^{35}$S] methionine. C100 cells were grown in 150-$cm^2$ dishes in ME medium/5% delipidated fetal bovine serum/225 μM compactin. On day 6, 5 ml of growth medium containing [$^{35}$S]methionine (25 μCi/ml) was added to each dish. Thirty minutes (lanes 1–3) or 24 hr (lanes 4–6) later, cells were harvested and immunoprecipitated. Lanes: 1 and 4, C100 total cell extract; 2 and 5, C100 total cell extract together with HMG-CoA reductase antiserum; 3 and 6, C100 total cell extract together with nonimmune rabbit serum. The gel was fluorographed for 2 days at −70°C. $^{35}$S radioactivity applied: lane 2, 3,220 dpm; lane 5, 5,218 dpm.

Using a similar antiserum, Chin *et al.* (27) recently identified a single $M_r$ 62,000 [$^{35}$S]methionine-labeled polypeptide from a compactin-resistant Chinese hamster ovary-derived cell line that overproduces HMG-CoA reductase. We found that we also could detect a single band of $M_r$ 63,000 if we heated the immunoprecipitate in gel sample buffer for 3 min at 90°C, in preparation for $NaDodSO_4$ gel electrophoresis as done by Chin *et al.* (27) (Fig. 3, lane 3). Also, we observed that the relative amounts of the $M_r$ 92,000 and 63,000 proteins immunoprecipitated from C100(+) enzyme changed with increasing time at 90°C (Fig. 3, compare lanes 1, 2, and 3). The $M_r$ 92,000 species gradually disappeared; however, there was not a corresponding increase in intensity of the $M_r$ 63,000 band. Instead, labeled material appeared at the top of the gel, indicative of aggregated protein. In addition, there seems to be a small amount of a species formed with an apparent $M_r$ 170,000. The origin and significance of this band is not clear.

To further investigate the relationship between the $M_r$ 92,000 and 63,000 proteins recognized by the HMG-CoA reductase antiserum, we compared the relative amounts of these proteins in total cell extracts of C100(+) cells labeled with [$^{35}$S]methionine for 30 min and 24 hr. We chose to compare total cell extracts rather than membrane preparations to minimize manipulations prior to analysis. The 30-min labeling showed that [$^{35}$S]methionine is incorporated rapidly into the $M_r$ 92,000 protein (Fig. 4, lane 1). Immunoprecipitation of the total cell extract revealed that, of the two proteins, only the $M_r$ 92,000 one was synthesized within a relatively short labeling time (lane 2). However, both proteins were present when C100(+) cells were grown in [$^{35}$S]methionine-containing medium for 24 hr (lane 5). Clearly, the steady-state concentration of the $M_r$ 92,000 protein is much greater than that of the $M_r$ 63,000 protein. This result suggests that the relationship between the two proteins is not a simple precursor–product one.

## DISCUSSION

We propose that the $M_r$ 92,000 protein detected in C100(+) cell extracts and microsomes is the intact protomer of HMG-CoA reductase. Our conclusion is supported by several lines of evi-

dence, the most convincing of which are that upregulation of the $M_r$ 92,000 protein coincides with the increase in enzymatic activity in the C100(+) cells, that this protein solubilizes with enzymatic activity, and that it is specifically precipitated under a wide range of stringencies with an antiserum raised against the purified $M_r$ 52,000 protein of the rat liver enzyme. Perhaps the most conclusive evidence is that the $M_r$ 92,000 protein disappears in the absence of protease inhibitors concomitant with appearance of the $M_r$ 52,000 protein, the previously reported subunit $M_r$ of HMG-CoA reductase (22). We also detect a $M_r$ 38,000 proteolysis product that is either the other fragment comprising the $M_r$ 92,000 protein or the result of further proteolysis of the $M_r$ 52,000 protein. Furthermore, we have shown that the immunoprecipitated translation product from HMG-CoA reductase mRNA has a $M_r$ of 92,000 (unpublished data). This reduces the possibility that the $M_r$ 92,000 protomer reported here is itself a degradation product, although that remains a possibility since we (Fig. 3, lane 2) and others (22, 23, 38) have noted higher molecular weight species in antireductase immunoprecipitates.

The susceptibility of HMG-CoA reductase to proteolysis has been noted by others (27, 28). Since we could not detect significant steady-state amounts of either the $M_r$ 52,000 or 38,000 proteolysis products (Fig. 4, lane 4), we propose that the enzyme is exposed to proteases during solubilization that do not participate in physiological *in vivo* processing or degradation of HMG-CoA reductase. It is possible that the $M_r$ 63,000 protein may be a product of *in vivo* processing or the normal degradation process. Our data suggest that the relationship between these two proteins is not one of simple physiological precursor–product. This is evident from the relative amounts of the $M_r$ 92,000 and 63,000 proteins after a 24-hr labeling of C100(+) cells (Fig. 4, lane 5). In C100(−) cells, which display the parental cell level of enzymatic activity, not only do the absolute levels of the two diminish in the absence of compactin, but the relative amounts also change (Fig. 2, compare lanes 7 and 9). Clearly, it is important to examine the structural and temporal relationships between these two proteins to determine whether or not they are distinct forms of HMG-CoA reductase or whether the $M_r$ 63,000 protein is simply a nonphysiological degradation product of the $M_r$ 92,000 protomer. The various cell lines we have described, C100(+), C100(−), and wild type, should prove useful in elucidating this relationship.

**Note Added in Proof.** While this manuscript was in press Chin *et al.* (39) reported that the undegraded protomer of HMG-CoA reductase is a $M_r$ 90,000 protein, in agreement with our results.

We would like to thank Pat Jones for helpful discussions. This investigation was supported by Public Health Service Grant HL26502. E.C.H. was a National Institutes of Health Predoctoral Fellow.

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