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Biochemistry

Regulation of synthesis and degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by low density lipoprotein and 25-hydroxycholesterol in UT-1 cells

(compactin/drug resistance/cholesterol metabolism/mevalonate/crystalloid endoplasmic reticulum)

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ABSTRACT **UT-1 cells are a clone of Chinese hamster ovary cells that were selected to grow in the presence of compactin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34]. These cells have 100- to 1,000-fold more immunoprecipitable reductase than normal. The enzyme activity is rapidly decreased when low density lipoprotein (LDL) or 25-hydroxycholesterol is added to the culture medium. In this current study, a quantitative immunoprecipitation assay was used to determine whether LDL and 25-hydroxycholesterol inhibit the synthesis or stimulate the degradation of reductase in UT-1 cells. Each of these agents inhibited the incorporation of [^{35}S]methionine into immunoprecipitable reductase by more than 98%. Pulse–chase experiments showed that reductase was degraded with a half-life of 10–13 hr in UT-1 cells and that the rate of degradation of preformed enzyme was increased 3-fold by the addition of either LDL or 25-hydroxycholesterol. We conclude that the predominant mechanism by which LDL and 25-hydroxycholesterol decrease reductase activity in UT-1 cells is a profound suppression of synthesis of the enzyme.**

3-Hydroxy-3-methylglutaryl-coenzyme A reductase [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] is a regulated membrane-bound enzyme that controls the synthesis of cholesterol and other polyisoprenoid compounds in animal cells (1). In cultured cells, the activity of the reductase is suppressed by cholesterol that enters through the receptor-mediated endocytosis of plasma low density lipoprotein (LDL) (1). Enzyme activity also can be suppressed by addition of 25-hydroxycholesterol or other oxygenated sterols dissolved in solvents (2). Studies of the turnover of reductase by indirect methods, such as cycloheximide and actinomycin treatment, suggested that LDL and oxygenated sterols decrease reductase activity largely by suppressing synthesis of the enzyme (3–5). Recently, Sinensky *et al.* (6) showed directly that 25-hydroxycholesterol inhibits incorporation of [^{35}S]methionine into immunoprecipitable reductase in Chinese hamster ovary cells.

Quantitative studies of the regulation of synthesis and degradation of reductase in ordinary cultured cells are difficult because reductase accounts for less than 0.01% of total cell protein, even when maximally induced. This problem can be circumvented by the use of cell lines with marked increases in reductase activity (7, 8). Such cells have been obtained by gradual adaptation to growth in increasing concentrations of compactin, a competitive inhibitor of reductase (9, 10). One of these lines, designated UT-1, has a 100- to 1,000-fold increase in reductase (8). To accommodate the increased amounts of reductase, UT-1 cells have developed a marked proliferation of tubular smooth endoplasmic reticulum that is packed in crystalloid hexagonal arrays (8). Despite their marked increase in reductase, UT-1 cells respond to LDL and 25-hydroxycholesterol with a rapid reduction in enzyme activity and a disappearance of the crystalloid membrane (8).

The current studies were designed to determine whether LDL suppresses reductase by altering the rate of synthesis or by increasing the rate of degradation of the enzyme in UT-1 cells.

METHODS

Materials. We obtained L-[^{35}S]methionine and D,L-3-hydroxy-3-methyl[3-^{14}C]glutaryl-CoA from New England Nuclear; leupeptin, phenylmethylsulfonyl fluoride, and bovine albumin (RIA grade, no. A7888) were from Sigma; Zwittergent 3-14 and Pansorbin (*Staphylococcus aureus* cells bearing protein A, no. 507858) were from Calbiochem–Behring; NaDodSO$_4$ was from BDH Biochemicals; Ham's F-12 medium (no. LM-259) was from KC Biologicals; and methionine- and leucine-free Ham's F-12 medium was from GIBCO. Human LDL (ρ, 1.019–1.063 g/ml) was prepared by ultracentrifugation (3). Newborn calf lipoprotein-deficient serum was prepared either by ultracentrifugation ($\rho > 1.215$ g/ml) or by removal of lipoproteins with 2% (wt/vol) Cab-o-sil (8). Rabbit anti-reductase IgG is a monospecific antibody directed against reductase purified from the livers of colestipol-fed rats (8). Compactin was kindly provided by A. Endo.

UT-1 Cells. UT-1 cells were derived from CHO-K1 cells that were selected for compactin resistance (8). Stock flasks of UT-1 cells were grown in monolayer in a 5% CO_2/95% air incubator at 37°C in medium A (Ham's F-12 medium containing 10% lipoprotein-deficient serum, 100 units of penicillin, and 100 μg of streptomycin per ml, 2 mM glutamine, and 40 μM compactin). On day 0, cells from stock flasks were dissociated with 0.05% trypsin/0.02% EDTA, and 4 to 5 $\times 10^4$ cells were seeded into each Petri dish (35 $\times$ 10 mm) containing 2 ml of medium A; the medium was renewed on days 2 and 4. The experiments were begun on day 3, 4, or 5 as indicated in legends. Cells were pulse-labeled with [^{35}S]methionine in 0.75 ml of medium B [Ham's F-12 medium with decreased concentrations of methionine (6 μM) and leucine (20 μM), 10% dialyzed lipoprotein-deficient serum, penicillin, streptomycin, 2 mM glutamine, and 40 μM compactin].

^{35}S-Labeled Cell Extracts. After incubation with [^{35}S]methionine, each cell monolayer was washed three times at 4°C

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Abbreviation: LDL, low density lipoprotein.

with 50 mM Tris·HCl, pH 7.4/155 mM NaCl. The cells were then processed by either of two methods.

Method 1. Cells were harvested with a rubber policeman in 50 mM Tris·HCl, pH 7.4/155 mM NaCl/20 μM leupeptin (1.4 ml per dish). One-half of each cell suspension was centrifuged at 800 × *g* for 5 min at 4°C, and the pellet was used for assay of reductase (see below). The other half was centrifuged at 12,500 × *g* for 5 min at 4°C, and the pellet was suspended in 75 μl of buffer C (10 mM sodium phosphate, pH 7.0/1 mM EDTA/0.1 mM leupeptin/10 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/0.25% dimethyl sulfoxide) and incubated for 30 min at 4°C. After sonication (10 sec), 75 μl of 4% Zwittergent 3-14 in buffer C was added. After Vortex mixing and incubation for 30 min at 4°C, the extract was centrifuged at 12,500 × *g* for 5 min at 4°C. Aliquots of the supernate were removed for measurements of protein content, trichloroacetic acid-precipitable ^{35}S-labeled protein, and immunoprecipitable ^{35}S-labeled reductase (see below).

Method 2. Cell monolayers were solubilized *in situ* by addition of 0.5 ml of buffer C containing 2% Zwittergent 3-14 and by agitation for 30 min at room temperature; the cell suspension was removed with a Pasteur pipette. After centrifugation at 12,500 × *g* for 5 min at room temperature, aliquots of the supernate were removed for measurement of reductase activity, protein content, trichloroacetic acid-precipitable radioactivity, and immunoprecipitable ^{35}S-labeled reductase (see below).

Immunoprecipitation of ^{35}S-Labeled Reductase. All procedures were performed at room temperature, and all centrifugations were at 12,500 × *g* for 1–5 min. Aliquots of the solubilized cell extracts (representing 5–10% of one dish of cells) were diluted to a final volume of 550 μl in buffer D (10 mM sodium phosphate, pH 7.0/0.5% Zwittergent 3-14/0.5 M NaCl/10 mM dithiothreitol/20 μM leupeptin) supplemented with bovine serum albumin at 5 mg/ml and 0.15% $NaDodSO_4$. The extracts were precleared by incubation for 10 min with 60 μl of 10% (wt/vol) Pansorbin followed by centrifugation. Aliquots of the supernate (500 μl) were incubated for 30 min with 25 μl of a 1 mg/ml solution of nonimmune IgG or anti-reductase IgG. Then, 25 μl of 10% Pansorbin was added for 10 min (just prior to use, the Pansorbin was washed twice with buffer D containing 0.15% $NaDodSO_4$ and resuspended in the same buffer). After centrifugation, the immune complexes were washed three times by vigorous Vortex mixing in 0.8 ml of buffer D containing 0.1% $NaDodSO_4$. After washing, the immunoprecipitates were resuspended in 175 μl of buffer E [62.5 mM Tris·HCl, pH 6.8/10% (vol/vol) glycerol/5% (vol/vol) 2-mercaptoethanol/100 mM dithiothreitol/2.3% (wt/vol) $NaDodSO_4$]. After incubation for 30 min, the suspension was heated at 85°C for 3 min and cooled to room temperature. After centrifugation, aliquots of the supernate were used for scintillation counting (80 μl in 10 ml of Aquasol) and for $NaDodSO_4$/polyacrylamide gel electrophoresis. Each value was corrected for ^{35}S radioactivity precipitated by nonimmune IgG, as measured in parallel incubations. In maximally induced UT-1 cells, the radioactivity precipitated by anti-reductase IgG was 5- to 10-fold higher than that precipitated by nonimmune IgG. Each value in the figures represents the mean of duplicate incubations.

Other Assays. The conversion of D,L-3-hydroxy-3-methyl[3-^{14}C]glutaryl-CoA to [^{14}C]mevalonate was measured in detergent-solubilized cell extracts as described (3) except that Kyro EOB was omitted from the assay of extracts solubilized by method 2. $NaDodSO_4$/polyacrylamide gel electrophoresis in 7% slab gels was carried out as described (8). Protein was measured by a modified Lowry procedure after precipitation with 10% trichloroacetic acid in the presence of 0.125% sodium deoxycholate (11). To measure trichloroacetic acid-precipitable ^{35}S-labeled cell protein, an aliquot (5–20 μl) of the solubilized cell extract was diluted to 200 μl with phosphate-buffered saline containing bovine serum albumin at 1 mg/ml, 0.125% sodium deoxycholate, and 0.625 mM methionine; 1 ml of 10% trichloroacetic acid was then added. After incubation for 18 hr at 4°C, the proteins were collected by centrifugation and washed with 1 ml of 10% trichloroacetic acid. The pellet was dissolved in 0.5 ml of 0.5 M KOH, and an aliquot (100 μl) was added to 10 ml of Aquasol. Glacial acetic acid (50 μl) was added just prior to scintillation counting.

RESULTS

To study the synthesis of reductase, we established a protocol in which UT-1 cells were pulse-labeled with [^{35}S]methionine. Solubilized cell extracts were incubated with anti-reductase IgG which was then precipitated with protein A. To validate this assay, we incubated cell extracts with increasing amounts of anti-reductase IgG and measured the amount of reductase activity that remained in the supernate and the amount of ^{35}S radioactivity that was precipitated. The amount of IgG required to precipitate 50% of reductase activity was the same as that required to precipitate 50% of the maximal amount of ^{35}S-labeled protein (8 μg per tube) (Fig. 1).

Fig. 2 shows autoradiograms of the immunoprecipitates after $NaDodSO_4$ gel electrophoresis. Precipitates in lanes 1–4 were prepared from cells that were harvested by scraping and pelleted by centrifugation before detergent solubilization (method 1). Cells in lane 1 had been grown in the absence of LDL. Cells in lanes 2–4 had been incubated for 24 hr in the presence of LDL at concentrations up to 50 μg/ml as indicated. In all cases, the major protein precipitated by the antibody had a M_r of 62,000. We previously had found that reductase subunits from UT-1 cells have a M_r of 62,000 when solubilized in hypotonic buffers in the presence of the protease inhibitor leupeptin (8). The amount of immunoprecipitable material declined markedly after LDL treatment. Lane 5 shows an immunoprecipitate pre-

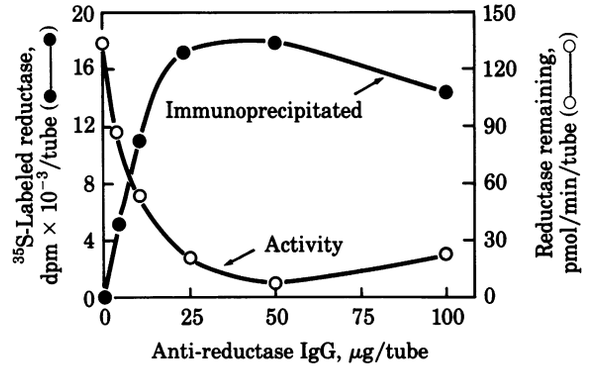

FIG. 1. Coordinate precipitation of reductase activity and ^{35}S-labeled protein by anti-reductase IgG. On day 3, each monolayer received 0.75 ml of medium B. Twenty-four hours later, cells were pulse-labeled for 1 hr at 37°C with [^{35}S]methionine at 125 μCi/ml. Solubilized extracts from seven dishes were prepared by method 2 and pooled. Each tube contained ≈1.8 × 10^6 dpm of trichloroacetic acid-precipitable cell protein and reductase activity equivalent to 143 pmol/min in 600 μl of buffer D supplemented with bovine serum albumin (5 mg/ml) but no $NaDodSO_4$. The tubes were precleared with Pansorbin, and the indicated amounts of immune or nonimmune IgG were added. After incubation and centrifugation, an aliquot (75 μl) of the supernate from each tube was removed and assayed for reductase activity (○). The pellet was solubilized and subjected to scintillation counting (●). The amount of radioactivity precipitated with nonimmune IgG was subtracted from each of the values obtained with the corresponding amount of immune IgG.

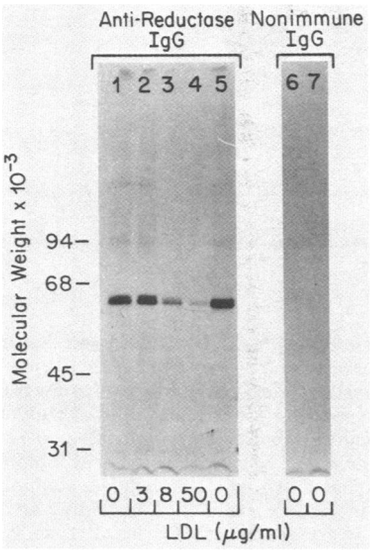

FIG. 2. $NaDodSO_4$ gel electrophoresis of ^{35}S-labeled immunoprecipitates from UT-1 cells incubated with various concentrations of LDL. On day 5, each monolayer received 0.75 ml of medium B containing the indicated amount of LDL. After 24 hr, the cells were pulse-labeled for 1 hr with [^{35}S]methionine (110 μCi/ml). Solubilized extracts were prepared by method 1 (lanes 1–4 and 6) or method 2 (lanes 5 and 7) and immunoprecipitated with either anti-reductase IgG (lanes 1–5) or nonimmune IgG (lanes 6 and 7). The immunoprecipitates were subjected to gel electrophoresis. Fluorography was conducted for 46 hr (8). ^{35}S radioactivity applied to gel: lane 1, 5,420 dpm; 2, 4,840 dpm; 3, 2,300 dpm; 4, 910 dpm; 5, 7,080 dpm; 6, 610 dpm; 7, 1,130 dpm.

pared from cells grown in the absence of LDL and solubilized *in situ* with a hypotonic solution containing Zwittergent (method 2). This method also gave a major immunoprecipitable protein of M_r 62,000, and the two methods of harvest were used interchangeably in the current studies. Lanes 6 and 7 show immunoprecipitates prepared with nonimmune IgG from cells solubilized after scraping (lane 6) or *in situ* (lane 7). Scintillation counting of gel slices showed that the reductase band at M_r 62,000 accounted for >90% of the total immunoprecipitated radioactivity. For routine assays, we estimated the amount of ^{35}S-labeled reductase by measuring the radioactivity precipitated by anti-reductase and nonimmune IgG and subtracting the latter from the former.

Incorporation of [^{35}S]methionine into immunoprecipitable reductase increased linearly for 4 hr, after which the cells reached a steady state in which immunoprecipitable reductase constituted approximately 0.8% of the total ^{35}S-labeled cell protein (Fig. 3*B*). The reductase activity of these cells averaged 35 nmol/min per mg of protein (Fig. 3*A*). If it is assumed that purified reductase from these cells has a specific activity of ≈6,000 nmol/min per mg of protein (8), the measured activity suggests that about 0.5% of the cell protein is reductase, a value similar to the value, 0.8%, derived from the immunoprecipitation data.

To study the effects of LDL on the rate of synthesis of reductase, we used a pulse-labeling time of 1 hr, which was within the linear range for isotope incorporation into reductase and total protein. Fig. 4 shows an experiment in which the effects of LDL on reductase activity and on the rate of synthesis of reductase were determined at intervals after addition of LDL to the culture medium; after a lag period the reductase activity declined precipitously (Fig. 4*A*). The rate of incorporation of [^{35}S]methionine into reductase (as measured by a 1-hr pulse) declined sharply after incubation with LDL for 5 hr (Fig. 4*B*). The 5-hr lag seen in this experiment was not observed in other experiments in which LDL was added to UT-1 cells.

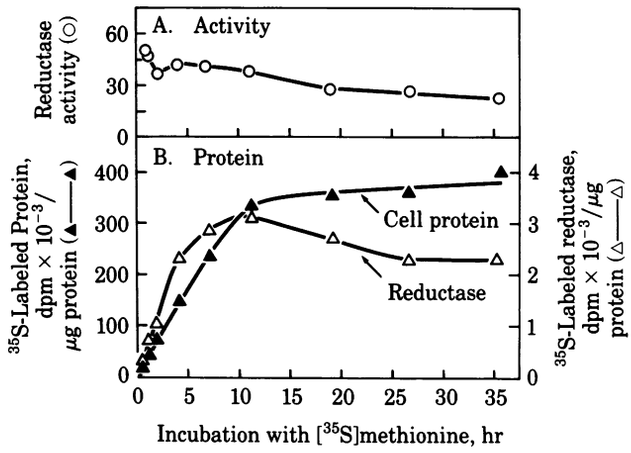

FIG. 3. Time course of incorporation of [^{35}]methionine into reductase in UT-1 cells. On day 5 (36 hr prior to harvest), each monolayer was switched to 0.75 ml of medium B. Additions of [^{35}S]methionine (71 μCi/ml) were made in a staggered fashion so that all cells could be harvested at the same time on day 6 after incubation with [^{35}S]methionine for the indicated times. Extracts were prepared by method 1 and used for measurements of reductase activity (shown as nmol/min per mg of protein) (○), total ^{35}S-labeled cell protein (▲), and immunoprecipitable ^{35}S-labeled reductase (△).

When UT-1 cells were incubated with increasing concentrations of LDL, reductase activity and the rate of synthesis of reductase declined in parallel (Fig. 5). LDL produced maximal suppression at about 50 μg of protein per ml, which is the saturating level for the LDL receptor in these cells.

25-Hydroxycholesterol also produced a marked reduction in the rate of synthesis of reductase (Fig. 6*A*) at the same concentrations that suppressed reductase activity (Fig 6*B*). Neither 25-hydroxycholesterol nor LDL reduced incorporation of [^{35}S]methionine into total cell protein (data not shown). The addition of mevalonate (13 mM) also inhibited synthesis of ^{35}S-labeled reductase by >95% without affecting incorporation of [^{35}S]methionine into total cell protein (data not shown).

Fig. 7 shows a pulse–chase experiment designed to measure

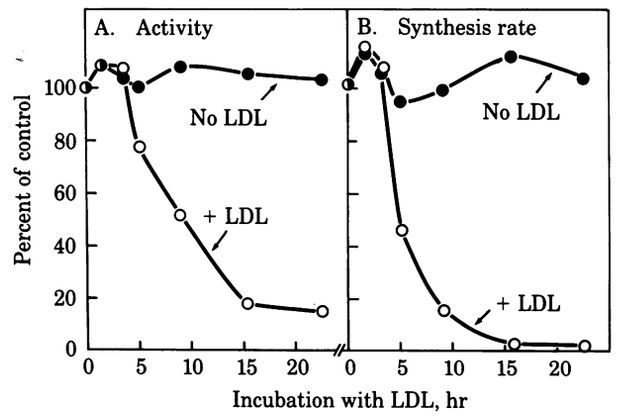

FIG. 4. Suppression of activity (*A*) and rate of synthesis (*B*) of reductase in UT-1 cells after incubation with LDL for various times. On day 4, monolayers were switched to 0.75 ml of medium B. Additions of LDL (125 μg/ml) were made in a staggered fashion so that all cells could be harvested at the same time on day 5 after incubation with LDL for the indicated times. One hour prior to harvest, all monolayers were pulse-labeled with [^{35}S]methionine (110 μCi/ml). Extracts were prepared by method 1 and used for measurements of reductase activity (*A*) and immunoprecipitable ^{35}S-labeled reductase (*B*). The "100% of control" values were 23 nmol/min per mg of protein for reductase activity (*A*) and 3,900 dpm/hr per μg of protein for rate of synthesis (*B*).

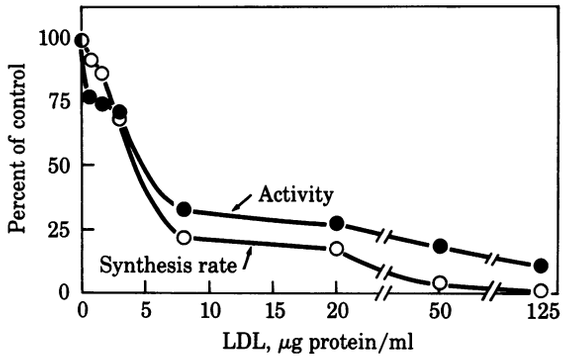

FIG. 5. Suppression of activity (●) and rate of synthesis (○) of reductase in UT-1 cells as a function of the concentration of LDL. On day 5 of cell growth, each monolayer received 0.75 ml of medium B containing the indicated amount of LDL. After 24 hr, each monolayer was pulse-labeled for 1 hr with [^{35}S]methionine (110 μCi/ml). Extracts were prepared by method 1 and used for measurements of reductase activity (●) and immunoprecipitable ^{35}S-labeled reductase (○). The "100% of control" values were 12 nmol/min per mg of protein for reductase activity (●) and 3,300 dpm/hr per μg of protein for synthesis rate (○).

the effect of LDL on the rate of degradation of reductase previously labeled by incubation of UT-1 cells with [^{35}S]methionine. In the absence of LDL, total reductase activity was maintained at a steady-state level (Fig. 7*A*) and the ^{35}S-labeled reductase was catabolized with a half-life of 10 hr (Fig. 7*B*). Addition of LDL produced a rapid decrease in reductase activity (Fig. 7*A*) and it also accelerated the rate of degradation of ^{35}S-labeled reductase, reducing the half-life to 4 hr (Fig. 7*B*). LDL had no effect on the turnover of total ^{35}S-labeled cell protein (Fig. 7*C*). Similar results were obtained in five other pulse–chase experiments in which the mean half-lives for ^{35}S-labeled reductase in the absence and presence of LDL were 13 and 3.6 hr, respectively. We also observed that 25-hydroxycholesterol enhanced the rate of degradation of preformed reductase by 3-fold (data not shown).

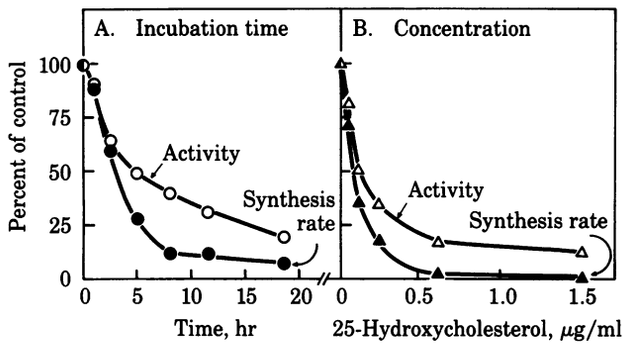

FIG. 6. Suppression of activity (○, △) and rate of synthesis (●, ▲) of reductase in UT-1 cells after incubation with 25-hydroxycholesterol. (*A*) On day 5, each monolayer received 0.75 ml of medium B. Additions of 25-hydroxycholesterol (at 0.5 μg/ml, added in 3 μl of ethanol) were made in a staggered fashion so that all cells could be harvested at the same time on day 6 after incubation with 25-hydroxycholesterol for the indicated times. At 1 hr prior to harvest, cells were pulse-labeled with [^{35}S]methionine (140 μCi/ml). Extracts were prepared by method 1. The "100% of control" values were 11 nmol/min per mg of protein for reductase activity (○) and 5,300 dpm/hr per μg of protein for synthesis rate. (*B*) On day 4, each monolayer received 1 ml of medium B containing the indicated concentration of 25-hydroxycholesterol plus cholesterol at 10 μg/ml (added in 5 μl of ethanol). The control cells received 5 μl of ethanol. After incubation for 19 hr, each monolayer was pulse-labeled for 1 hr with [^{35}S]methionine (178 μCi/ml). Extracts were prepared by method 2. The "100% of control" values were 62 nmol/min per mg of protein for reductase activity (△) and 3,750 dpm/hr per μg of protein for synthesis rate (▲), respectively.

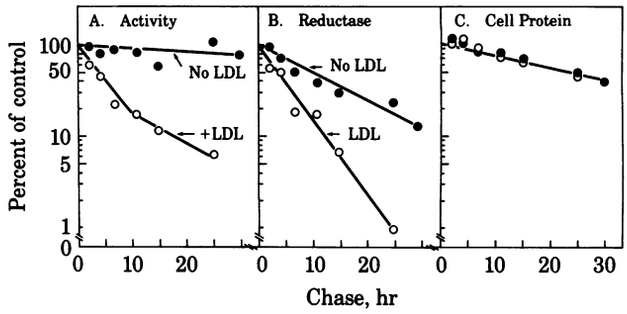

FIG. 7. Time course of LDL-mediated suppression of reductase activity (*A*), turnover of ^{35}S-labeled reductase (*B*), and turnover of total ^{35}S-labeled cell protein (*C*) in UT-1 cells. On day 5, each monolayer was pulse-labeled with [^{35}S]methionine (38 μCi/ml) added in 0.8 ml of modified medium B containing 15 μM methionine and 100 μM leucine. After 15 hr with [^{35}S]methionine, the medium was removed and the cells were chased in 1 ml of medium B containing 300 μM unlabeled methionine, no [^{35}S]methionine, and either no LDL (●) or LDL at 125 μg/ml (○). At the indicated times of chase, monolayers were harvested by method 1 and measurements were made. The "100% of control" values were 40 nmol/min per mg of protein for reductase activity, 925 dpm/μg of protein for ^{35}S-labeled reductase, and 96,000 dpm/μg of protein for total ^{35}S-labeled cell protein.

DISCUSSION

In the current experiments, immunoprecipitable hydroxymethylglutaryl-CoA reductase accounted for 0.5–2% of the proteins synthesized from [^{35}S]methionine by UT-1 cells. This high level made it possible to perform quantitative measurements of the rates of synthesis and degradation of the enzyme. The addition of LDL to UT-1 cells suppressed the synthesis of immunoprecipitable reductase by >98%. 25-Hydroxycholesterol and high concentrations of externally added mevalonate had a similar effect. The profound suppression of enzyme synthesis appears to constitute the major mechanism by which these agents decrease reductase activity in UT-1 cells. These findings are similar to those of Sinensky *et al.* who found that 25-hydroxycholesterol suppresses incorporation of [^{35}S]methionine into immunoprecipitable reductase in Chinese hamster ovary cells (6).

Pulse–chase experiments showed that the half-life of immunoprecipitable reductase protein in UT-1 cells grown in the absence of LDL was 10–13 hr. This is similar to the 13-hr half-life for the decline of reductase activity after addition of cycloheximide to Chinese hamster ovary cells grown in lipoprotein-depleted serum, as reported by Chang *et al.* (12). The addition of LDL or 25-hydroxycholesterol accelerated the rate of degradation of immunoprecipitable reductase in UT-1 cells by 3-fold. Using assays of enzyme activity, Chang *et al.* also observed that these agents accelerate reductase degradation in the hamster cells (12). The 3-fold increase in the rate of degradation of reductase elicited by LDL and 25-hydroxycholesterol may contribute to the lowering of the enzyme activity, but clearly the most important effect of these agents is the 98% decrease in the rate of synthesis of the enzyme.

In some of the current experiments, small amounts of reductase activity persisted in UT-1 cells long after synthesis of immunoprecipitable enzyme apparently had been totally suppressed by LDL (Fig. 4) or 25-hydroxycholesterol (Fig. 6*B*). This residual activity represented authentic reductase because it was totally inhibitable *in vitro* by compactin. Moreover, the residual reductase activity could be immunoprecipitated when incubated with anti-reductase IgG *in vitro*. It is likely that syn-

thesis of small amounts of reductase continued in the presence of LDL or 25-hydroxycholesterol, but the amount of this synthesis was too small to be differentiated reliably from the nonimmune blank value for ^{35}S incorporation in the immunoprecipitation assay.

As in our previous study (8), we noted a wide variation (10-fold) in reductase activity when UT-1 cells were studied on different days under apparently similar experimental conditions. Reductase activity in other types of cells is known to vary widely, depending strongly on the growth rate of cells (3). In UT-1 cells, we have consistently observed that reductase activity is higher (100–300 nmol/min per mg of protein) when the cells are grown in roller bottles than when grown in Petri dishes (10–100 nmol/min per mg of protein). In contrast to this day-to-day variation, on any single day the reductase activity in replicate dishes or roller bottles is constant.

Reductase in many cell types is known to exist in a phosphorylated (inactive) as well as in a nonphosphorylated (active) form (13). Dephosphorylation occurs during cell homogenization and this is blocked by inclusion of fluoride in the homogenization buffer (14). We have not yet performed a detailed study of phosphorylation of reductase in UT-1 cells. In preliminary experiments, we observed that reductase activity is decreased up to 90% when UT-1 cells are harvested in NaF and that the activity can be restored partially when the cells are incubated with a preparation of phosphoprotein phosphatase from rat liver (14). The degree of apparent phosphorylation was similar when the cells were grown in the absence or presence of LDL. Under the harvesting conditions used in the current studies (i.e., in the absence of NaF), reductase is fully activated (i.e., incubation with hepatic phosphatase causes no further increase) and therefore the measurements in this paper relate to the total amount of enzyme in the cells (i.e., phosphorylated plus nonphosphorylated) (15).

Throughout the current experiments, reductase was solubilized in the presence of the protease inhibitor leupeptin (8, 16). The immunoprecipitated reductase appeared as a band at M_r 62,000 on NaDodSO$_4$/polyacrylamide gels. Minor bands at $M_r \approx 94{,}000$ and $\approx 160{,}000$ were also observed (Fig. 2). The native structure of reductase within membranes of the endoplasmic reticulum is unknown. In the current experiments we found that UT-1 cells must be solubilized in hypotonic buffer in order for the immunoprecipitated reductase to migrate as a single M_r 62,000 band. When solubilization was performed at higher ionic strength, much of the immunoprecipitated reductase did not enter the NaDodSO$_4$ gels. It is possible that the true M_r of reductase subunits is >62,000 and that proteolysis occurs when cells are harvested in hypotonic buffer.

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