

Regulation of squalene epoxidase in HepG2 cells

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Abstract Regulation of squalene epoxidase in the cholesterol biosynthetic pathway was studied in a human hepatoma cell line, HepG2 cells. Since the squalene epoxidase activity in cell homogenates was found to be stimulated by the addition of Triton X-100, enzyme activity was determined in the presence of this detergent. Incubation of HepG2 cells for 18 h with L-654,969, a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, increased squalene epoxidase activity dose-dependently. On the other hand, low density lipoprotein (LDL) and 25-hydroxycholesterol decreased the enzyme activity. These results demonstrate that squalene epoxidase is regulated by the concentrations of endogenous and exogenous sterols. The affinity of the enzyme for squalene was not changed by treatment with L-654,969. Cytosolic (S_{105}) fractions, prepared from HepG2 cells treated with or without L-654,969, had no effect on microsomal squalene epoxidase activity of HepG2 cells, in contrast to the stimulating effect of S_{105} fractions from rat liver homogenate. Mevalonate, LDL, and oxysterol treatment abolished the effect of L-654,969. Simultaneous addition of cycloheximide and actinomycin D also prevented enzyme induction in HepG2 cells. From these results, the change in squalene epoxidase activity is thought to be caused by the change in the amount of enzyme protein. It is further suggested that squalene epoxidase activity is suppressed only by sterols, not by nonsterol derivative(s) of mevalonate, in contrast to the regulation of HMG-CoA reductase.—Hidaka, Y., T. Satoh, and T. Kamei. Regulation of squalene epoxidase in HepG2 cells. *J. Lipid Res.* 1990. 31: 2087–2094.

Supplementary key words cholesterol synthesis • HMG-CoA reductase • low density lipoprotein • mevalonate • cytosolic fraction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [EC 1.1.1.34] is the major rate-limiting enzyme in the cholesterol biosynthetic pathway (1). In cultured cells, HMG-CoA reductase has been shown to be controlled through multivalent feedback regulation (2). Full suppression of the reductase activity is mediated by sterols and nonsterol substance(s) derived from mevalonate.

Several other enzymes in the cholesterol biosynthetic pathway, such as HMG-CoA synthase, mevalonate kinase, and squalene synthetase, have also been reported to be regulated by sterols (3, 4). Squalene epoxidase [EC 1.14.99.7], which is a membrane-associated enzyme in the middle stage of the sterol biosynthetic pathway, catalyzes the conversion of squalene to 2,3-oxidosqualene. The properties of squalene epoxidase

have been extensively studied by Yamamoto and Bloch (5), Tai and Bloch (6), and Ono and Bloch (7) using rat liver microsomes. This enzyme requires cytosolic (S_{105}) fractions, which can be replaced by Triton X-100 for activation. There are a few reports that squalene epoxidase might play an important role in cholesterol biosynthesis (8, 9). Little is known about the detailed regulatory mechanism of squalene epoxidase, especially in hepatic cells. HepG2 cells, a human hepatoma cell line, maintain liver-specific cell functions and are thought to be a suitable model to investigate lipid synthesis and metabolism in human liver (10, 11).

In this report, we examine the regulatory mechanism of squalene epoxidase using HepG2 cells. L-654,969, an active β -hydroxy acid form of simvastatin and a potent competitive inhibitor of HMG-CoA reductase (12), was used to investigate the inhibitory effect of cholesterol synthesis on squalene epoxidase activity in HepG2 cells. 25-Hydroxycholesterol, which is one of the potent HMG-CoA reductase suppressors (13), and low density lipoprotein (LDL) were used as negative regulators in cultured cells. HMG-CoA reductase has been reported to be regulated by sterols and nonsterol substance(s) derived from mevalonate. Sterol-mediated control of reductase activity is exerted at the transcriptional level, whereas nonsterol substance(s) regulate at the enzyme level (14, 15). Therefore, we investigated whether or not squalene epoxidase activity could be regulated by mevalonate-derived substance(s), and we have uncovered new information about the regulatory mechanism of squalene epoxidase activity in HepG2 cells.

MATERIALS AND METHODS

Materials

L-654-969, [1S-[1 α (β S*, δ S*), 2 α ,6 β ,8 β ,8 $\alpha\beta$]-1,2,6,7,8,8 α -Hexahydro- β , δ -dihydroxy-2,6-dimethyl-8-(2,2-dimethyl-1-oxobutoxy)-1-naphthalene-heptanoic acid, was prepared in

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LDL, low density lipoproteins; LPDS, lipoprotein-deficient serum; FBS, fetal bovine serum; DMSO, dimethylsulfoxide; PBS, phosphate-buffered saline.

Merck Sharp and Dohme Research Laboratories (Rahway, NJ). [4,8,12,13,17,21-³H]Squalene (24.6 Ci/mmol) was obtained from New England Nuclear, (Boston, MA). 3-Hydroxy-3-methyl[3-¹⁴C]glutaryl coenzyme A (HMG-CoA, 52 mCi/mmol) was purchased from Amersham International plc. (Buckinghamshire, England). 2,3-Oxidosqualene was synthesized in our laboratory according to the method of Nadeau and Hanzlik (16). Human low density lipoprotein (d 1.020–1.063 g/ml) and lipoprotein-deficient serum (LPDS) (d > 1.215 g/ml) were prepared by ultracentrifugation as described by Brown, Dana, and Goldstein (17). AMO 1618 was obtained from Calbiochem (La Jolla, CA). All other chemicals used were standard commercial high purity materials.

Cell cultures

The established HepG2 cell line, derived from human hepatoma, was obtained from American Type Culture Collection (Rockville MD). Cell stocks were grown in 80-cm² flasks containing medium A (Eagles modified minimum essential medium, (MEM Flow Laboratories, McLean, VA) supplemented with penicillin G, 100 U/ml; streptomycin, 100 µg/ml; and pyruvate, 1 mM) with 10% heat inactivated fetal bovine serum (FBS), and incubated in a humidified incubator (5% CO₂) at 37°C. On day 0, cells were seeded in 21-cm² dishes at 7.5 × 10⁵ cells in 2.5 ml of medium A with 10% FBS. On day 4, the medium was exchanged for 4 ml of fresh medium. On day 6, the cells were incubated with each compound in fresh medium A containing 10% LPDS for the indicated time. L-654,969 and 25-hydroxycholesterol were dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO did not exceed 0.4% (v/v). Under these conditions, DMSO had no effect on squalene epoxidase and HMG-CoA reductase activities in HepG2 cells. After incubation, the cells were washed twice with medium A and incubated in medium A for another 15 min at 37°C to remove intracellular compounds. The cells were washed three times with cold phosphate-buffered saline (PBS), pH 7.5, and scraped with a policeman. After centrifugation (1,000 g, 5 min at 4°C), the cell pellet was frozen and kept at –80°C until use.

Enzyme preparation

Under the standard assay conditions, squalene epoxidase activity was measured in cell homogenate in the presence of 0.1% Triton X-100. After thawing and rupture of the cells by sonication (Bronson sonifier B-100, for 5 sec at 0°C) in 0.1 M Tris-HCl, pH 7.5, 1 mM EDTA (buffer A), the cell homogenate was mixed with one fourth volume of 2% Triton X-100 (final concentration of detergent, 0.4%). The mixture stood at 0°C for 20–60 min and aliquots of the mixture were assayed for squalene epoxidase activity.

Assay of squalene epoxidase

Squalene epoxidase activity was determined according to the method of Tai and Bloch (6) with some modifica-

tions. Aliquots of the mixture described above (0.72–0.9 mg of protein) were incubated for 90 min at 37°C in a final volume of 0.3 ml containing 0.1 M Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM NADPH, 0.1 mM FAD, 0.4 mM AMO 1618 (an inhibitor of 2,3-oxidosqualene lanosterol cyclase) (18), 0.1% Triton X-100, and 8 µM [³H]squalene (45,000 dpm) dispersed in 0.075% Tween 80. The reaction was stopped by the addition of 0.3 ml of 10% methanolic KOH. After incubation for 60 min at 75°C, nonsaponifiable materials were extracted twice with 2 ml of petroleum ether. The combined extracts were evaporated under a nitrogen stream. The residue, taken up in a small volume of diethylether, was spotted on a thin-layer Silica Gel G plate (Art5583, E. Merck, Darmstadt, West Germany) which was then developed in benzene-ethyl acetate 99.5:0.5. The band corresponding to authentic 2,3-oxidosqualene (*R_f* 0.5) was scraped into a vial (*R_f* value of squalene was 0.95) and the radioactivity was counted with a liquid scintillation counter (TRI-CARB 2000CA, Packard Instrument Co., Downers Grove, IL). In routine assays, ergosterol acetate (*R_f* 0.6) was used as a convenient ultraviolet-visible marker for 2,3-oxidosqualene. Squalene epoxidase activity is expressed in pmol of 2,3-oxidosqualene formed per mg of cell protein per min.

Assay of HMG-CoA reductase

HMG-CoA reductase activity was determined as described by Brown et al. (17) with some modifications. HepG2 cells were suspended in buffer containing 0.1 M potassium phosphate, pH 7.5, 5 mM EDTA, 0.2 M KCl, 0.25% Brij 96, and then centrifuged at 12,000 g for 15 min at 4°C. Aliquots of the supernatant (1–50 µg protein) were incubated for 1 h at 37°C in a final volume of 100 µl containing 0.1 M potassium phosphate, pH 7.4, 20 mM glucose 6-phosphate, 2.5 mM NADP⁺, 1.0 unit of glucose 6-phosphate dehydrogenase, 4 mM dithiothreitol, and 30 µM [¹⁴C]HMG-CoA (50,000 dpm). The enzyme activity was terminated by the addition of 20 µl 2 M HCl. After the mixture stood for 15 min at 37°C for lactonization of mevalonic acid, [¹⁴C]mevalonolactone was separated from [¹⁴C]HMG-CoA on a small column packed with ion exchange resin (AG 1-X8, formate form, Bio-Rad, Richmond, CA) (19). The enzyme activity was determined under conditions in which the inhibitory effect of L-654,969 was overcome by dilution.

Preparation of microsomes and supernatant fractions from HepG2 cells

The cell pellet (ca. 2 × 10⁸ cells) was suspended in 3 ml of buffer A and homogenized in a glass-teflon homogenizer. The homogenate was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was recentrifuged at 105,000 g for 60 min to give S₁₀₅ fractions. The microsomes were washed once with buffer A. All fractions were kept at –80°C until use.

Preparation of rat liver supernatant fraction

Female SD rats (125–145 g) were maintained under a reverse illumination cycle and fed powdered food supplemented with 5% (w/w) cholestyramine for 10 days. Animals were killed 4 h after onset of the dark period. Rat liver S_{105} fraction was prepared as described by Yamamoto and Bloch (5).

Protein determination

The protein concentrations were determined according to the method of Lowry et al. (20) using bovine serum albumin as a standard.

RESULTS

Measurement of squalene epoxidase activity of HepG2 cells

Rat squalene epoxidase activity has been reported to be stimulated by the addition of Triton X-100 (7). Therefore, we examined the effect of Triton X-100 on the enzyme activity of HepG2 cells. As shown in Fig. 1a, squalene epoxidase in the cell homogenate was activated by the addition of Triton X-100 in a concentration-dependent manner. The optimum Triton X-100 concentration depended on the cell

protein concentration (data not shown). Enzyme activity was optimally activated by 0.1–0.15% Triton X-100 at a protein concentration of 3 mg/ml (Fig. 1a). In the presence of 0.1% Triton X-100, the reaction was linear with cellular protein concentrations from 1.0 to 3.5 mg/ml (Fig. 1b, nonlinearity at low protein concentrations was due to the inhibitory effect of Triton X-100) and with incubation times at least up to 150 min (Fig. 1c). In the following experiments, unless otherwise stated, the enzyme activity was measured with 2.4–3.0 mg/ml protein concentrations and a 90-min incubation time in the presence of 0.1% Triton X-100.

Effect of cholesterol concentration on squalene epoxidase activity

Squalene epoxidase activity in HepG2 cells was increased time-dependently when the cells were incubated in 10% LPDS to exclude the supply of exogenous cholesterol (Fig. 2). L-654,969, which depletes the endogenously synthesized cholesterol, further increased the enzyme activity. After 18 h treatment, the enzyme activity in LPDS-grown cells was about twofold higher than the control value (at zero time), and the activity of cells treated with L-654,969 was fourfold higher than that in the control.

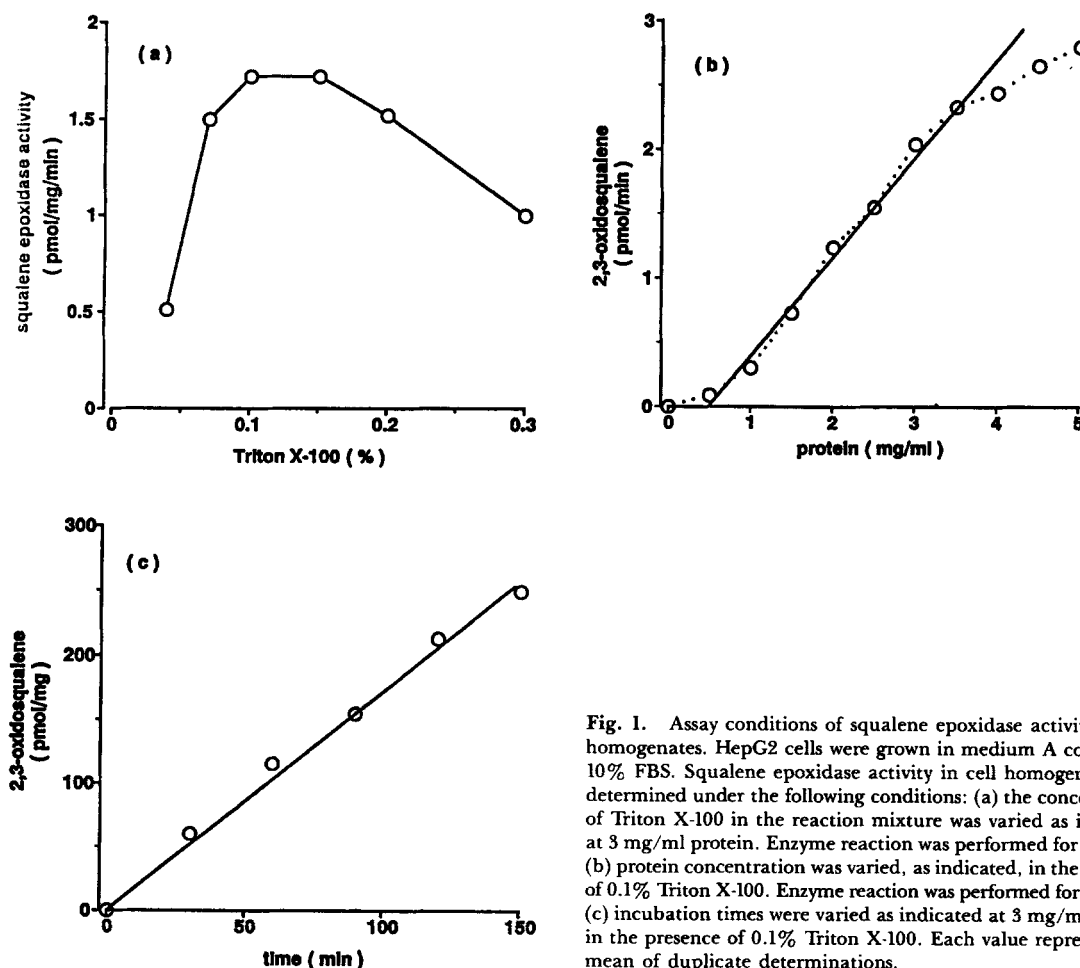


Fig. 1. Assay conditions of squalene epoxidase activity in cell homogenates. HepG2 cells were grown in medium A containing 10% FBS. Squalene epoxidase activity in cell homogenates was determined under the following conditions: (a) the concentration of Triton X-100 in the reaction mixture was varied as indicated at 3 mg/ml protein. Enzyme reaction was performed for 90 min.; (b) protein concentration was varied, as indicated, in the presence of 0.1% Triton X-100. Enzyme reaction was performed for 90 min.; (c) incubation times were varied as indicated at 3 mg/ml protein in the presence of 0.1% Triton X-100. Each value represents the mean of duplicate determinations.

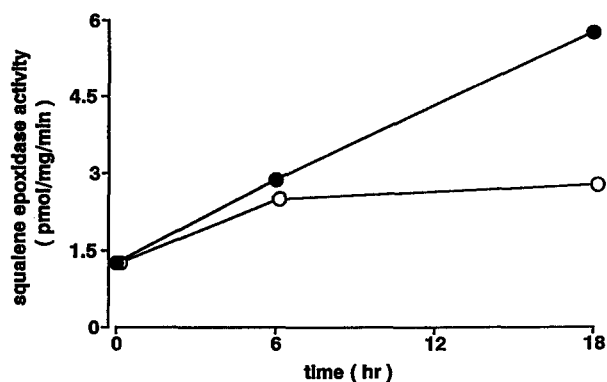


Fig. 2. Time course of squalene epoxidase induction in HepG2 cells. The cells were incubated for the indicated time in medium A containing 10% LPDS in the presence (●) or absence (○) of 1 μ M L-654,969. Squalene epoxidase activity in HepG2 cells was determined under standard assay conditions as described in Materials and Methods. Each value represents the mean of duplicate determinations.

HepG2 cells were incubated for 18 h with various concentrations of L-654,969, LDL, and 25-hydroxycholesterol. As shown in Fig. 3, L-654,969 increased the squalene epoxidase activity in a concentration-dependent manner. On the other hand, LDL and 25-hydroxycholesterol decreased the enzyme activity. L-654,969 had no effect on squalene epoxidase activity directly, at least up to 50 μ M (data not shown).

Properties of squalene epoxidase induced by L-654,969

In order to examine whether these changes in squalene epoxidase activity were due to the change in the amount of the enzyme protein, the enzymatic properties of squalene epoxidase induced by L-654,969 were investigated. Fig. 4 shows the saturation curves for squalene epoxidase activity with respect to the concentration of squalene. From the Lineweaver-Burk plot analysis (Fig. 4, inset), the V_{max} value of enzyme activity of cells treated with L-654,969 was increased from 1.67 to 3.97 pmol/mg per min, while the K_m value was constant (3 μ M). These results indicate that the increase in enzyme activity produced by L-654,969 was not due to a change in the affinity of the enzyme for squalene.

The induced squalene epoxidase activity of cells treated with LPDS plus L-654,969 was stimulated by Triton X-100 in the same manner as noninduced enzyme (data not shown). A 0.1% concentration of Triton X-100, used as a standard assay condition, was also optimum for induced enzyme to give full activation.

Effect of S_{105} fractions on microsomal squalene epoxidase activity from HepG2 cells

It has been reported that squalene epoxidase activity in rat liver microsomes is remarkably stimulated by the addition of the hepatic S_{105} fractions (6). Therefore, we examined the effect of S_{105} fractions on enzyme activity in HepG2 cells. Fig. 5 shows the effect of S_{105} fractions on microsomal

squalene epoxidase activity of FBS-grown cells. S_{105} fractions prepared from both FBS-grown cells and LPDS plus L-654,969-treated cells slightly activated the microsomal enzyme activity. These results suggest that the S_{105} fraction in HepG2 cells does not contribute to the regulation of squalene epoxidase activity. In contrast, the S_{105} fraction from rat liver remarkably enhanced the microsomal enzyme activity in HepG2 cells (Fig. 5).

Effect of various compounds on the enzyme induction by L-654,969

As shown in Table 1, mevalonate, which is synthesized by HMG-CoA reductase and converted to cholesterol via a series of enzymes, blocked the increase in squalene epoxidase activity produced by L-654,969. LDL and 25-hydroxycholesterol also abolished the effect of L-654,969 completely. Since the effects of those drugs were not examined on enzyme preparations from cells incubated with 10% FBS, it may be difficult to decide whether those drugs interfered with the induction of the enzyme by L-654,969 only, with the induction elicited by 10% LPDS, or with the basal (noninduced) levels of the enzyme (25-hydroxycholesterol is thought to influence the basal enzyme activity, Fig. 3). However, as an inducible effect of L-654,969 was not found in the presence of any drugs, it seems that the induction of squalene epoxidase activity by L-654,969 is due to cholesterol depletion within the cells through the inhibition of HMG-CoA reductase. Treatment with cycloheximide and actinomycin D produced remarkable suppression of squalene epoxidase activity and also prevented the induction by L-654,969. These results suggest that the half-life of squalene epoxidase is less than 18 h under the experimental conditions used and the induction of this enzyme requires protein and RNA synthesis.

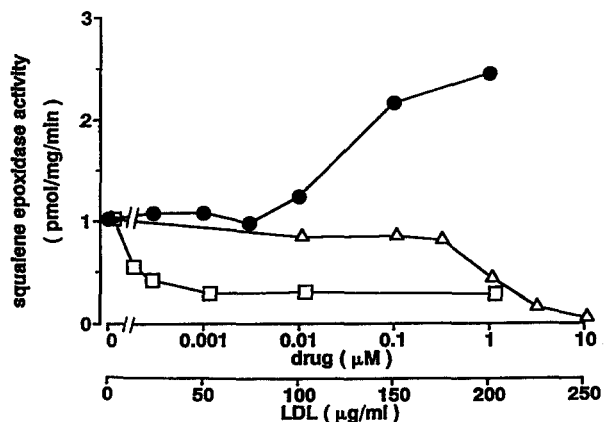


Fig. 3. Effect of various compounds on squalene epoxidase activity in HepG2 cells. The cells were incubated for 18 h in medium A containing 10% LPDS with the indicated concentrations of L-654,969 (●), 25-hydroxycholesterol (Δ), and LDL (\square). After the cells were washed and harvested, squalene epoxidase activity was determined as described in Materials and Methods. Each value represents the mean of duplicate determinations.

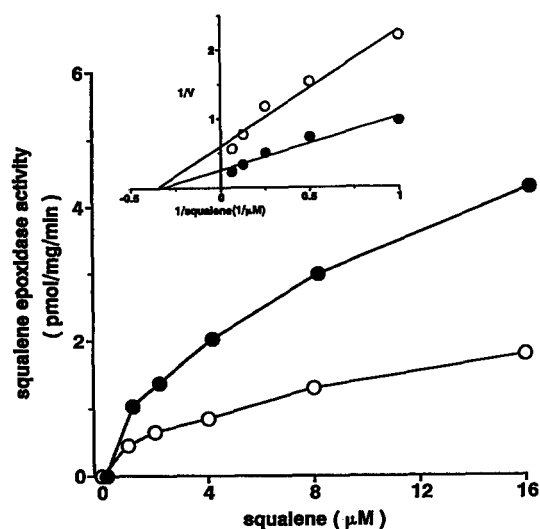


Fig. 4. Effect of squalene concentration on squalene epoxidase activity in HepG2 cells grown in the presence or absence of L-654,969. HepG2 cells were incubated for 18 h in medium A containing 10% LPDS in the presence (●) or absence (○) of $1 \mu\text{M}$ L-654,969. After the cells were harvested, squalene epoxidase activity was assayed under standard conditions except for the squalene concentration as indicated. Each value represents the mean of duplicate determinations. The inset shows the Lineweaver-Burk plot of this data.

Effect of mevalonate-derived substance(s) on enzyme activity

HMG-CoA reductase has been reported to be regulated by sterol as well as nonsterol substance(s) derived from mevalonate (2, 21). To examine whether mevalonate-derived substance(s) are involved in the regulation of squalene epoxidase, the suppressive effect of LDL and mevalonate on enzyme activity was investigated. As shown in Fig. 6a, the increase in HMG-CoA reductase activity produced by L-654,969 was suppressed by the addition of LDL. However, LDL did not suppress the reductase activity completely. The activity remained relatively high at a concentration of $200 \mu\text{g/ml}$ LDL, which is sufficient to supply cholesterol to the cells. Co-addition of mevalonate with LDL suppressed the induction of the reductase activity completely. These data show that unidentified metabolite(s) derived from mevalonate play an additional role in the regulation of reductase. On the other hand, squalene epoxidase activity was suppressed to the control level (in the absence of L-654,969) by the addition of LDL only (Fig. 6b). In the presence of $100 \mu\text{g/ml}$ LDL, L-654,969 did not induce squalene epoxidase activity.

DISCUSSION

Many studies have been carried out on the regulatory mechanism of HMG-CoA reductase (22), a major rate-limiting step in the cholesterol biosynthetic pathway. Several other enzymes in this pathway, such as HMG-CoA synthase and prenyltransferase, have been reported to be coordinately controlled by sterols (23). However, little is known about

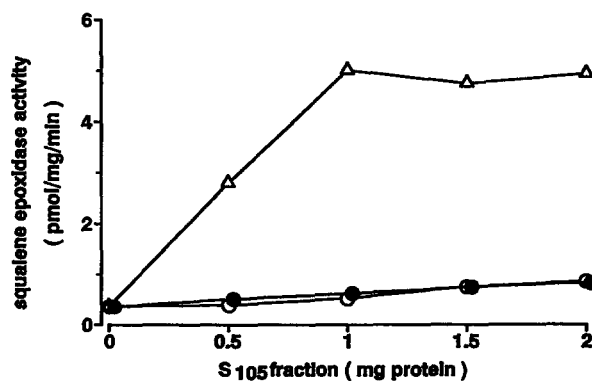


Fig. 5. Effect of supernatant cytosolic (S_{105}) fractions on microsomal squalene epoxidase activity from HepG2 cells. Microsomes were prepared from HepG2 cells grown in medium A containing 10% FBS. S_{105} fractions were prepared from rat liver (Δ) and HepG2 cells grown in either 10% FBS (○) or 10% LPDS plus $1 \mu\text{M}$ L-654,969 (●) for 18 h. Squalene epoxidase activity was determined at 1 mg of microsomal protein in the presence of increasing concentrations of each S_{105} fraction. The assay was performed under the same conditions as in cell homogenates as described in Materials and Methods (Triton X-100 was removed from the reaction mixture).

the regulation of squalene epoxidase, which catalyzes the reaction at the middle stage of the cholesterol synthetic pathway, because an assay system for this enzyme has not yet been established.

A nonionic detergent, Triton X-100, has been shown to stimulate squalene epoxidase activity in rat liver microsomes (7). Chin and Bloch (24) reported that squalene epoxidase may be less restricted conformationally and more accessible to substrate by the disruption of the microsomal membrane.

In this report, we showed that squalene epoxidase activity in HepG2 cells was also stimulated by the addition of Triton X-100. In the presence of 0.1% Triton X-100 (a relatively low concentration), the reaction was linear with

TABLE 1. Effect of mevalonate, LDL, oxysterol, protein, and RNA synthesis inhibitors on the increase of squalene epoxidase activity by L-654,969

Drug	Squalene Epoxidase Activity	
	Control	L-654,969 ($1 \mu\text{M}$)
	<i>pmol/mg/min</i>	
None	1.62	3.36
Mevalonate (5 mM)	0.75	0.77
LDL (200 $\mu\text{g/ml}$)	0.61	0.55
25-Hydroxycholesterol (3 μM)	0.31	0.28
Cycloheximide (10 μM)	0.08	0.05
Actinomycin D (5 $\mu\text{g/ml}$)	0.04	0.05

HepG2 cells were incubated for 18 h in medium A containing 10% LPDS with each drug in the presence or absence of L-654,969. After the cells were harvested and homogenized, squalene epoxidase activity was determined as described in Materials and Methods. Each value represents the mean of duplicate determinations.

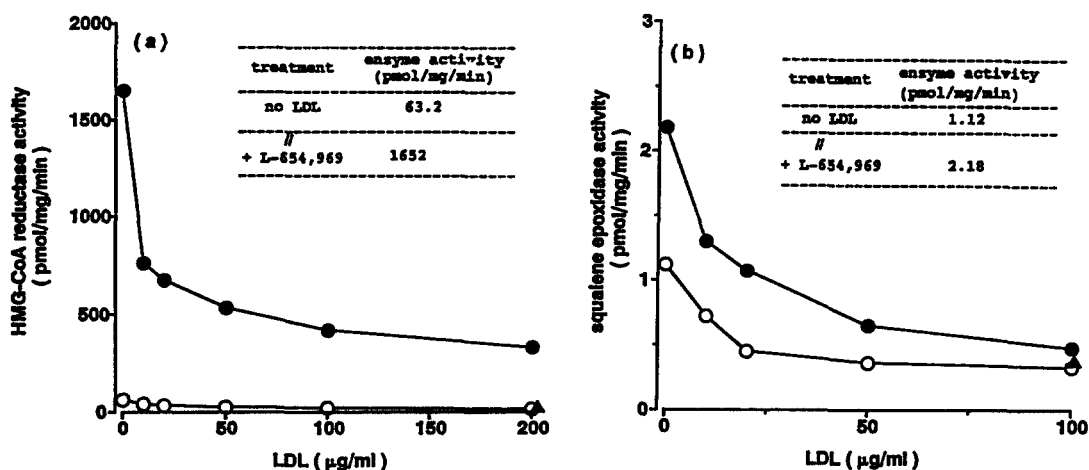


Fig. 6. Suppression of HMG-CoA reductase and squalene epoxidase activities by LDL and mevalonate. HepG2 cells were incubated for 18 h in medium A containing 10% LPDS in the presence (●) or absence (○) of $1 \mu\text{M}$ L-654,969 with the indicated concentrations of LDL. The cells were also incubated for 18 h in 10% LPDS in the presence of $1 \mu\text{M}$ L-654,969 with 100 or 200 $\mu\text{g/ml}$ LDL plus 5 mM mevalonolactone (▲). After the cells were washed and harvested, (a) HMG-CoA reductase and (b) squalene epoxidase activities were determined as described in Materials and Methods. Each inset shows the value of enzyme activities in the absence of LDL.

protein concentrations and incubation times. The regulatory mechanism of squalene epoxidase was investigated in HepG2 cells under conditions where the linearity was observed between protein concentrations and enzyme activity.

In the present study, incubation of HepG2 cells with L-654,969 was found to increase squalene epoxidase activity dose-dependently. On the other hand, LDL and 25-hydroxycholesterol decreased the activity. The increase in enzyme activity by L-654,969 was not due to a change in the optimum concentration of detergent. The K_m value for squalene was not changed by treatment with L-654,969. S_{105} fractions, prepared from HepG2 cells with or without L-654,969, had no different effect on microsomal squalene epoxidase activity in HepG2 cells. In the presence of mevalonate, LDL, or oxysterol, L-654,969 did not induce epoxidase activity. Simultaneous addition of cycloheximide or actinomycin D also prevented the induction of the enzyme activity in HepG2 cells.

From these results, we conclude that squalene epoxidase is subject to feedback regulation mediated by endogenous and exogenous sterols. The regulatory mechanism of this enzyme is thought to be due to the change in the amount of enzyme protein, though we have not examined whether this is caused by the change in protein synthesis rate or degradation rate.

Squalene epoxidase in rat liver microsomes requires cytosolic (S_{105}) fractions (6). As shown in Fig. 5, S_{105} fractions from rat liver homogenate markedly enhanced the microsomal squalene epoxidase activity from HepG2 cells. On the other hand, homologous S_{105} fractions from HepG2 cells showed only a slight activating effect. Similar results have been reported in the case of Chinese hamster ovary cells (9). The reason for this observation is not clear at present. However, it seems that these differences are not due to differences in the ori-

gin of tissues used (both S_{105} fractions are of liver origin), but rather to differences in species, or tissue, or use of cultured cells. During cell culture, some activating factor in the cytosolic fraction may be lost.

HMG-CoA reductase has been reported to be regulated by sterols as well as nonsterol metabolite(s) derived from mevalonate (2). The nonsterol metabolite(s) regulate exclusively at the enzyme level, whereas sterols regulate at the mRNA level (14, 15). The activity of other enzymes in the cholesterol biosynthetic pathway has also been shown to be suppressed by sterols. However, there are no reports that mevalonate-derived substance(s) could regulate the activity of the enzymes in the cholesterol synthetic pathway, except for HMG-CoA reductase (25, 26). Squalene epoxidase activity was also thought to be suppressed only by sterols, not by nonsterol derivative(s) of mevalonate. the nonsterol suppressor(s) may be specific for the regulation of HMG-CoA reductase.

Cultured cells have been known to synthesize several isoprenoid compounds, such as ubiquinone (27), and dolichol (28), in addition to cholesterol. Since farnesyl pyrophosphate is the last common intermediate in the cholesterol and isoprenoid pathway (29), the regulation of enzyme activity distal to squalene, formed from two molecules of farnesyl pyrophosphate, probably influences ubiquinone and dolichol synthesis. Faust, Goldstein, and Brown (4) suggested that the regulation of squalene synthetase activity could play a role in the formation of such side-products of the cholesterol biosynthetic pathway. However, since squalene epoxidase is located after this last common regulatory step in the sterol pathway, the regulation of this enzyme was not thought to influence isoprenoid synthesis directly. The regulation of squalene epoxidase was considered only to maintain cholesterol homeostasis in the cells.

Cohen, van Miert, and Griffioen (25) have recently reported that squalene synthetase activity in HepG2 cells was increased by the addition of compactin and decreased by LDL. However, the maximum effect induced by compactin was only about 1.4-fold higher than the control activity (without inhibitor). The addition of LDL resulted in only 20% suppression (25). On the other hand, in our study, squalene epoxidase activity in HepG2 cells was shown to be increased more than 2-fold by treatment with L-654,969 and it was suppressed more than 70% by the addition of LDL (Fig. 3). Squalene epoxidase activity is thought to be more strictly regulated by both exogenously delivered cholesterol and endogenously synthesized cholesterol than the activity of squalene synthetase.

Astruc et al. (30) reported that the level of squalene epoxidase is very low especially in noncholesterogenic tissues. In our assay system, squalene epoxidase activity in HepG2 cells was also very low. Compared with the specific activity of HMG-CoA reductase, the velocity of squalene epoxidase is about $\frac{1}{60}$ – $\frac{1}{800}$ of the rate of HMG-CoA reductase under the same conditions (Fig. 6a, b, inset), even though each molecule of 2,3-oxidosqualene is derived from six molecules of mevalonate. Gonzalez, Carlson, and Dempsey (8) reported that in human renal cancer cells exogenous cholesterol inhibited the conversion of mevalonate to cholesterol, resulting in the accumulation of squalene.

From these observations, it is concluded that squalene epoxidase is a secondary rate-limiting enzyme in the middle stage of the cholesterol synthetic pathway, and squalene that accumulates as a result of squalene epoxidase regulation may play an unknown physiological role within the cells.

HMG-CoA reductase, LDL receptor, and HMG-CoA synthase activity have been reported to be suppressed by sterols (3, 31). Recent studies of these three proteins, using cloned genes, have shown that this negative feedback regulation is mediated at the transcriptional level and that each gene has consensus octanucleotide sequences in the 5' flanking regions which are necessary for sterol-mediated suppression (32–34). From the current studies, we hypothesize that the squalene epoxidase gene also has the consensus sequence in 5' flanking region and that the enzyme activity is mediated through a common feedback mechanism. However, nothing is known about the gene for squalene epoxidase. In addition to examining squalene epoxidase protein using a specific antibody, isolation of squalene epoxidase cDNA probe and mRNA determination will be necessary for further studies. ■

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