Purification and characterization of recombinant squalene epoxidase

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Abstract Recombinant rat squalene epoxidase (rSE) was expressed in *E. coli* and purified to an apparent homogeneity. This expression system was constructed using squalene epoxidase (SE) cDNA in which nucleotides coding 99 amino acids in the N-terminal were deleted and nucleotides coding hexahistidine in the C-terminal were added. Purification was carried out using Ni-chelate affinity agarose and Cibacron Blue Sepharose column chromatography. Purification was achieved 100-fold over the crude *E. coli* extract with a yield of about 50%. The purified enzyme demonstrated a single band on SDS-polyacrylamide gel electrophoresis. The enzyme showed no distinct absorption spectrum in the visible regions. The properties of rSE were compared with those of rat liver microsomal SE. The requirement of the co-factors, the S_{105} fraction or Triton X-100, and NADPH-cytochrome c reductase, the pH dependency for enzyme activity, and the sensitivity to NB-598 seen with both enzymes suggest that rSE has properties very similar to rat microsomal SE. 2,3-Oxidosqualene (OSQ) and 2,3;22,23-dioxidosqualene (DOSQ) were formed by rSE in a completely reconstituted system. It is suggested that recombinant squalene epoxidase catalyzes the conversion of squalene to 2,3-oxidosqualene and of 2,3-oxidosqualene to 2,3;22,23-dioxidosqualene.—**Nagumo, A., T. Kamei, J. Sakakibara, and T. Ono.** Purification and characterization of recombinant squalene epoxidase. *J. Lipid Res.* 1995. **36:** 1489–1497.

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Squalene epoxidase (SE) [EC 1.14.99.7], a membrane-associated enzyme in the middle stage of the sterol biosynthetic pathway, catalyzes the conversion of squalene to 2,3-oxidosqualene. SE was purified to homogeneity by us (1, 2) Bai and Prestwich (3) reported the partial purification of pig liver SE and the effects of several substrate analog on enzymatic activity. We described (4, 5) the mechanism of regulation of SE in vitro and in vivo. Cholesterol feeding decreased hepatic SE activity, but the administration of an HMG-CoA reductase inhibitor and cholestyramine increased enzyme activity in rat. These results show that SE is regulated by the intracellular level of cholesterol. From our studies, SE appears to be a secondary rate-limiting enzyme that plays an important role in the maintenance of cholesterol homeostasis.

The properties of SE have been studied (6–8) using rat liver microsomes. This enzyme requires cytosolic (S_{105}) fractions, which can be replaced by Triton X-100, molecular oxygen, NADPH-cytochrome c reductase, NADPH, and FAD. We recently isolated SE cDNA from a rat liver cDNA library using specific SE inhibitors (terbinafine and NB-598) (9). This led us to investigate the precise properties of SE.

Oxysterols are reported to influence vital activities such as de novo sterol biosynthesis, membrane function, DNA synthesis, cell growth and proliferation, and atherosclerosis (10). However, the formation process of oxysterols in vivo is unknown. Recently, Spencer (11) reported the squalene diepoxide pathway for oxysterol formation. The epoxidation of squalene is known to be the first step among several oxygenase-catalyzed reactions in cholesterol biosynthesis (12). It is possible that SE catalyzes the conversion of 2,3-oxidosqualene (OSQ) to 2,3:22,23-dioxidosqualene (DOSQ).

In this paper, we describe the construction of a rat SE expression system in *E. coli* and the purification of the enzyme. We compared the enzymatic properties of recombinant squalene epoxidase (rSE) with those of rat liver microsomal SE. Furthermore, we investigated whether rSE could form DOSQ in a completely reconstituted system.

Abbreviations: SE, squalene epoxidase; rSE, recombinant SE; OSQ, 2,3-oxidosqualene; DOSQ, 2,3;22,23-dioxidosqualene; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; PCR, polymerase chain reaction; TLC, thin-layer chromatography; GC–MS, gas chromatography–mass spectrometry.

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MATERIALS AND METHODS

Materials

NB-598, (E)-N-ethyl-N-(6,6-dimethyl-2-hepten-4-ynyl)-3-[(3,3′-bithiophen-5-yl)methoxyl]benzenemethanamine, was synthesized in our laboratory. OSQ and DOSQ were prepared by the method of Field and Holmlund (13). Simvastatin was obtained from Merck Sharp & Dohme Research Laboratories (Rahway, NJ). [4,8,12,13,17,21-^{3}H]squalene was purchased from DuPont New England Nuclear (Boston, MA). Ni-NTA agarose was obtained from FUNAKOSHI (Tokyo, Japan). All other chemicals used were standard commercial high purity materials.

Bacterial expression of recombinant squalene epoxidase

We synthesized two primers, 5′-GAATTCCATATG-GAAGTCAATCTGTCAGAGAC-3′ and 5′-GAATTCG-GATCCTCAATGGTGATGGTGATGGTGAACCAGA-TACTTCATTT-3′, which contained sequences corresponding to amino acids 100–105 and 568–573 of rat SE, respectively. Polymerase chain reaction (PCR) was carried out using these primers and pUCTbl as a template. The PCR product was digested with Bam HI and Nde I, and this fragment was ligated into the Bam HI-Nde I large fragment of pET3a (9). This expression vector (pETRSEΔ99His) was sequenced and introduced into *E. coli* (DE3). The transformants containing pETRSEΔ99His were grown overnight at 37°C in LB medium containing 50 μg/ml ampicillin. The cells were then diluted 1:100 in LB medium and further incubated at 37°C until the A_{600nm} reached 0.6. The expression of rSE was induced by adding isopropyl-β-D(−)-thiogalactopyranoside (IPTG) at a final concentration of 0.4 mM and continuing incubation for another 4 h. The cells were harvested by centrifugation at 5000 *g* for 15 min and stored at −80°C until use.

Preparation of rat microsomes

Female Sprague-Dawley rats aged 6 weeks were given 0.5% simvastatin and 5% cholestyramine in their diet. Microsomal SE from rat liver was prepared according to Yamamoto and Bloch (6) with some modifications. Rats under ether anesthesia were bled from the heart and the livers were perfused with 50 ml of cold 0.1 M Tris-HCl (pH 7.5) via the portal vein, minced, and homogenized with 2 volumes of the same buffer. The homogenate was centrifuged twice at 9750 *g* for 10 min and the supernatant was recentrifuged at 105,000 *g* for 60 min. The microsomes were washed once with 0.1 M Tris-HCl buffer (pH 7.5) and used for the assay of SE. The 105,000 *g* supernatant was used as the S_{105} fraction. All of the fractions were kept at −80°C until use.

Enzyme assay

SE activity was determined according to the method of Tai and Bloch (7) with some modifications. The reaction mixture was incubated for 30 min at 37°C in a final volume of 0.3 ml containing 67 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM NADPH, 0.1 mM FAD, 0.1% Triton X-100, and 8 μM [^{3}H]squalene (20000 dpm) dispersed in 0.075% Tween 80. The enzyme 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 cyclohexane. 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 (Art 5583, E. Merck, Darmstadt, Germany) that was then developed on benzene–ethyl acetate 99.5:0.5. The band corresponding to authentic OSQ (R_f = 0.5) was scraped into a vial and the radioactivity was counted with a liquid scintillation counter (TRI-CARB 2500RT, Packard Instrument Co., Downers Grove, IL).

The NADPH-cytochrome c reductase reaction was performed in a temperature-controlled cell at 37°C using a spectrophotometer (U-3210, Hitachi, Tokyo, Japan). For determining enzyme activity, enzyme (1–10 μg protein) was added to 0.1 M potassium phosphate buffer (pH 7.5) containing 0.02 μmol cytochrome c and 0.1 μmol NADPH in a final volume of 1.0 ml. NADPH was added to initiate the reaction. One unit of the activity represented 1 μmol of cytochrome c reduction per min.

Determination of protein concentrations

Protein concentrations were determined according to the method of Smith et al. (14) using bovine serum albumin as a standard.

RESULTS

Purification of recombinant squalene epoxidase (rSE)

We previously isolated rat SE cDNA and expressed it in *E. coli* (9). Rat SE polypeptide deduced from the nucleotide sequence was thought to consist of 573 amino acids. The amino acid sequence revealed one potential transmembrane domain, a hydrophobic segment (Leu^{27} to Tyr^{43}) in the N-terminal. This region also contained a basic amino acid cluster (Lys^{95} to Lys^{99}). The expression vector of full-length rat SE (pETRSE) was transformed into *E. coli*. Although significant SE activity was observed in pETRSE containing transformant, the visible band of SE was not detected on SDS-PAGE. Therefore, we constructed two kinds of vectors, pETRSEΔ49 and pETRSEΔ99, which produced

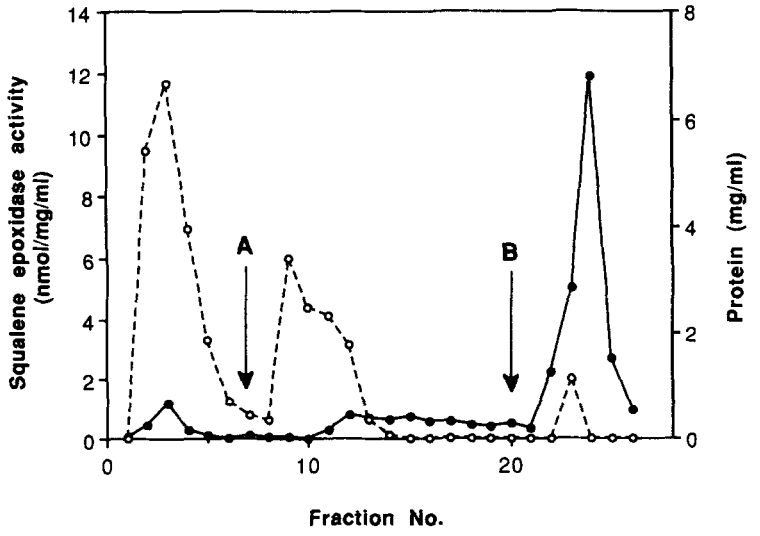


Fig. 1. Ni-chelate affinity chromatography of crude *E. coli* extract. The dialyzed *E. coli* extract (0.5 g protein) was fractionated in an Ni-NTA agarose column as described in the text. Elution was performed with (A) 50 mM glycine and (B) 100 mM imidazole. Seven-milliliter fractions were collected; (○) protein; (●) squalene epoxidase activity.

Cys^{45}–His^{573} and Glu^{100}–His^{573} fragments, respectively. These fragments showed significant SE activity. A visible band of rSE was observed on SDS-PAGE in pETRSEΔ99 transformant. We selected pETRSEΔ99 for the expression of rSE. Furthermore, a hexahistidine tag was added in the C-terminal of the Glu^{100}–His^{573} fragment for simple purification using a chelate affinity column.

An *E. coli* cell pellet (5 g) that was transformed with pETRSEΔ99His was suspended in 10 ml of lysis buffer (50 mM Tris-HCl, pH 8.0, containing 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, and 1 mg/ml lysozyme) and stirred for 1 h at 4°C. The cells were then lysed by freezing and thawing twice and by sonication. $MgCl_2$, $MnCl_2$, and DNaseI were added to final concentrations of 10 mM, 1 mM, and 50 μg/ml, respectively, and the mixture was stirred for 30 min at 4°C. The lysate was clarified by centrifugation at 12,000 *g* for 30 min. The supernatant was dialyzed against buffer A (20 mM Tris, pH 8.0, containing 0.5 M NaCl, 5% glycerol, and 0.5% Triton X-100) and then applied to an Ni-NTA (nitrilotriacetic acid) agarose column (1.8 × 12 cm) equilibrated with at least 5 column volumes of buffer A. After all the crude *E. coli* extract had entered the column, the column was washed with 2 volumes of buffer B (buffer A containing 1.0 M NaCl instead of 0.5 M NaCl) and 8 volumes of buffer B containing 50 mM glycine. As shown in **Fig. 1,** the elution of rSE was remarkably retarded on an Ni-NTA agarose column, while most of the contaminating *E. coli* proteins were eliminated after elution with 50 mM glycine. The bound rSE was eluted with buffer B containing 100 mM imidazole. Active fractions were dialyzed against buffer C (50 mM Tris, pH 7.4, containing 1 mM EDTA, 1 mM DTT, 5% glycerol and 0.5% Triton X-100). The dialyzed enzyme was applied to a Blue Sepharose CL-6B column (bed volume, 2 ml). After extensive washing of the column with buffer C containing 0.2 M KCl, rSE was eluted with buffer C containing 1.0 M KCl. Typical experimental results of the purification of rSE from crude *E. coli* extract are summarized in **Table 1.** The specific activity of the final preparation was about 170 nmol OSQ formed per mg protein per min, which corresponded to 100-fold purification with a yield

TABLE 1. Purification of recombinant squalene epoxidase

Step	Protein	Squalene Epoxidase		
		Total Activity	Specific Activity	Yield
	mg	*nmol/min*	*nmol/mg/min*	%
Extract	360.9	614	1.7	100
Ni-NTA-agarose	7.2	464	64	76
Blue Sepharose CL-6B	1.9	323	170	53

The enzyme assay of squalene epoxidase is described in Materials and Methods. The final concentration of Triton X-100 was adjusted 0.1%.

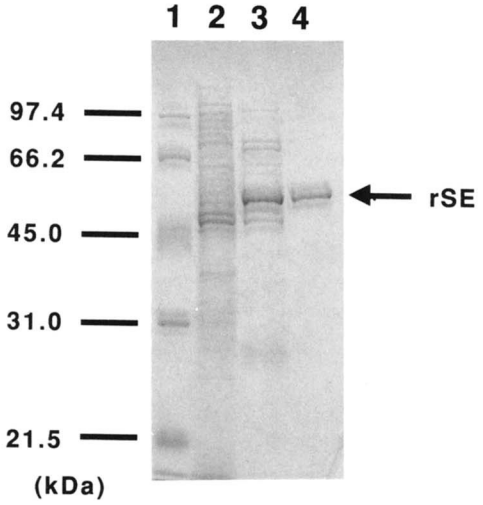


Fig. 2. SDS-PAGE analysis of recombinant squalene epoxidase fractions. SDS-polyacrylamide gel was stained with Coomassie brilliant blue; lane 1, molecular weight marker; lane 2, crude *E. coli* extract; lane 3, Ni-NTA-agarose fraction; lane 4, Blue Sepharose CL-6B fraction.

of about 52.7% on the basis of the crude *E. coli* extract. The patterns of SDS-PAGE in each step are shown in **Fig. 2.** Based on SDS-PAGE, the rSE was almost homogeneous and the apparent molecular weight was about 60,000.

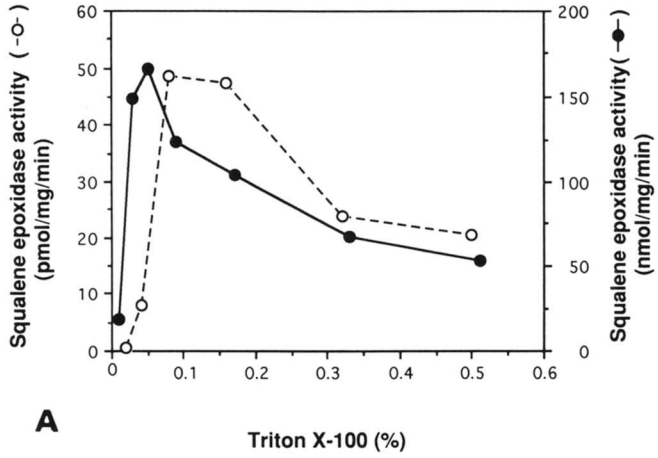
Properties of rSE

The properties of rSE were compared with those of rat microsomal SE. Rat SE in microsomes has been reported to be activated by S_{105} fraction and Triton X-100 (6, 7). As shown in **Table 2,** S_{105} fraction and Triton X-100 activated microsomal and recombinant SE in a concentration-dependent manner (**Fig. 3A**). In the case of the recombinant enzyme, some SE activity was detected even in the absence of Triton X-100, as the purified enzyme fraction contained a small amount of

TABLE 2. Effects of cytosolic (S_{105}) fraction on microsomal and recombinant squalene epoxidase

Enzyme	Additions	Squalene Epoxidase Activity	
		pmol/mg/min	*fold*
Rat microsome	none	1.5	(1.0)
	+S_{105} fraction	12.6	(8.5)
	+Triton X-100 (0.1%)	52.7	(36)
rSE	none	2680	(1.0)
	+S_{105} fraction	7960	(3.0)
	+Triton X-100 (0.05%)	91800	(34)

Squalene epoxidase activity was assayed at 250 μg/ml (microsomal enzyme) and 4.8 μg/ml (recombinant enzyme) protein in the presence of S_{105} fraction (1 mg protein) or Triton X-100.



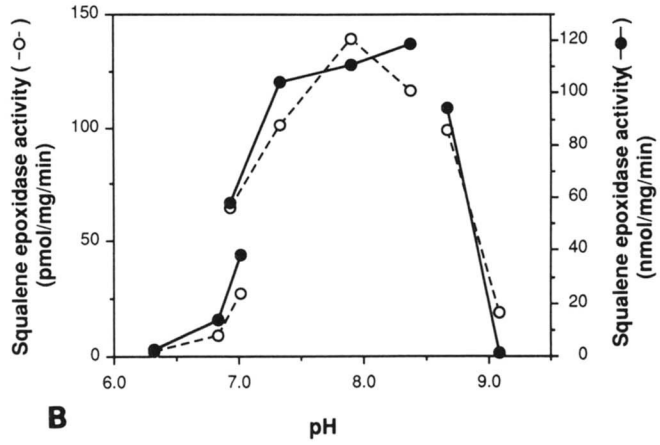


Fig. 3. Effects of Triton X-100 (A) and pH (B) on microsomal and recombinant squalene epoxidase activity. (A) Triton X-100 concentrations in the reaction mixture were varied as indicated. Microsomal enzyme (250 μg/ml) or recombinant enzyme (1.2 μg/ml) was incubated in the presence of 0.05 units of NADPH-cytochrome c reductase, 1 mM NADPH, and 0.1 mM FAD for 30 min. (B) Squalene epoxidase activity was assayed in 120 mM potassium phosphate buffer (pH 6–7), 120 mM Tris-HCl buffer (pH 7–8.5), or 120 mM glycine-NaOH buffer (pH 8.5–9.5). Each value represents the mean of duplicate determinations; (○) microsomes; (●) rSE.

Triton X-100. The enzyme activities were optimally activated by 0.05–0.15% Triton X-100. In the presence of 0.1% Triton X-100, the enzyme reaction was linear from 250 to 1100 μg/ml (microsomal enzyme) and 0.4 to 4 μg/ml (recombinant enzyme) (**Fig. 4A**). At 500 μg/ml (microsomal enzyme) and 1.6 μg/ml (recombinant enzyme) protein, OSQ formation was linear with an incubation time of up to 60 min in both enzyme preparations (Fig. 4B). The effects of pH on microsomal and recom-

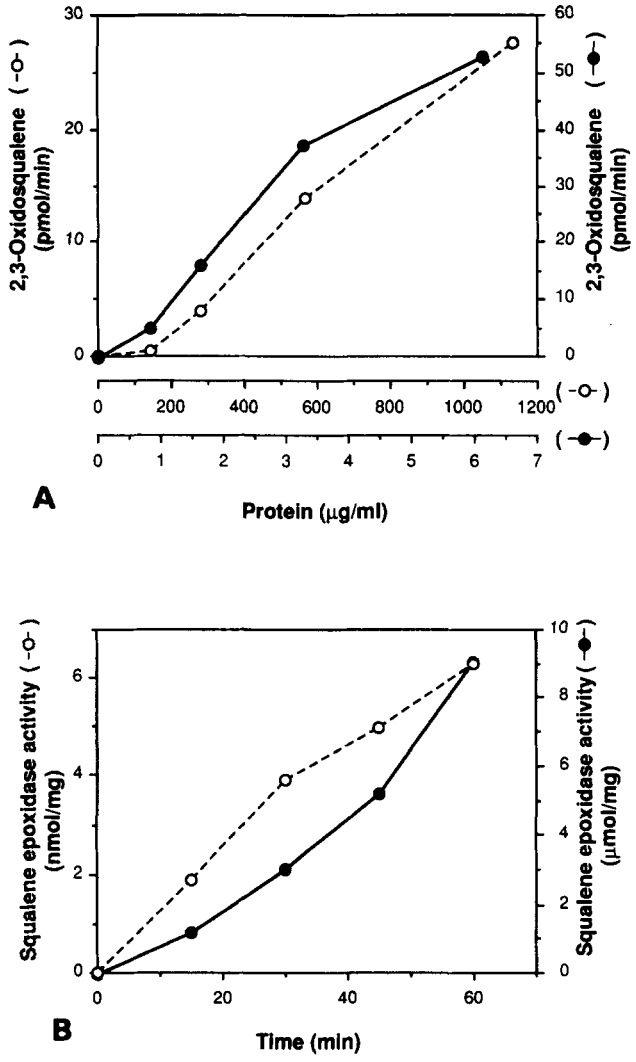


Fig. 4. Assay conditions of microsomal and recombinant squalene epoxidase activity. (A) Protein concentrations in the reaction mixture were varied as indicated. The enzyme reaction was performed with 0.1% Triton X-100. Other assay conditions were the same as in Fig. 3. (B) Incubation times were varied as indicated. Microsomal enzyme (500 μg/ml) or recombinant enzyme (1.6 μg/ml) was used in the enzyme reaction; (○) microsomes; (●) rSE.

binant SE are shown in Fig. 3B. Both enzymes showed bell-shaped pH-dependency, and the optimum pH was 7.5–8.5. NADPH cytochrome c reductase was required for rSE activity in a concentration-dependent manner (**Fig. 5**). NADPH and FAD were required for microsomal and recombinant SE activity in a similar manner (**Fig. 6**). Neither FMN nor riboflavin could be replaced by FAD (data not shown). Microsomal and recombinant SE had very similar properties although in rSE nucleotides coding 99 amino acids in the N-terminal were deleted and those coding hexahistidine in the C-terminal were added.

Effects of NB-598 on SE activity

The inhibitory activity of NB-598 was determined using microsomal and recombinant SE (**Fig. 7**). NB-598 was found to inhibit microsomal and recombinant SE with IC_{50} values of 3.2 and 1.9 nM, respectively. **Figure 8** shows the Lineweaver-Burk analysis of rSE by NB-598 over a range of squalene concentrations. The enzyme reaction was started by adding NADPH. NB-598 inhibited rSE in a competitive manner with respect to squalene. The K_m for squalene was found to be 3.6 μM, and the K_i for NB-598 was 0.41 nM.

Production of 2,3;22,23-dioxidosqualene (DOSQ) by recombinant squalene epoxidase (rSE)

Corey et al. (15) reported that DOSQ accumulated along with larger amounts of OSQ when the cyclization of OSQ by partially purified hog liver OSQ:lanosterol cyclase was inhibited by its inhibitor (2,3-iminosqualene). As shown in **Fig. 9** (A, lane 2), squalene was converted to lanosterol and cholesterol by rat liver microsomes. As indicated by Corey et al. (15) in the presence of AMO-1618 (an inhibitor of OSQ:lanosterol cyclase) (Fig. 9A, lane 3), the production of lanosterol and cholesterol was reduced and DOSQ was formed in rat liver microsomes. To examine the conversion of OSQ to DOSQ by SE, a completely reconstituted SE system was prepared using purified rSE and NADPH-cytochrome c reductase. Figure 9 (A, lane 4) showed that rSE formed OSQ and DOSQ in the reconstituted enzyme system. DOSQ was confirmed with an authentic sample using TLC and GC–MS. The time course of the formation of reaction products by rSE is shown in Fig. 9 (B). Squalene disappeared time-dependently in the

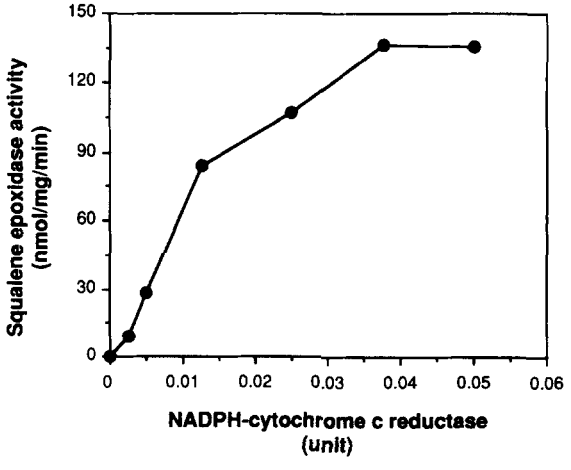
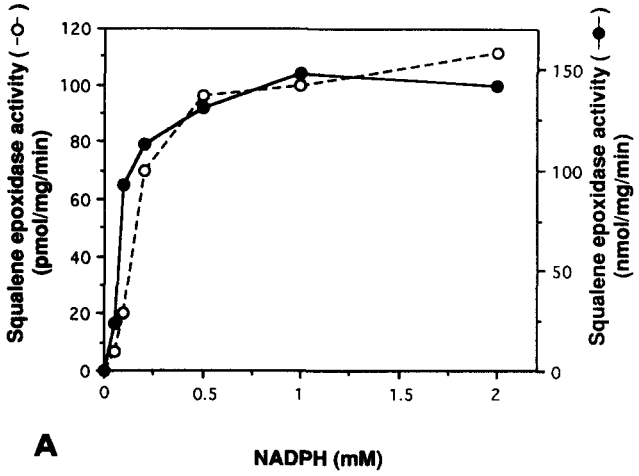


Fig. 5. Effects of NADPH-cytochrome c reductase on recombinant squalene epoxidase activity. NADPH-cytochrome c reductase concentrations in the reaction mixture were varied as indicated. Protein (1.6 μg/ml) was used in the enzyme reaction. Other assay conditions were the same as in Fig. 4.



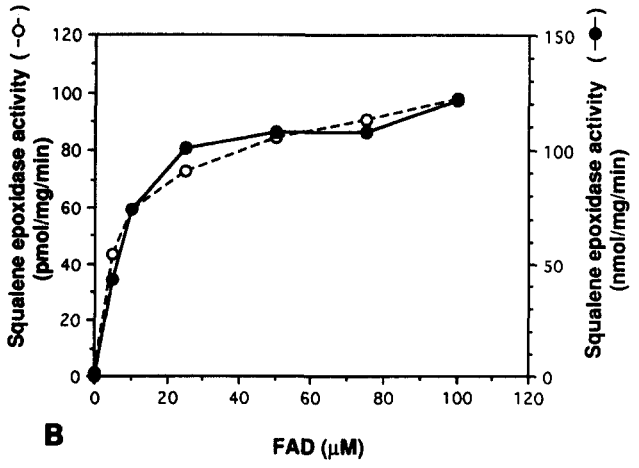


Fig. 6. Effects of co-factors on microsomal and recombinant squalene epoxidase activity. NADPH (A) and FAD (B) concentrations in the reaction mixture were varied as indicated. Other assay conditions were the same as in Fig. 4; (○), microsomes; (●), rSE.

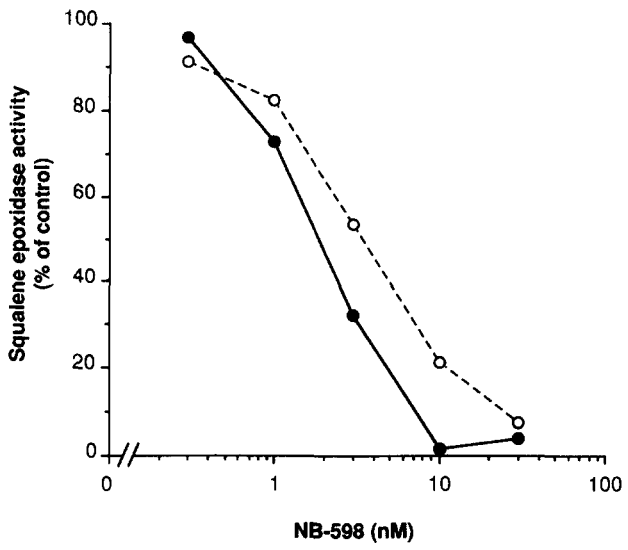


Fig. 7. Effects of NB-598 on microsomal and recombinant squalene epoxidase activity. NB-598 concentrations in the reaction mixture were varied as indicated. Other conditions were the same as in Fig. 4; (○) microsomes; (●) rSE.

reconstituted system. OSQ appeared in a time-dependent manner, reached a plateau at 5 h, and then decreased. DOSQ developed after 3 h of incubation and increased time-dependently. In the absence of SE, OSQ and DOSQ were not detected. These results indicated that DOSQ was converted from OSQ by rSE.

DISCUSSION

Many attempts have been made to purify microsomal electron transport components associated with mixed-function oxygenase activity (16). We previously purified SE from rat liver microsomes (1, 2) using Cibacron Blue Sepharose and a chromatofocusing column. However, this procedure was very complicated and recovery was low. An exceedingly low level of activity and the instability of SE make it difficult to purify the enzyme from rat liver microsomes. Therefore, until now there were no applicable procedures for the purification of SE.

We recently isolated and sequenced rat SE cDNA that contained 2230 bp nucleotides coding 573 amino acids (9). For the efficient expression and simple purification of the enzyme, an expression system was constructed in *E. coli* using SE cDNA in which nucleotides coding 99 amino acids in the N-terminal were deleted and nucleotides coding hexahistidine in the C-terminal were added. The hexahistidine in the C-terminal made it easy to purify rSE using a chelate affinity column. Immobilized metal ion affinity chromatography for the purification of naturally occurring proteins was discovered by Porath et al. (17). The proteins bind to the metal ion(s) through the side chains of amino acid residues capable of donating electrons. Potential electron donor groups are cysteine and histidine. Ni-NTA, a metal chelate adsorbent, was introduced by Hochuli, Dobeli, and Schacher (18) as a resin that bound the metal ion tightly. Therefore, Ni-chelate affinity chromatography in combination with the hexahistidine affinity tail provides a universal purification method for recombinant proteins. The purification method described here is a rapid two-step procedure based on Ni-chelate affinity chromatography followed by dye-ligand chromatography and results in the isolation of homogenous enzyme with a greater than 50% yield.

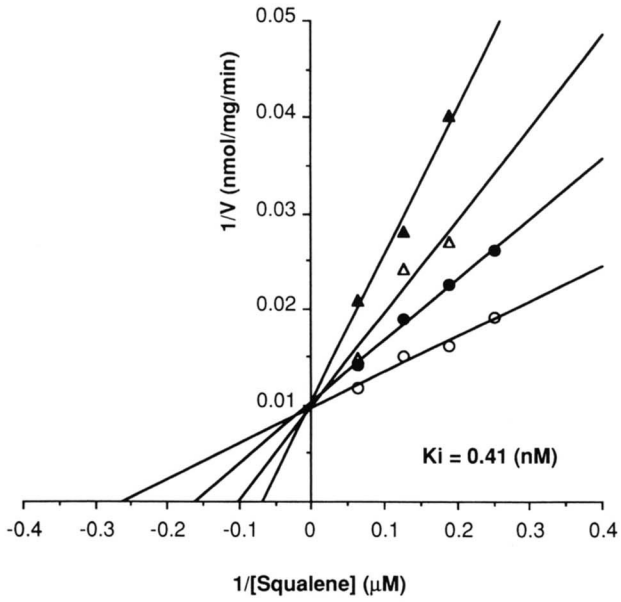


Fig. 8. Lineweaver-Burk plot of recombinant squalene epoxidase inhibition by NB-598. Squalene epoxidase activity was assayed under standard conditions. The enzyme reaction was performed in (○) vehicle control or in the presence of NB-598 (●) 1 nM; (;to) 3 nM; and (▲) 5 nM.

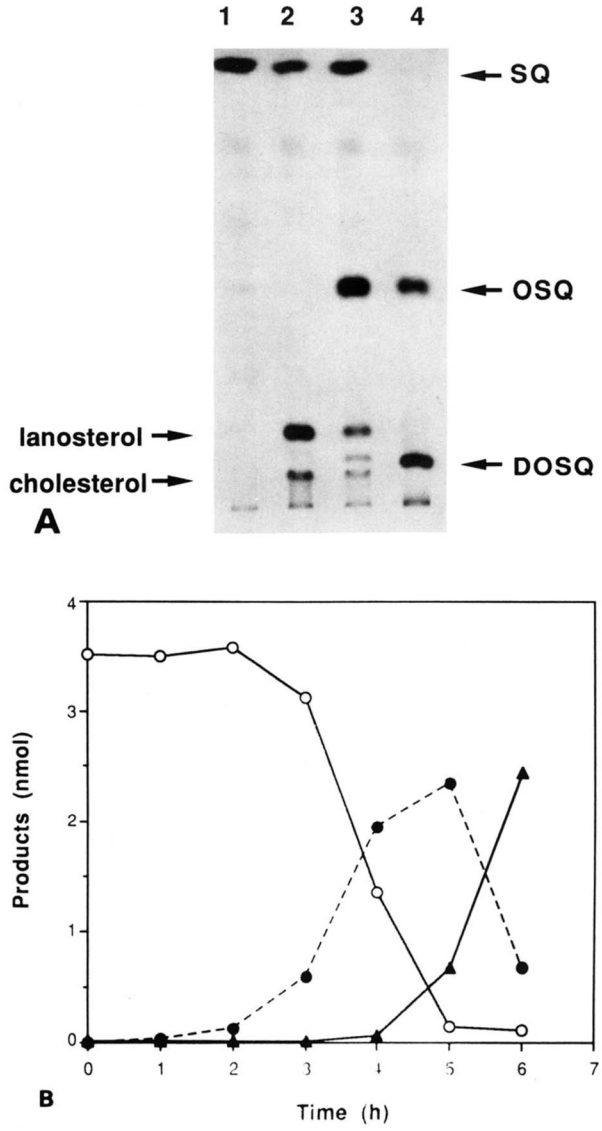


Fig. 9. Reaction products from squalene indicated by recombinant squalene epoxidase. (A) The enzyme reaction was performed with 1 mg/ml (microsomal enzyme) or 2 μg/ml (recombinant enzyme) protein. The reaction products were separated by TLC using benzene/ethyl acetate (99.5:0.5) and identified by TLC and GC–MS with authentic samples. Lane 1, blank; lane 2, microsomes (in the absence of AMO 1618); lane 3, microsomes (in the presence of 30 mM AMO 1618); lane 4, rSE. (B) Incubation times were varied as indicated. The enzyme reaction was performed with 1 mg/ml (microsomal enzyme) or 2 μg/ml (recombinant enzyme) protein; (○) squalene; (●) 2,3-oxidosqualene; and (▲) 2,3;22,23-dioxidosqualene. Other conditions were the same as in Fig. 4.

SE is believed to be a microsomal membrane-bound protein. Rat SE requires FAD, NADPH-cytochrome c reductase, NADPH, and S_{105} fraction for its activity (2, 6–8). S_{105} fraction can be replaced by supernatant protein factor (SPF) and phospholipids (7). These two cytoplasmic components can be displaced by Triton X-100 (8). Activation of the enzyme by Triton X-100 is thought to occur via a conformational change in the enzyme, an increased rate of enzyme diffusion, an association between the enzyme and substrate, or an effect on enzyme kinetic parameters.

The properties of purified rSE were compared with those of rat liver microsomal SE. The requirement of the co-factors, S_{105} fraction or Triton X-100 and NADPH-cytochrome c reductase, the pH dependency of enzyme activity and the sensitivity to NB-598 of both enzymes suggest that rSE has properties very similar to rat microsomal SE. The purest preparation showed no distinct absorption spectrum in the visible regions (data not shown). Recombinant SE is not thought to be an enzyme containing tightly bound FAD. As SE requires FAD for activity and probably has an FAD binding sequence (9), purified rSE may be thought of as an apoenzyme with easily dissociable FAD as its prosthetic group. The specific activity of recombinant enzyme was much higher than that of native enzyme reported by us (2). The introduction of a hexahistidine tag in rSE may affect the reaction system of epoxidation and increase the specific activity of recombinant enzyme.

The synthesis of DOSQ from mevalonate and 24(S),25-epoxycholesterol from diepoxide by rat liver homogenates was reported by Nelson, Steckbeck, and

Spencer (19). Because oxygenated sterols such as 25-hydroxycholesterol are known to be potent regulators of cholesterol biosynthesis in cultured cells (20, 21), it is important to determine an enzyme system that will generate oxygenated sterols. Recently, Gupta, Sexton, and Rudney (22) demonstrated the existence of a pathway for the formation of oxysterol via the conversion of OSQ to DOSQ. Also, recently, Bai, Xiao, and Prestwich (23) reported that partially purified SE from pig liver converted OSQ to DOSQ with approximately one-half the efficiency of the epoxidation of squalene. We demonstrated here that purified rSE formed DOSQ from squalene in a completely reconstituted system. Kameda, Ono, and Imai (24) suggested that hydrogen peroxide produced from superoxide anions was decomposed to form hydroxyl radicals by the action of the Fe-EDTA complex in the lipid peroxidation system, and that the hydroxyl radical is a trigger of lipid peroxidation. As catalase, superoxide dismutase, and mannitol had no effect on squalene epoxidation, active oxygen species are not thought to participate in the SE system. Therefore, the conversion of squalene to OSQ and of OSQ to DOSQ was catalyzed specifically by SE.

Many studies have examined the regulatory mechanism of HMG-CoA reductase (25), 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 oxysterol (26). However, little is known about the regulation of SE, which catalyzes the reaction in the middle stage of the cholesterol biosynthetic pathway, because there is little information about the structure and function of SE. Isolation of the genomic clone of SE will allow studies of the genomic regulation of SE.

HMG-CoA reductase inhibitors block mevalonate production, which is part of an early step in the cholesterol synthetic pathway (27). Therefore, these compounds are thought to inhibit the synthesis of other biologically important substances derived from mevalonate. However, SE occurs in the middle of the cholesterol synthetic pathway. We previously reported that NB-598 increased the number of LDL receptors to a remarkable extent but did not increase HMG-CoA reductase activity as compared an with HMG-CoA reductase inhibitor in HepG2 cells (28). HMG-CoA reductase has been reported to be controlled through multivalent regulation (29). Full suppression of reductase activity is mediated by sterols and non-sterol substance(s) derived from mevalonate. Sterol-mediated control of reductase activity is exerted at the transcriptional level, whereas non-sterol substance(s) are thought to operate primarily at the post-transcriptional level (30–32). SE inhibitor did not prevent the synthesis of the non-sterol product of mevalonate that has been thought to regulate HMG-CoA reductase activity post-transcriptionally (28). The non-sterol suppressor is considered to be an intermediate or metabolite between mevalonate and squalene in the cholesterol biosynthetic pathway. On the other hand, SE inhibitor is thought to increase the intracellular level of squalene. However, squalene has been reported to be a safe substance in feeding experiments (33). Therefore, SE should be a good target for developing useful lipid-lowering agents. Mass production of SE using this recombinant system will help the development of new SE inhibitors.

In the present study, we developed a simple purification method for rSE and presented some properties of the purified enzyme. Using purified SE, we also demonstrated that the enzyme catalyzes the conversion of squalene to OSQ and DOSQ. To examine the structure and function of SE, crystallization of rSE will be necessary for X-ray diffraction studies.

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