JOURNAL OF BACTERIOLOGY, Jan. 1996, p. 19–23 Vol. 178, No. 1
0021-9193/96/$04.00+0

3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase from *Haloferax volcanii*: Purification, Characterization, and Expression in *Escherichia coli*†

KENNETH M. BISCHOFF‡ AND VICTOR W. RODWELL*

Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907-1153

Received 17 May 1995/Accepted 23 October 1995

Prior work from this laboratory characterized eukaryotic (hamster) and eubacterial (*Pseudomonas mevalonii*) 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductases. We report here the characterization of an HMG-CoA reductase from the third domain, the archaea. HMG-CoA reductase of the halobacterium *Haloferax volcanii* was initially partially purified from extracts of *H. volcanii*. Subsequently, a portion of the *H. volcanii* lovastatin (formerly called mevinolin) resistance marker *mev* was subcloned into the *Escherichia coli* expression vector pT7-7. While no HMG-CoA reductase activity was detectable following expression in *E. coli*, activity could be recovered after extracts were exposed to 3 M KCl. Following purification to electrophoretic homogeneity, the specific activity of the expressed enzyme, 24 μU/mg, equaled that of homogeneous hamster or *P. mevalonii* HMG-CoA reductase. Activity was optimal at pH 7.3. K_ms were 66 μM (NADPH) and 60 μM [(*S*)-HMG-CoA]. (*R*)-HMG-CoA and lovastatin inhibited competitively with (*S*)-HMG-CoA. *H. volcanii* HMG-CoA reductase also catalyzed the reduction of mevaldehyde {optimal activity at pH 6.0; V_{max} 11 μU/mg; K_ms 32 μM (NADPH), 550 μM [(*R*,*S*)-mevaldehyde]} and the oxidative acylation of mevaldehyde {optimal activity at pH 8.0; V_{max} 2.1 μU/mg; K_ms 350 μM ($NADP^+$), 300 μM (CoA), 470 μM [(*R*,*S*)-mevaldehyde]}. These properties are comparable to those of hamster and *P. mevalonii* HMG-CoA reductases, suggesting a similar catalytic mechanism.

The genetic and biochemical properties of living organisms have led to their classification into three domains: the eukaryotes, the eubacteria or true bacteria, and the archaea (26). The representatives of the third domain, the archaea, inhabit extreme ecological niches such as the Dead Sea, hot springs, and oceanic thermal vents. The gross morphological features of the archaea resemble those of the eubacteria, and in both archaea and eubacteria DNA replication, transcription, and protein synthesis occur in the cytosol. However, the proteins of the archaea exhibit closer homology to eukaryotic proteins than to their eubacterial counterparts (7, 27).

A striking characteristic of the archaea is that their membrane lipids consist primarily of mevalonate-derived isoprenoid glycerol ethers rather than the fatty acid esters characteristic of eubacteria and eukaryotes (8, 16). In eukaryotes, mevalonate is formed in a reaction catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; EC 1.1.1.34). This enzyme reductively deacylates the thioester group of HMG-CoA to the primary alcohol group of mevalonate in a reaction that consumes 2 equivalents of NADPH and proceeds without the dissociation of intermediates, viz.: HMG-CoA + 2 NADPH + 2 H^+→mevalonate + CoA + 2 $NADP^+$.

In eukaryotes, this reaction is rate limiting for the biosynthesis of isoprenoids and hence serves as the target enzyme for cholesterol-lowering drugs such as lovastatin (formerly called mevinolin), a potent inhibitor of eukaryotic HMG-CoA reductase (1). A considerable body of literature documents the properties of HMG-CoA reductases from lower and higher eukaryotes. Although no truly biosynthetic HMG-CoA reductase has ever been detected in eubacteria, an NAD^+-dependent, biodegradative HMG-CoA reductase from *Pseudomonas mevalonii* has been extensively studied (2, 6, 13–15, 25) and its crystal structure has been solved at 3 Å (0.3 nm) resolution (21). HMG-CoA reductase activity has been detected in extracts of the archaeon *Halobacterium halobium* (4). However, there are no reports of the purification or characterization of an archaeal HMG-CoA reductase.

Recently, the gene for a presumptively biosynthetic HMG-CoA reductase from the archaeon *Haloferax volcanii* was cloned by selecting for its ability to confer resistance to lovastatin (19). Lovastatin resistance was shown to result from the overexpression of a gene, present on the resistance determinant *mev*, that encodes a 403-aminoacyl-residue protein that exhibits 40% primary structural identity to mammalian HMG-CoA reductases (19).

To complement our study of the catalysis and regulation of the activity of HMG-CoA reductases and to extend our analyses to all three domains, we report here the expression in *Escherichia coli*, purification, and characterization of the first biosynthetic HMG-CoA reductase from a noneukaryote, that of the halophilic archaeon *H. volcanii.*

(The data in this report were derived in part from the Ph.D. thesis of Kenneth M. Bischoff [2a]).

MATERIALS AND METHODS

Materials. Purchased materials included NADPH, $NADP^+$, (*R*,*S*)-HMG-CoA, coenzyme A, phenylmethylsulfonyl fluoride, mevaldehyde precursor, anti-rabbit immunoglobulin G alkaline phosphatase conjugate, 5-bromo-4-chloro-3-indolylphosphate, and nitroblue tetrazolium chloride (Sigma); hydroxylapatite, DEAE cellulose (Cellex D), and both prestained and unstained protein molecular mass standards for sodium dodecyl sulfate-polyacrylamide gel electrophore-

* Corresponding author. Phone: (317) 494-1608 or (317) 494-1606. Fax: (317) 494-7897. Electronic mail address: rodwell@biochem.purdue.edu.

† This is journal paper 14571 from the Purdue University Agricultural Experiment Station.

‡ Present address: Dept. of Biochemistry and Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

sis (SDS-PAGE) (Bio-Rad); Sepharose CL-4B, Sephadex G-150, and Sephadex G-50 (Pharmacia); isopropyl thiogalactoside (U.S. Biochemicals); and a PM-30 ultrafiltration membrane (Amicon). (*S*)-HMG-CoA and (*R*)-HMG-CoA were prepared as previously described (3). Lovastatin, a gift from Al Alberts of Merck & Co., Rahway, N.J., was dissolved in dimethylsulfoxide for use. Polyclonal antibodies to rat liver HMG-CoA reductase were a gift from Joe Papiez and David Gibson, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Ind. *H. volcanii* WFD11 (5) and the *E. coli-H. volcanii* shuttle vector pWL102, which contains the lovastatin resistance determinant *mev* for selection in *H. volcanii* (18), were gifts from Wan Lam and W. Ford Doolittle of the Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia. Hamster HMG-CoA reductase, the homogeneous catalytic domain of Syrian hamster HMG-CoA reductase (11), was provided by Joe Ross, Department of Chemistry, Central State University, Wilberforce, Ohio. All other materials were from previously listed sources (6).

Buffered solutions. Buffer A contained 3.0 M KCl, 5.0 mM dithiothreitol (DTT), and 50 mM K_xPO_4 (pH 6.6 or 7.3). Buffer B contained 5.0 mM DTT and 25 mM K_xPO_4 (pH 6.6). Buffer C contained 3.0 M KCl, 5.0 mM DTT, 50 mM Tris, 50 mM K_xPO_4, and 50 mM glycine (adjusted either to pH 6.5 or 8.5). DTT was added to solutions just prior to use.

Growth media. One liter of ML medium (17) for the growth of *H. volcanii* contained 5 g of tryptone, 5 g of yeast extract, 125 g of NaCl, 50 g of $MgCl_2 \cdot 6H_2O$, 5 g of K_2SO_4, and 0.12 g of $CaCl_2$, pH 7.5. One liter of LB_{amp} medium for the growth of *E. coli* contained 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 0.1 g of ampicillin.

Assay of HMG-CoA reductase activities. Three reactions were assayed, each in a final volume of 200 μl. Oxidation or reduction of NADP(H) was monitored at 340 nm in a Hewlett-Packard model 8452 diode array spectrophotometer equipped with a cell holder maintained at 37°C. For the reductive deacylation of HMG-CoA, standard assays contained 0.2 mM NADPH and 0.5 mM (*R*,*S*)-HMG-CoA in Buffer A (pH 7.3). For the reduction of mevaldehyde, standard assays contained 0.2 mM NADPH, 3.0 mM (*R*,*S*)-mevaldehyde, and 1.0 mM coenzyme A in Buffer C (pH 6.5). While not a stoichiometric reactant, coenzyme A greatly stimulated mevaldehyde reduction. For the oxidative acylation of mevaldehyde, standard assays contained 3.5 mM $NADP^+$, 5.0 mM CoA, and 3.0 mM (*R*,*S*)-mevaldehyde in Buffer C (pH 8.5). Except where otherwise noted, all assays were initiated by the addition of substrate (HMG-CoA or mevaldehyde). For all three reactions, 1 enzyme unit (μU) represents the turnover, in 1 min, of 1 μmol of NADP(H).

Data for calculation of kinetic parameters employed assays conducted under standard conditions except for the concentration of the varied substrate. Varied substrate concentrations were as follows: (*S*)-HMG-CoA, 0.04 to 0.2 mM; (*R*,*S*)-mevaldehyde, 0.2 to 1.0 mM; CoA, 0.1 to 0.5 mM; NADPH, 0.03 to 0.1 mM; and $NADP^+$, 0.25 to 1.0 mM. V_{max} and K_m values were then calculated by the statistical evaluation of Lineweaver-Burk plots of initial velocity versus substrate concentration.

Western blotting. Western blotting (immunoblotting) employed rabbit polyclonal antibodies raised against rat liver HMG-CoA reductase as the primary antibody and anti-rabbit immunoglobulin G alkaline phosphatase conjugate as the secondary antibody. The blot was developed with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium chloride (24).

Partial purification of HMG-CoA reductase from *H. volcanii*. *H. volcanii* was grown at 45°C, with shaking at 250 rpm, in 2.8-liter Fernbach flasks containing 1 liter of ML medium. At a culture density of about 300 Klett units (red filter), cells were harvested by centrifugation (4,000 × *g*, 20 min, 4°C), suspended in Buffer A, and again centrifuged. The washed cells were suspended in Buffer A (15 ml/liter of culture), ruptured by passage twice through a French pressure cell operated at 10,000 lb/in^2, and centrifuged (100,000 × *g*, 30 min, 15°C). The supernatant liquid, the cytosol fraction, was dialyzed overnight at 4°C against 1.7 M $(NH_4)_2SO_4$ in Buffer B and then centrifuged (100,000 × *g*, 30 min, 15°C). The precipitated protein was discarded. The $(NH_4)_2SO_4$ concentration of the supernatant liquid was then adjusted to 2.5 M by the addition of solid $(NH_4)_2SO_4$. After 60 min at room temperature (25°C), additional precipitated protein was removed by centrifugation and discarded. The supernatant liquid was retained as the ammonium sulfate fraction.

The ammonium sulfate fraction was heated to and maintained at 70°C for 10 min, allowed to cool to 25°C, and centrifuged. The supernatant liquid, the heat fraction, was applied to a Sepharose CL-4B column (1.5 by 22 cm) equilibrated with 2.5 M $(NH_4)_2SO_4$ in Buffer B and washed in with 100 ml of the same solution. The column was then eluted with 500 ml (total volume) of a decreasing gradient of 2.5 to 0.5 M $(NH_4)_2SO_4$ in Buffer B. Fractions containing HMG-CoA reductase activity were combined and concentrated by ultrafiltration through an Amicon PM-30 membrane to give the Sepharose fraction.

To facilitate buffer exchange, the Sepharose fraction was passed through a Sephadex G-50 column (2.5 by 17 cm) equilibrated in Buffer A and then applied to a hydroxylapatite column (1.5 by 15 cm) in Buffer A. The hydroxylapatite column was washed with 3 column volumes of Buffer A and then eluted with 400 ml (total volume) of 3.0 M KCl in an increasing gradient of 50 to 300 mM K_xPO_4, pH 6.6. Active fractions were combined and concentrated by ultrafiltration through a PM-30 membrane to give the hydroxylapatite fraction.

The hydroxylapatite fraction was applied to a Sephadex G-150 superfine column (1.5 by 24 cm) in Buffer A and eluted with Buffer A. Active fractions were combined and concentrated by ultrafiltration to give the G-150 fraction, which was stored in liquid nitrogen. Table 1 summarizes a typical purification.

TABLE 1. Purification of HMG-CoA reductase from extracts of *H. volcanii*[a]

Fraction	Activity (μU)	Protein (mg)	Sp act (μU/mg)	Enrichment (fold)	Yield (%)
Cytosol	16	1,140	0.014	1.0	100
Sepharose	17.5	230	0.077	5.5	100
Hydroxylapatite	6.8	25	0.27	19	43
G-150	3.85	3.4	1.2	83	24

[a] The data are for the purification of native enzyme from 7 liters of *H. volcanii* culture.

Subcloning of the *H. volcanii* HMG-CoA reductase gene from pWL102 into the expression vector pT7-7 and expression of *H. volcanii* HMG-CoA reductase in *E. coli*. The 3.5-kbp lovastatin resistance marker *mev* was excised from pWL102 (18) by digestion with *Sph*I and *Xba*I and ligated into the polylinker region of M13mp18, forming M13-mev^r. An Amersham oligonucleotide-directed mutagenesis kit was then used to change the AGCATG, which contains the translation start site of the HMG-CoA reductase gene, to CATATG. This change created an *Nde*I site at the start of the HMG-CoA reductase gene and formed M13KB-1. The 3.5-kbp *mev* resistance fragment excised from M13KB-1 with *Sph*I and *Bam*HI was digested with *Nde*I, and the 1.4-kbp fragment that encodes HMG-CoA reductase was subcloned into the *Nde*I and *Bam*HI sites of pT7-7 to create the expression plasmid pT7-Vred, in which the *H. volcanii* HMG-CoA reductase gene is under the control of the T7 promoter.

Cells harboring pT7-Vred were grown in LB_{amp} medium at 37°C to a culture density of 80 Klett units (red filter). Following the addition of isopropyl thiogalactoside (final concentration, 0.5 mM), cell growth was continued to a culture density of 200 Klett units. Cells were harvested by centrifugation, washed with 0.9% NaCl, suspended in 150 mM KCl–5 mM DTT–1 mM phenylmethylsulfonyl fluoride–10% (vol/vol) glycerol–20 mM Tris (pH 8.3) (15 ml/g of dry cell equivalent), and ruptured by passage twice through a French pressure cell. Following centrifugation of the cell lysate (100,000 × *g*, 30 min, 4°C), the supernatant liquid was retained as the cytosol fraction, which was catalytically inactive.

The activation of the cytosol fraction was achieved by the addition of solid KCl to a concentration of 3.0 M. NaCl or $(NH_4)_2SO_4$ also activated the enzyme (Fig. 1). Maximal activity was attained after approximately 6 h at 4°C. Following centrifugation to remove precipitated protein, the supernatant liquid, i.e., the activated cytosol, was dialyzed against 20 volumes of 1.9 M $(NH_4)_2SO_4$ in Buffer B. After centrifugation to remove precipitated protein, the supernatant liquid was retained as the ammonium sulfate fraction.

The ammonium sulfate fraction was heated to and maintained at 70°C for 10 min, allowed to cool to room temperature, and centrifuged to remove denatured protein. The supernatant liquid, the heat fraction, was applied to a DEAE-

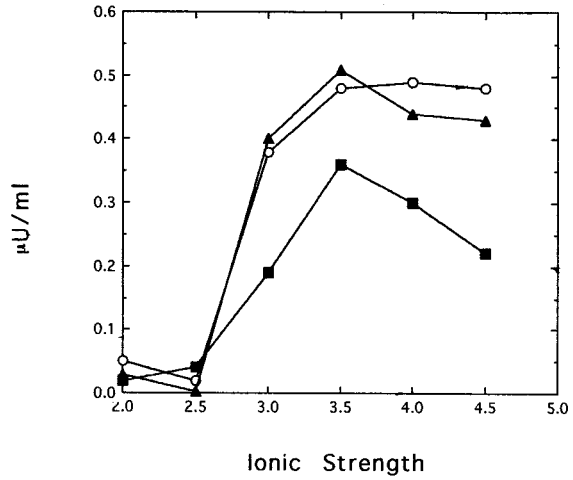

FIG. 1. Activation by different salts. $(NH_4)_2SO_4$ (■), NaCl (○), or KCl (▲) was added to the cytosol fraction of the expressed enzyme at the indicated ionic strengths. Following mild agitation to dissolve the salt, the tubes were incubated at 4°C for 15 h. Following centrifugation to remove precipitated protein, the supernatant liquid was assayed for HMG-CoA reductase activity.

TABLE 2. Purification of *H. volcanii* HMG-CoA reductase expressed in *E. coli*[a]

Fraction	Activity (μU)	Protein (mg)	Sp act (μU/mg)	Enrichment (fold)	Yield (%)
Cytosol	0	209			
Activated cytosol	23.1	160	0.14	1	100
Ammonium sulfate	21.6	40	0.54	4	93
Heat	31.4	14	2.2	16	136
DEAE	14.8	0.6	24	170	64

[a] The data are for the purification of enzyme from 1 liter of *E. coli* BL21(DE3)/pT7-Vred culture. Since no activity was detectable in unactivated cytosol, the recovery of enzyme units is based on the activated cytosol fraction.

cellulose column (1.5 by 6.5 cm) equilibrated with 1.9 M $(NH_4)_2SO_4$ in Buffer B and washed in with 100 ml of the same solution. Most of the HMG-CoA reductase, but few *E. coli* proteins, bound to this support. The column was eluted with 100 ml of 1.5 M $(NH_4)_2SO_4$ in Buffer B. Active fractions were combined and concentrated by ultrafiltration through a PM-30 membrane to give the DEAE fraction. Table 2 and Fig. 2 summarize the purification.

RESULTS

Optimal activity requires KCl. The maximal activity of HMG-CoA reductase from extracts of *H. volcanii* was observed only in the presence of high concentrations of salts. KCl was more effective than NaCl (Fig. 3). In the presence of 3 M KCl, optimal activity for the reductive deacylation of HMG-CoA occurred at pH 7.3 and K_m values for (*S*)-HMG-CoA and NADPH were 46 μM and 66 μM, respectively (data not shown).

Subunit size and cross-reactivity with antibodies raised against rat liver HMG-CoA reductase. SDS-PAGE of the G-150 fraction revealed a prominent band which cross-reacted with polyclonal antibodies specific for rat HMG-CoA reductase and had an apparent size of 52 kDa, a value greater than the anticipated size of 41.2 kDa predicted from the DNA-derived amino acid sequence (19) (Fig. 2, lanes G). Since intrinsic charge can lead to anomalous migration on SDS-polyacrylamide gels (9, 22), this difference may reflect the acidic nature of *H. volcanii* HMG-CoA reductase, which has a pI estimated by the Isoelectric program of the Wisconsin Genetics Computer Group package of 4.5.

Reductive deacylation of HMG-CoA. The optimal activity of

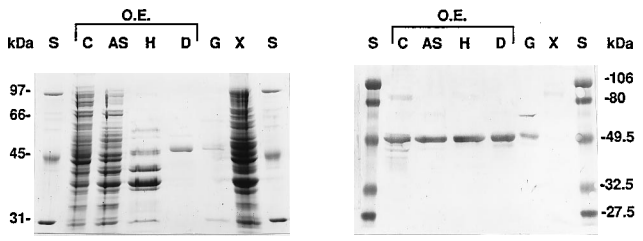

FIG. 2. SDS-PAGE and Western blotting of *H. volcanii* HMG-CoA reductase. Left panel: gel stained with Coomassie blue. Lane S contains the unstained standard proteins phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (31 kDa). Right panel: Western blot analysis using antibodies raised against rat HMG-CoA reductase. Lane S contains prestained standard proteins of the indicated apparent mass: phosphorylase *b* (106 kDa), bovine serum albumin (80 kDa), ovalbumin (49.5 kDa), carbonic anhydrase (32.5 kDa), and soybean trypsin inhibitor (27.5 kDa). Shown for the expressed enzymes (O.E.) are data for the activated cytosol (lanes C), ammonium sulfate fraction (lanes AS), heat fraction (lanes H), and DEAE fraction (lanes D). Lanes G represent the G-150 fraction of the enzyme purified from *H. volcanii*, and lanes X contain an extract of BL21(DE3) cells harboring plasmid pT7-7, which lacks the insert which encodes *H. volcanii* HMG-CoA reductase.

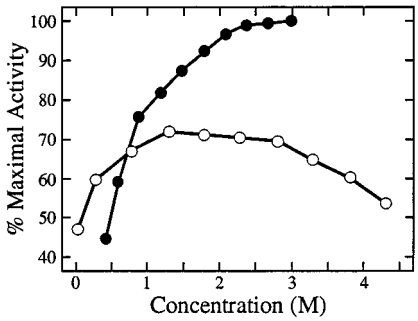

FIG. 3. Optimal activity is expressed in the presence of 3 M KCl. The G-150 fraction of HMG-CoA reductase from extracts of *H. volcanii* was assayed in the presence of the indicated concentrations of KCl (●) or NaCl (○) under otherwise standard conditions. Data are expressed relative to the maximal observed activity in 3.0 M KCl (1.2 μU/mg).

HMG-CoA reductase expressed in *E. coli* for the reductive deacylation of HMG-CoA to mevalonate occurred at pH 7.3 (Fig. 4) with a V_{max} of 34 μU/mg. K_m values for the overall reaction were 66 μM for NADPH and 60 μM for (*S*)-HMG-CoA. In addition to the overall reaction, *H. volcanii* HMG-CoA reductase catalyzed two half-reactions of the putative enzyme-bound intermediate mevaldehyde.

Reduction of mevaldehyde to mevalonate. For the reduction of mevaldehyde to mevalonate, optimal activity occurred at pH 6.0 with a V_{max} of 11 μU/mg. K_m values were 550 μM for (*R*,*S*)-mevaldehyde and 32 μM for NADPH. When K_m equals K_i, the apparent K_m estimated from a plot of 1/velocity versus 1/total racemic substrate concentration equals the true K_m determined with only the physiologically active isomer (3). K_m values determined with (*R*,*S*)-mevaldehyde thus probably reflect K_m values for the physiologic isomer.

Oxidative acylation of mevaldehyde to HMG-CoA. Optimal activity for the oxidative acylation of mevaldehyde to HMG-CoA occurred at pH 8.0 (Fig. 4) with a V_{max} of 2.1 μU/mg. K_m values were 470 μM for (*R*,*S*)-mevaldehyde, 350 μM for $NADP^+$, and 300 μM for CoA.

Inhibition by (*R*)-HMG-CoA. *H. volcanii* HMG-CoA reduc-

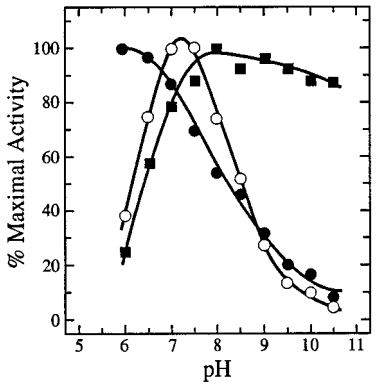

FIG. 4. Effect of pH on reactions catalyzed by *H. volcanii* HMG-CoA reductase expressed in *E. coli*. Shown are data for the reductive deacylation of HMG-CoA to mevalonate (○), the reduction of mevaldehyde to mevalonate (●), and the oxidative acylation of mevaldehyde to HMG-CoA (■) catalyzed by the DEAE fraction of the expressed enzyme. Assays were conducted in Buffer C at the indicated pH under otherwise standard conditions. Data are expressed relative to the maximal observed activity for each reaction (21 μU/mg for reductive deacylation of HMG-CoA at pH 7.5; 7.6 μU/mg for reduction of mevaldehyde at pH 6.0; and 2.4 μU/mg for oxidative acylation of mevaldehyde at pH 8.0).

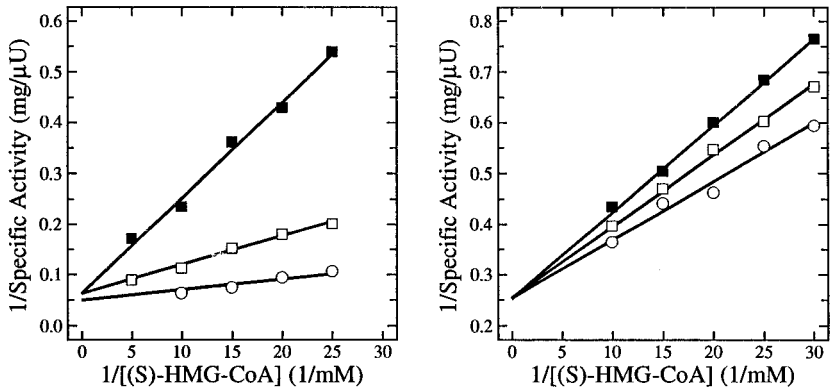

FIG. 5. Double reciprocal plots for inhibition by the lactone and free acid forms of lovastatin. The initial rate of reductive deacylation of (*S*)-HMG-CoA to mevalonate was measured at pH 7.3 at the indicated concentrations of (*S*)-HMG-CoA in the presence of 0 μM (○), 25 μM (□), or 50 μM (■) lovastatin lactone (left panel) or of 0 nM (○), 5 nM (□), or 10 nM (■) lovastatin, free acid form (right panel). Reactions were initiated by the addition of NADPH. As previously noted for rat liver HMG-CoA reductase (1), erratic results were obtained when assays were initiated by adding HMG-CoA. Reproducible results and competitive inhibition were, however, observed with lovastatin when assays were initiated by adding NADPH.

tase exhibited absolute stereospecificity for the (*S*)-diastereomer of HMG-CoA. The enzyme was competitively inhibited by (*R*)-HMG-CoA, with a K_i of 80 μM.

Inhibition by lovastatin. We assayed the susceptibilities of homogeneous *H. volcanii* HMG-CoA reductase expressed in *E. coli* and, as a control, hamster HMG-CoA reductase (11) to inhibition by lovastatin. Both the lactone and the free acid forms of lovastatin were competitive inhibitors with respect to (*S*)-HMG-CoA, with K_is of 15,000 nM (lactone) and 15 nM (free acid), respectively (Fig. 5). For the hamster enzyme, the K_i for the free acid of lovastatin was 5 nM (data not shown).

DISCUSSION

Our initial attempts to obtain catalytically active *H. volcanii* HMG-CoA reductase by the expression in *E. coli* of the portion of the lovastatin resistance marker *mev* thought to encode HMG-CoA reductase met with only partial success. Following expression, cell extracts contained a protein that reacted with polyclonal antibodies to rat HMG-CoA reductase but which lacked catalytic activity. Since the *mev* resistance gene encodes a 403-residue HMG-CoA reductase which is smaller than any known HMG-CoA reductase (19), we were concerned that, despite its 40% primary structural identity with mammalian HMG-CoA reductases, the short *mev* resistance gene product might possess significantly altered catalytic properties. To address this issue, we first extracted and partially purified the enzyme from *H. volcanii*. Like many halobacterial enzymes (10, 20, 23), *H. volcanii* HMG-CoA reductase is most stable and active in solutions of high ionic strength. Optimal activity and stability were observed in 3 M KCl. Since the requirement for a high-ionic-strength environment precluded the use of certain chromatographic purification techniques, we employed salt-insensitive protocols to purify the enzyme from extracts of *H. volcanii* to a specific activity of 1.2 μU/mg, a value we subsequently were able to infer represented about 10% homogeneity.

On the basis of the hypothesis that the expression of a halophilic enzyme in the low-salt environment of the *E. coli* cytosol yielded an inappropriately folded protein, we next investigated conditions which might convert the inactive enzyme expressed in *E. coli* into a catalytically active form. Following denaturation in 6 M urea, extensive dialysis against 3.0 M KCl was accompanied by the appearance of catalytic activity. Subsequently, we learned that denaturation and dialysis could be replaced by the addition of 3.0 M KCl followed by several hours of incubation at 4°C. We then employed a modified version of the protocol for purification of the enzyme from *H. volcanii* cell extracts to purify the enzyme expressed in *E. coli* to a specific activity of 24 μU/mg, an activity comparable to that of any purified HMG-CoA reductase (11, 14).

As may be inferred from its 40% primary structural identity to mammalian HMG-CoA reductases, the enzymic properties of *H. volcanii* HMG-CoA reductase parallel those of the enzyme from Syrian hamster (3). Consistent with its postulated biosynthetic role, the halophilic enzyme used NADPH as the reductant and exhibited absolute stereospecificity for (*S*)-HMG-CoA. (*R*)-HMG-CoA was a competitive inhibitor with a K_i/K_m ratio of about unity, suggesting that both HMG-CoA diastereomers bind at the same site and with comparable affinities.

The gene that confers lovastatin resistance on *H. volcanii* contains an up-promoter mutation, resulting in increased levels of transcripts of the wild-type HMG-CoA reductase gene (19). Lovastatin resistance presumably results from the biosynthesis of high concentrations of an active, lovastatin-sensitive HMG-CoA reductase. We therefore investigated the sensitivity of *H. volcanii* HMG-CoA reductase to lovastatin. The purified enzyme expressed in *E. coli* retained sensitivity to lovastatin, with a K_i of 15 nM, a value comparable to those for the rat (1) and hamster enzymes.

In addition to the reductive deacylation of HMG-CoA to mevalonate, the halophilic enzyme catalyzed the reduction of mevaldehyde to mevalonate and the oxidative acylation of mevaldehyde to HMG-CoA. These reactions proceeded at rates comparable to those catalyzed by hamster HMG-CoA reductase (Table 3). As for hamster (3) and *P. mevalonii* HMG-CoA reductases (6), the pH-activity profile for the overall reaction suggests participation in catalysis of at least two ionizable groups, the protonated form of one functioning in the reduction of mevaldehyde and the deprotonated form of another participating in the oxidative acylation of mevaldehyde.

As one of only a small set of highly purified archaeal enzymes, the properties of *H. volcanii* HMG-CoA reductase may ultimately provide insight into the adaptations which permit enzymes to function in high-salt environments. Comparisons with other HMG-CoA reductases should also enhance our knowledge of catalysis by HMG-CoA reductases in general.

TABLE 3. Comparison of *H. volcanii* and hamster HMG-CoA reductase for catalysis of all three reactions[a]

Reaction	V_{max} (μU/mg) for HMG-CoA reductase from:	
	H. volcanii	Hamster
Reductive deacylation of HMG-CoA	34	29
Oxidative acylation of mevaldehyde	2.1	1.2
Reduction of mevaldehyde	11	6.5

[a] Shown, for catalysis of the indicated reactions, are V_{max} values for homogeneous, expressed hamster HMG-CoA reductase (12) and *H. volcanii* HMG-CoA reductase expressed in *E. coli.*

The archaeal and mammalian enzymes have many common features. That the enzymic properties of the archaeal enzyme closely parallel those of the HMG-CoA reductase of higher eukaryotes is reassuring, given its 40% primary structural identity to the mammalian enzyme. Despite a vast evolutionary distance and membership in different phylogenetic domains, the archaeal and eukaryotic HMG-CoA reductases should ultimately be shown to share many more characteristics, including, at a minimum, a common catalytic mechanism.

ACKNOWLEDGMENTS

We thank Wan Lam and W. Ford Doolittle for generous gifts of *H. volcanii* WFD11 and the shuttle vector pWL102, David Gibson and Joe Papiez for a generous gift of polyclonal antibody to rat HMG-CoA reductase, Joe Ross for a gift of hamster HMG-CoA reductase, and our colleagues Dan Bochar, Jon Friesen, Kenneth Frimpong, Kenneth Rogers, Scott Rosenthal, Ronald Somerville, and Henry Weiner for helpful suggestions.

This work was funded in part by National Institutes of Health grant HL 47113.

REFERENCES

1. **Alberts, A. W., J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch, and J. Springer.** 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc. Natl. Acad. Sci. USA **77:**3957–3961.
2. **Beach, M. J., and V. W. Rodwell.** 1989. Cloning, sequencing, and overexpression of *mvaA*, which encodes *Pseudomonas mevalonii* 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J. Bacteriol. **171:**2994–3001.

2a. **Bischoff, K. M.** 1995. Ph.D. thesis. Purdue University, West Lafayette, Ind.

3. **Bischoff, K. M., and V. W. Rodwell.** 1992. Biosynthesis and characterization of (*S*)- and (*R*)-3-hydroxy-3-methylglutaryl coenzyme A. Biochem. Med. Metab. Biol. **48:**149–158.
4. **Cabrera, J. A., J. Bolds, P. E. Shields, C. M. Havel, and J. A. Watson.** 1986. Isoprenoid synthesis in *Halobacterium halobium*. Modulation of 3-hydroxy-3-methylglutaryl-coenzyme A concentration in response to mevalonate availability. J. Biol. Chem. **261:**3578–3583.
5. **Charlebois, R. L., W. L. Lam, S. W. Cline, and W. F. Doolittle.** 1987. Characterization of pHV2 from *Halobacterium volcanii* and its use in demonstrating transformation of an archaebacterium. Proc. Natl. Acad. Sci. USA **84:**8530–8534.
6. **Darnay, B. G., Y. Wang, and V. W. Rodwell.** 1992. Identification of the catalytically important histidine of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J. Biol. Chem. **267:**15064–15070.
7. **Dennis, P. P.** 1986. Molecular biology of archaebacteria. J. Bacteriol. **168:** 471–478.
8. **De Rosa, M., A. Gambacorta, and A. Gliozzi.** 1986. Structure, biosynthesis, and physicochemical properties of archaebacterial lipids. Microbiol. Rev. **50:** 70–80.
9. **Dunker, A. K., and R. R. Rueckert.** 1969. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. **244:**5074–5080.
10. **Eisenberg, H., M. Mevarech, and G. Zaccai.** 1992. Biochemical, structural, and molecular genetic aspects of halophilism. Adv. Protein Chem. **43:**1–62.
11. **Frimpong, K., B. G. Darnay, and V. W. Rodwell.** 1993. Syrian hamster 3-hydroxy-3-methylglutaryl-coenzyme A reductase expressed in *Escherichia coli*: production of homogeneous protein. Protein Expr. Purif. **4:**337–344.
12. **Frimpong, K., and V. W. Rodwell.** 1994. Catalysis by Syrian hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase. Proposed roles of histidine 865, glutamate 558, and aspartate 766. J. Biol. Chem. **269:**11478–11483.
13. **Gill, J. F., Jr., M. J. Beach, and V. W. Rodwell.** 1985. Mevalonate utilization in *Pseudomonas* sp. M. Purification and characterization of an inducible 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Biol. Chem. **260:**9393–9398.
14. **Jordan-Starck, T. C., and V. W. Rodwell.** 1989. *Pseudomonas mevalonii* 3-hydroxy-3-methylglutaryl-CoA reductase. Characterization and chemical modification. J. Biol. Chem. **264:**17913–17918.
15. **Jordan-Starck, T. C., and V. W. Rodwell.** 1989. Role of cysteine residues in *Pseudomonas mevalonii* 3-hydroxy-3-methylglutaryl-CoA reductase. Site-directed mutagenesis and characterization of the mutant enzymes. J. Biol. Chem. **264:**17919–17923.
16. **Kates, M., M. K. Wassef, and D. J. Kushner.** 1968. Radioisotopic studies on the biosynthesis of glyceryl diether lipids of *Halobacterium cutirubrum*. Can. J. Biochem. **46:**971–977.
17. **Kauri, T., R. Wallace, and D. J. Kushner.** 1990. Nutrition of the halophilic archaebacterium, *Haloferax volcanii*. Syst. Appl. Microbiol. **13:**14–18.
18. **Lam, W. L., and W. F. Doolittle.** 1989. Shuttle vectors for the archaebacterium *Haloferax volcanii*. Proc. Natl. Acad. Sci. USA **86:**5478–5482.
19. **Lam, W. L., and W. F. Doolittle.** 1992. Mevinolin-resistant mutations identify a promoter and the gene for a eukaryote-like 3-hydroxy-3-methylglutaryl-coenzyme A reductase in the archaebacterium *Haloferax volcanii*. J. Biol. Chem. **267:**5829–5834.
20. **Lanyi, J. K.** 1974. Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriol. Rev. **38:**272–290.
21. **Lawrence, C. M., V. W. Rodwell, and C. V. Stauffacher.** 1995. Crystal structure of *Pseudomonas mevalonii* HMG-CoA reductase at 3.0 angstrom resolution. Science **268:**1758–1762.
22. **Monstadt, G. M., and A. W. Holldorf.** 1990. Arginine deiminase from *Halobacterium salinarium*. Biochem. J. **273:**739–745.
23. **Rao, J. K. M., and P. Argos.** 1981. Structural stability of halophilic proteins. Biochemistry **20:**6536–6543.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Wang, Y., B. G. Darnay, and V. W. Rodwell.** 1990. Identification of the principal catalytically important acidic residue of 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Biol. Chem. **265:**21634–21641.
26. **Woese, C. R., O. Kandler, and M. L. Wheelis.** 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucaya. Proc. Natl. Acad. Sci. USA **87:**4576–4579.
27. **Zillig, W., P. Palm, W. Reiter, F. Gropp, G. Puhler, and H. Klenk.** 1988. Comparative evaluation of gene expression in archaebacteria. Eur. J. Biochem. **173:**473–482.