Engineering of *Sulfolobus solfataricus* HMG-CoA Reductase to a Form Whose Activity Is Regulated by Phosphorylation and Dephosphorylation†

Dong-Yul Kim,‡ Cynthia V. Stauffacher,§ and Victor W. Rodwell*,‡

Departments of Biochemistry and Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Received July 28, 1999; Revised Manuscript Received December 5, 1999

ABSTRACT: There are two classes of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase: the class I enzymes of eukaryotes and some archaea, and the class II enzymes of certain eubacteria. The activity of the class I Syrian hamster HMG-CoA reductase is regulated by phosphorylation−dephosphorylation of Ser871. Phosphorylation apparently prevents the active site histidine, His865, from protonating the inhibitory coenzyme A thioanion prior to its release from the enzyme. Structural evidence for this hypothesis is, however, lacking. The HMG-CoA reductase of the thermophilic archaeon *Sulfolobus solfataricus*, whose stability recommends it for physical studies, lacks both a phosphoacceptor serine and a protein kinase recognition motif. Consequently, its activity is not regulated by phosphorylation. We therefore employed site-directed mutagenesis to engineer an appropriately located phosphoacceptor serine and cAMP-dependent protein kinase recognition motif. Substitution of serine for Ala406, the apparent cognate of hamster Ser871, and replacement of Leu403 and Gly404 by arginine created *S. solfataricus* mutant enzyme L403R/G404R/A406S. The general properties of enzyme L403R/G404R/A406S (K_m values, V_{max}, optimal pH and temperature) were essentially those of the wild-type enzyme. Exposure of enzyme L403R/G404R/A406S to [γ-^{32}P]ATP and cAMP-dependent protein kinase was accompanied by incorporation of $^{32}P_i$ and by a parallel decrease in catalytic activity. Subsequent treatment with a protein phosphatase released enzyme-bound $^{32}P_i$ and restored activity to pretreatment levels. The regulatory properties of enzyme L403R/G404R/A406S thus match those of the hamster enzyme. Solution of the three-dimensional structures of the phospho and dephospho forms of this mutant enzyme thus should reveal structural features critical for regulation of the activity of a class I HMG-CoA reductase.

The HMG-CoA[1] reductases of eukaryotes, archaea, and certain true bacteria catalyze the reductive deacylation of (*S*)-HMG-CoA to form the isoprenoid precursor (*R*)-mevalonate (*1*). Sequence comparisons have revealed the existence of two structural classes of HMG-CoA reductase (*2*, *3*). Class I includes all eukaryotic and several archaeal forms of the enzyme. The class II HMG-CoA reductases include those of *Pseudomonas mevalonii* and of several pathogenic eubacteria. The catalyst for the rate-limiting reaction of isoprenoid biosynthesis, HMG-CoA reductase, constitutes the target for control of cholesterol biosynthesis by the inhibitory "statin" drugs that lower blood cholesterol levels in human subjects. A four-electron oxidoreduction, the reaction proceeds in three stages, the first and third of which are reductive.

HMG-CoA reductase catalyzes three additional reactions. Reactions 2 and 3 of free mevaldehyde resemble the third stage and the reverse of the second and first stages of reaction 1, respectively. The enzyme also catalyzes reaction 4, the oxidative acylation of (*R*)-mevalonate to (*S*)-HMG-CoA (*1*).

The crystal structure of the class II HMG-CoA reductase of *P. mevalonii* (*4*, *5*) and site-directed mutagenesis of representatives of both classes of the enzyme have established that a conserved glutamate (*6*), aspartate (*7*), histidine (*7*−*9*), and lysine (*5*, *10*, *11*) participate in a mechanism of catalysis (Figure 1) that is common to both classes of the enzyme (*5*, *11*).

The activity of the class I HMG-CoA reductases of representative vertebrates (*12*) and plants (*13*) is regulated by phosphorylation. Phosphorylation attenuates and dephosphorylation restores catalytic activity. On the basis of primary structural similarities, phosphorylation-mediated regulation of activity probably is general for the HMG-CoA reductases of all higher eukaryotes. For man and hamster, down-regulation of activity involves phosphorylation of a single serine (*14*−*16*), which in the HMG-CoA reductases of higher eukaryotes is located precisely six residues on the C-terminal side of the active site histidine (Figure 2).

The 90% decrease in activity that accompanied substitution of aspartate for the regulatory serine of hamster HMG-CoA reductase suggested that the introduction of negative charge that accompanies phosphorylation may play a major role in regulation of catalytic activity (*16*). Phosphorylation was proposed to impair the ability of the active site histidine,

† Funded by National Institutes of Health Grants HL 47113 (V.W.R.) and HL 52115 (C.V.S.). The data are from the Ph.D. thesis of D.Y.K. This is journal paper 16018 from the Purdue University Agricultural Experiment Station.

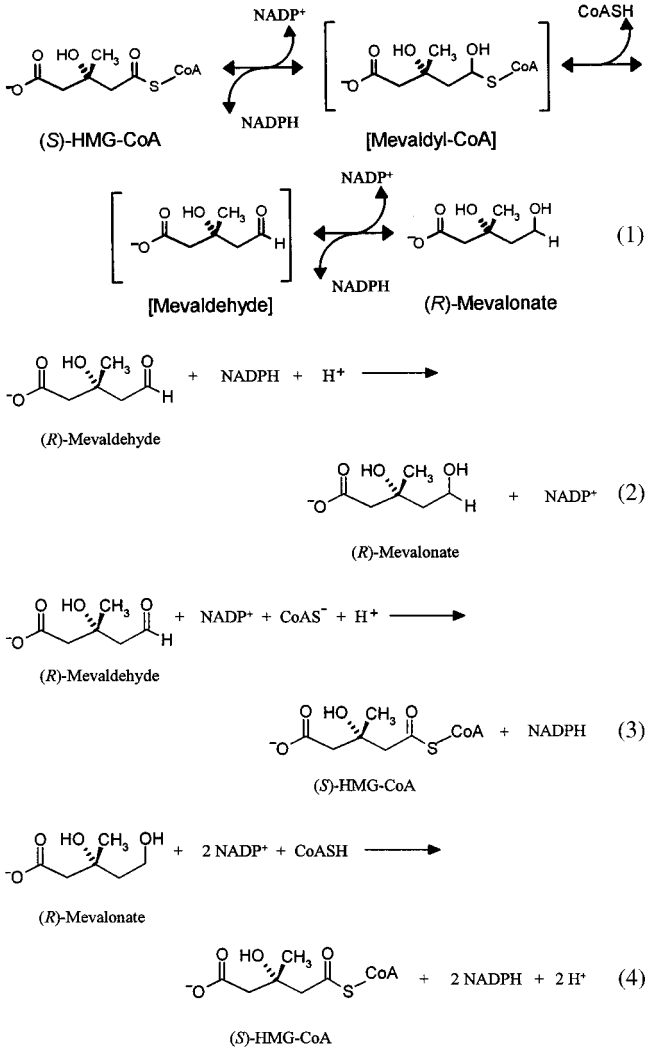
* To whom correspondence and requests for reprints should be addressed: Phone: (765) 494-1608. Fax: (765) 494-7897. E-mail: vrodwell@purdue.edu.

‡ Department of Biochemistry.

§ Department of Biological Sciences.

[1] Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; CoA, coenzyme A.

10.1021/bi991749t CCC: $19.00 © 2000 American Chemical Society
Published on Web 02/12/2000



His865, to perform its established function, protonation of the inhibitory coenzyme A thioanion prior to its departure from the enzyme (*7*). Attenuation of activity was proposed to result from a direct interaction between the phosphate of phosphoserine and the imidazole of His865 (Figure 3). Supportive evidence for this hypothesis was obtained using hamster enzyme H865Q, in which glutamine replaces the active site histidine. Enzyme H865Q catalyzed reaction 2, but not reaction 1, establishing that the active site histidine plays no role in catalysis of reaction 2. Phosphorylation of Ser871 of the wild-type enzyme was accompanied by attenuation of its ability to catalyze both reactions 1 and 2. By contrast, while phosphorylation of Ser871 of enzyme H865Q attenuated its ability to catalyze reaction 1, its ability to catalyze reaction 2 remained unaltered. Although this observation is consistent with a direct interaction between the phosphate of phosphoserine and the active site histidine, direct physical evidence for such an interaction is lacking. Solution of the three-dimensional structures of the phospho and dephospho forms of an HMG-CoA reductase should provide structural information that reveals why phosphorylation regulates activity. The class I HMG-CoA reductase of the thermophilic archaeon *Sulfolobus solfataricus* retains activity at above 80 °C. In addition to providing long-term stability, a feature desirable both for physical studies and for possible exploitation as an industrial biocatalyst, this enzyme therefore represents an attractive candidate for crystallographic investigation. Its activity is not, however, regulated by phosphorylation. We therefore have engineered *S. solfataricus* HMG-CoA reductase to a form whose activity now is regulated by phosphorylation−dephosphorylation.

EXPERIMENTAL PROCEDURES

Materials. Purchased reagents included β-NADPH, (*R*,*S*)-HMG-CoA, coenzyme A, β-NADP$^+$, mevaldic acid precursor, phenylmethylsulfonyl fluoride, and 2-(*N*-morpholino)-ethanesulfonic acid (Sigma); [α-^{35}S]dATP and [γ-^{32}P]ATP (Amersham); T4 DNA ligase (Promega); an Isotherm DNA sequencing kit (Epicentre Technologies); restriction enzymes (New England Biolabs, or Promega); Vent DNA polymerase (New England Biolabs); a Qiaex gel extraction kit, a Qiaprep spin plasmid miniprep kit, and a Qiaquick PCR purification kit (Qiagen); homogeneous λ protein phosphatase (New England Biolabs); and prestained low-range protein standards (Bio-Rad). Partially purified beef heart cAMP-dependent protein kinase was prepared as previously described (*17*). Determination of protein concentration employed the method

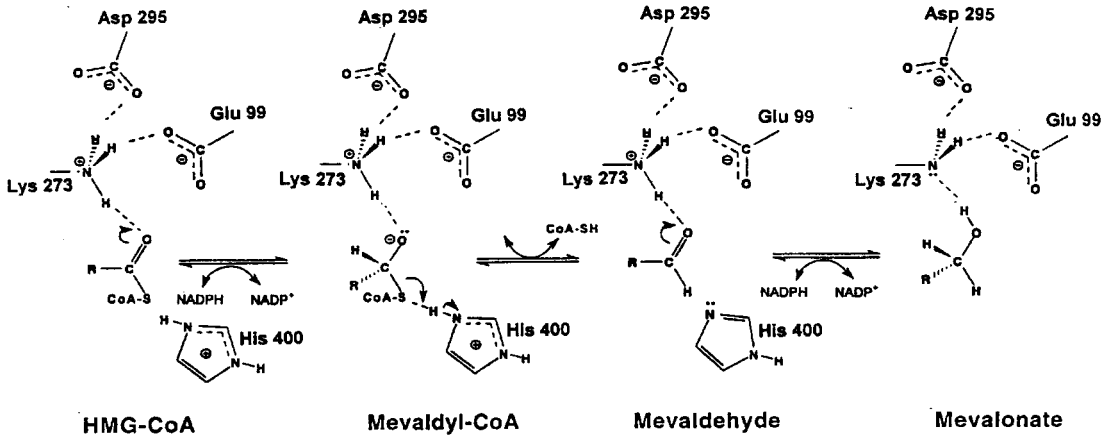


FIGURE 1: Proposed roles of active site residues during catalysis by *S. solfataricus* HMG-CoA reductase. For clarity, portions of the structures of substrates, products, intermediates, and catalytic residues have been omitted. Initial protonation of HMG-CoA by Lys273 of the Lys273-Asp295-Glu99 triad facilitates hydride transfer from NADPH, forming enzyme-bound mevaldyl-CoA. His400 next donates a proton to the coenzyme A thioanion released by abstraction of a proton from bound mevaldyl-CoA by Glu99. Protonated coenzyme A then departs from the enzyme. Finally, protonation of bound mevaldehyde by Lys273 facilitates a second hydride transfer, forming mevalonate. Redrawn from ref 5, with residue assignments based on the roles of cognate residues in both classes of HMG-CoA reductase.

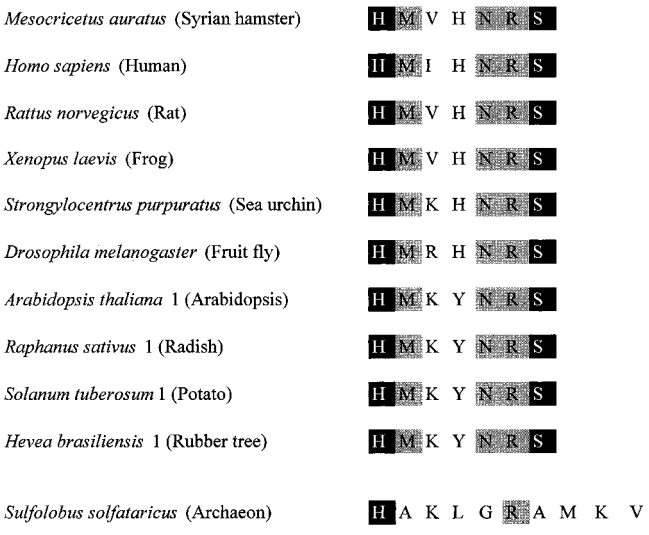


FIGURE 2: Sequences of selected eukaryotic HMG-CoA reductases and of the last 10 residues of the *S. solfataricus* enzyme. The active site histidines are highlighted in black. The target serines of the eukaryotic enzymes and all conserved sequences are highlighted in black and gray, respectively. Sequences were obtained from Genbank. Alignments were produced by the Pileup program of the Wisconsin package (*25*).

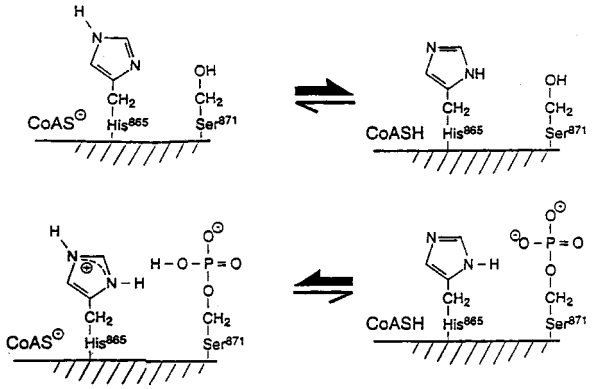


FIGURE 3: Proposed mechanism for phosphorylation-mediated attenuation of the catalytic activity of HMG-CoA reductase. Top: The imidazolium proton of the active site histidine of the unphosphorylated enzyme can protonate the potentially inhibitory coenzyme A thioanion released after the first reductive stage of the overall reaction. This permits the second reductive stage to proceed, completing the overall reaction. Bottom: Phosphorylation of the regulatory serine introduces adjacent negative charge that neutralizes the histidinium charge. The equilibrium now favors the coenzyme A thioanion, which can react with mevaldehyde, reversing stage 1 and removing substrate essential for stage 2 of the overall reaction. Redrawn from ref *16*.

of Bradford (*18*). Buffer A contained 10% (v/v) glycerol and 1 mM phenylmethylsulfonyl fluoride in 20 mM K_xPO_4, pH 7.3.

Vector and Bacteria. Expression vector pET-21b(sol)-HMGR has been previously described (*19*). *Escherichia coli* strain BL21(DE3) served as host during mutagenesis, cloning, and plasmid preparation. LB_{amp} medium, LB medium (*20*) plus 75 μg/mL ampicillin, was used for growth of *E. coli*.

DNA Manipulations and Site-Directed Mutagenesis. Oligonucleotides synthesized in the Purdue University Laboratory for Macromolecular Structure were purified prior to use by thin-layer chromatography on silica gel in 15 N NH_4-OH/2-propanol/1-propanol/H_2O/(35:28:28:9 (v/v)). Polymerase chain reaction-based oligonucleotide-directed mutagenesis was employed to construct genes that encode mutant enzymes A406D, A406S, and L403R/G404R/A406S (Table 1). All mutations were verified by double-stranded DNA sequencing (*21*) using an Isotherm DNA sequencing kit and [α-^{35}S]dATP.

Expression and Purification of Wild-Type and Mutant Enzymes. Genes encoding wild-type or mutant HMG-CoA reductases were expressed in *E. coli* BL21(DE3) cells. The resulting enzymes were then purified by the protocol developed for purification of the wild-type enzyme (*22*). Table 2 summarizes the purification of the wild-type enzyme and of enzyme L403R/G404R/A406S.

Assay of HMG-CoA Reductase Activities. Assays employed a Hewlett-Packard model 8452 diode array spectrophotometer. For assay of reactions 1 and 2, the instability of NADPH at 50 °C and an acidic pH required an initial concentration of NADPH too great to measure accurately at 340 nm ($\epsilon_{340} = 6220\ M^{-1}\ cm^{-1}$). Disappearance of NADPH was therefore monitored at 366 nm ($\epsilon_{366} = 3300\ M^{-1}\ cm^{-1}$). Assays were initiated by adding the substrate, HMG-CoA, mevaldehyde, or mevalonate. The absorbance at 366 nm (reactions 1 and 2) or at 340 nm (reactions 3 and 4) was then monitored, typically for 15−30 s. For all assays, 1 eu represents the turnover, in 1 min, of 1 μmol of NADP(H). This corresponds to turnover of 1 μmol of mevaldehyde or mevalonate, but 0.5 μmol of HMG-CoA. Unless otherwise stated, all assays were conducted at 50 °C. Standard conditions for assay of all catalyzed reactions were as follows:

Table 1: Oligonucleotides Used to Construct Mutant Genes[a]

	400	401	402	403	404	405	406	407	408	409
wild-type enzyme	His	Ala	Lys	Leu	Gly	Arg	Ala	Met	Lys	Val
	CAC	GCT	AAA	CTT	GGA	AGA	GCT	ATG	AAA	GTC
3'-primer for enzyme A406D						GAA	CCT	TCT	CTA	TAC TTT CAG ATT CTT AAG
3'-primer for enzyme A406S						GAA	CCT	TCT	TGA	TAC TTT CAG ATT CTT AAG
3'-primer for enzyme L403R/G404R/A406S				GAG	CGA	TTT	GCA GCT	TCT	TGA	TAC TTT CAG ATT CTT AAG

[a] Black indicates mutated codons. Gray indicates added *Eco*RI restriction sites. The listed mutagenic oligonucleotides and oligonucleotide 5′-GCTGACATATGAAAATTGATGAA-3′ were used to construct all mutant genes.

Table 2: Purification Summary for the Wild-Type and Mutant Enzyme L403R/G404R/A406S[a]

fraction	enzyme	total activity (eu)	total protein (mg)	specific activity (eu/mg)	enrichment (fold)	recovery (%)
heat fraction	wild-type	348	273	1.3	(1.0)	(100)
	L403R/G404R/A406S	209	239	0.90	(1.0)	(100)
butyl sepharose	wild-type	227	95	2.4	1.8	65
	L403R/G404R/A406S	167	77	2.2	2.4	79
red agarose	wild-type	205	42	4.9	3.8	59
	L403R/G404R/A406S	147	38	3.9	4.3	70

[a] The data are for the purification of enzyme from 3 L of culture. The unheated cytosol contained 1060 mg (wild-type) or 910 mg (enzyme L403R/G404R/A406S) of protein. Precipitation of nonreductase protein at 50 °C hindered accurate measurement of activity in unheated cytosol.

Reaction 1, Reductive Deacylation of HMG-CoA to Mevalonate. Assays contained, in 200 μL, 0.5 mM NADPH and 0.5 mM (*R*,*S*)-HMG-CoA in 100 mM 2-(*N*-morpholino)-ethanesulfonic acid, 100 mM potassium acetate, pH 5.5.

Reaction 2, Reduction of Mevaldehyde to Mevalonate. Assays contained, in 200 μL, 0.5 mM NADPH and 4.0 mM (*R*,*S*)-mevaldehyde in 100 mM 2-(*N*-morpholino)ethanesulfonic acid, 100 mM potassium acetate, pH 5.0.

Reaction 3, Oxidative Acylation of Mevaldehyde to HMG-CoA. Assays contained, in 200 μL, 5.0 mM $NADP^+$, 7.5 mM coenzyme A, and 3.5 mM (*R*,*S*)-mevaldehyde in 100 mM Tris, 100 mM K_xPO_4, 100 mM glycine, pH 9.0.

Reaction 4, Oxidative Acylation of Mevalonate to HMG-CoA. Assays contained, in 200 μL, 5.0 mM $NADP^+$, 7.5 mM coenzyme A, and 2.5 mM (*R*,*S*)-mevalonate in 100 mM Tris, 100 mM K_xPO_4, 100 mM glycine, pH 9.0.

RESULTS

Introduction of Negative Charge at Position 406 Attenuates Catalytic Activity. Consistent with the hypothesis that the attenuation of activity by phosphorylation results in part from the introduction of negative charge (*16*), mutant enzyme A406D exhibited less than 1% wild-type activity.

Construction of a Mutant Sulfolobus solfataricus HMG-CoA Reductase Whose Activity Is Regulated by Phosphorylation−Dephosphorylation. The regulatory serine of the HMG-CoA reductases of higher eukaryotes is located six residues on the C-terminal side of the active site histidine, a position that in *S. solfataricus* HMG-CoA reductase is occupied by alanine rather than by serine (Figure 2). Ala406 was therefore replaced by serine to introduce an appropriately located phosphorylation site. Mutant enzyme A406S, while fully active, was not phosphorylated by cAMP-dependent protein kinase. Using enzyme A406S as a scaffold, Leu403 and Gly404 were therefore next replaced by arginine to construct a suitably located recognition motif for cAMP-dependent protein kinase (Figure 4). The resulting mutant enzyme L403R/G404R/A406S was then purified to apparent homogeneity as judged by SDS−PAGE (Figure 5).

Exposure of enzyme L403R/G404R/A406S to [γ-^{32}P]ATP and cAMP-dependent protein kinase resulted in a time-dependent incorporation of $^{32}P_i$. Phosphorylation was accompanied by a parallel attenuation of activity for catalysis of reaction 1, the reductive deacylation of HMG-CoA to mevalonate. Exposure of phosphorylated enzyme L403R/G404R/A406S to a protein phosphatase both released bound $^{32}P_i$ and restored activity for catalysis of reaction 1 to its initial value (Figure 6). The ability of *S. solfataricus* enzyme L403R/G404R/A406S to catalyze reaction 2 was also attenuated by phosphorylation (Figure 7), and as for the wild-type hamster enzyme (*16*), the effect of phosphorylation was abolished by addition of desthiocoenzyme A. The regulatory properties of enzyme L403R/G404R/A406S thus are indistinguishable from the regulatory properties of the HMG-CoA reductase of a higher eukaryote.

enzyme		sequence										
Hamster	865	H	M	V	H	N	R	S	K	I	N	874
Wild-type *S. solfataricus*	400	H	A	K	L	G	R	A	M	K	V	409
Mutant L403R/G404R/A406S	400	H	A	K	R	R	R	S	M	K	V	409
Consensus cAMP-dependent protein kinase recognition motif				X	β	β	X	S	X			

FIGURE 4: Sequences of Syrian hamster HMG-CoA reductase, of the wild-type *S. solfataricus* enzyme, and of *S. solfataricus* mutant enzyme L403R/G404R/A406S. Valine 409 is the carboxy-terminal residue of the *S. solfataricus* enzymes. The hamster enzyme extends for an additional 14 residues. For the consensus sequence, β represents a basic amino acid and X represents any amino acid. The underlined basic residues in the sequence of the wild-type enzyme may form the hydrophilic face of an amphipathic helix.

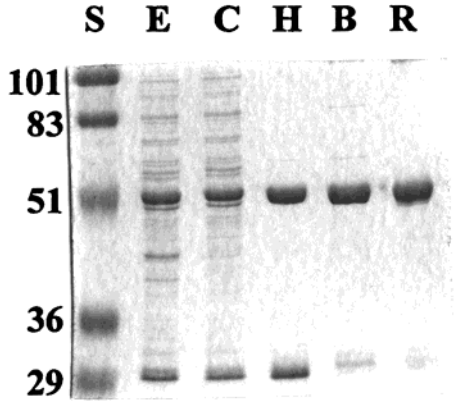


FIGURE 5: SDS−PAGE of purified enzymes. Approximately 5 μg portions of purified or mutant enzyme L403R/G404R/A406S were subjected to SDS−PAGE. The gel was stained with Coomassie blue. Lanes contained prestained protein standards (S) of the indicated molecular mass in kilodaltons, crude extract (E), cytosol (C), heat fraction (H), butylsepharose fraction (B), and red agarose fraction (R).

The Mutations Did Not Affect the Kinetic Properties of the Enzyme. To determine whether the three introduced mutations had altered the basic kinetic properties of the wild-type enzyme, we first examined the effects of pH and of temperature on the activity of enzyme L403R/G404R/A406S. With respect to both its optimal pH and temperature for activity, enzyme L403R/G404R/A406S was virtually indistinguishable from the wild-type enzyme (Figures 8 and 9). The same was true for the standard kinetic parameters.

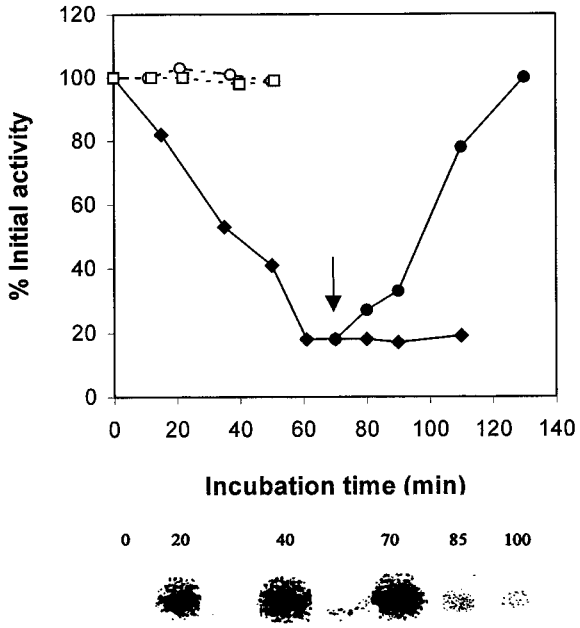


FIGURE 6: Phosphorylation attenuates and dephosphorylation restores activity to mutant enzyme L403R/G404R/A406S. The experiment employed three 200 μL nonradioactive incubations (tubes 1−3) and one 20 μL radioactive incubation (tube 4). Tubes 1−3 each contained 60 μL (300 μg) of purified mutant enzyme L403R/G404R/A406S in buffer A, plus where indicated 2 mM ATP (ATP), 950 μg of cAMP-dependent protein kinase (protein kinase), and 40 mM Tris·HCl/20 mM magnesium acetate, pH 7.4 (Tris/Mg). Additions were (tube 1 (complete)) ATP, protein kinase, and Tris/Mg (◆); (tube 2 (no kinase)) ATP and Tris/Mg (○); (tube 3 (no ATP)) protein kinase and Tris/Mg (□). Tube 4 (complete) contained, in 20 μL, 95 μg of protein kinase, 2 mM [γ-^{32}P]ATP (specific activity 40 Ci/mmol), and Tris/Mg. All four tubes were incubated at 30 °C. Portions were removed at the indicated times and were assayed for catalytic activity (tubes 1−3), or were subjected to SDS−PAGE for determination of incorporated radioactivity (tube 4). Incorporation of $^{32}P_i$ into bands whose mobility corresponded to that of an HMG-CoA reductase subunit was determined in a Packard instant imager. The indicated quantities of phosphatase (λ-protein phosphatase in 1 mM dithiothreitol, 4 mM $MnCl_2$, 10 mM Tris, pH 7.8) were added to an 80 μL portion of tube 1 (4800 units) (●) and tube 4 (1200 units) at 70 min (arrow), and incubation was continued at 30 °C. Top: Time course for attenuation and restoration of HMG-CoA reductase activity. Bottom: Autoradiogram of a time course for phosphorylation and subsequent dephosphorylation.

Enzyme L403R/G404R/A406S had K_m and V_{max} values for all four reactions similar to those of the wild-type enzyme (Table 3).

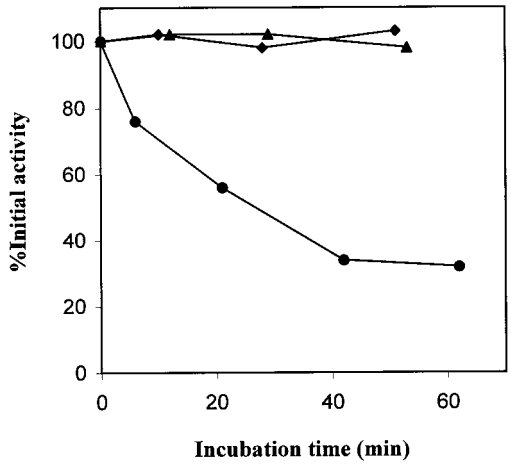


FIGURE 7: Attenuation of mevaldehyde reduction by phosphorylation. The experiment was conducted essentially as described in Figure 6. Portions of phosphorylated mutant enzyme L403R/G404R/A406S were removed at the indicated times and were assayed for catalysis of mevaldehyde reduction conducted in the absence of added coenzyme A (●) or in the presence of either 0.1 mM (◆) or 0.5 mM (▲) desthiocoenzyme A.

DISCUSSION

The ability of phosphorylation to regulate catalytic activity has been established for selected class I HMG-CoA reductases (*12*−*14*, *16*, *23*), and the ubiquitous presence of an appropriately positioned phosphoacceptor serine and protein kinase recognition motif suggests that this phenomenon is general for the HMG-CoA reductases of all higher eukaryotes. Since the class I HMG-CoA reductase of the thermophilic archaeon *S. solfataricus* lacks both these features, its activity is not regulated by phosphorylation. To facilitate future investigation of the structural features important for phosphorylation-mediated regulation of the activity of a class I HMG-CoA reductase, we therefore engineered a phosphoacceptor serine and a cAMP-dependent protein kinase recognition motif into *S. solfataricus* HMG-CoA reductase. These mutations had no adverse effects on catalytic activity, optimal pH, optimal temperature, or K_m values.

The regulatory properties of enzyme L403R/G404R/A406S accurately mimic those of hamster HMG-CoA reductase (*16*). As for the wild-type hamster enzyme, exposure of enzyme L403R/G404R/A406S to MgATP and cAMP-dependent protein kinase was accompanied by a time-dependent attenuation of activity for catalysis both of the

Table 3: Kinetic Parameters for Catalysis of the Indicated Reactions

		K_m values					
reaction and enzyme	V_{max}	HMG-CoA (μM)	NADPH (μM)	mevaldehyde (mM)	mevalonate (mM)	CoASH (mM)	$NADP^+$ (mM)
1: HMG-CoA → mevalonate							
wild-type enzyme	7.6	45	55				
enzyme L403R/G404R/A406S	5.0	76	83				
2: mevaldehyde → mevalonate							
wild-type enzyme	5.8		45	7.0			
enzyme L403R/G404R/A406S	3.2		82	13			
3: mevaldehyde → HMG-CoA							
wild-type enzyme	1.4			4.3		2.6	0.90
enzyme L403R/G404R/A406S	1.0			6.3		2.8	1.2
4: mevalonate → HMG-CoA							
wild-type enzyme	0.60				1.5	0.11	0.90
enzyme L403R/G404R/A406S	0.40				1.7	0.15	1.3

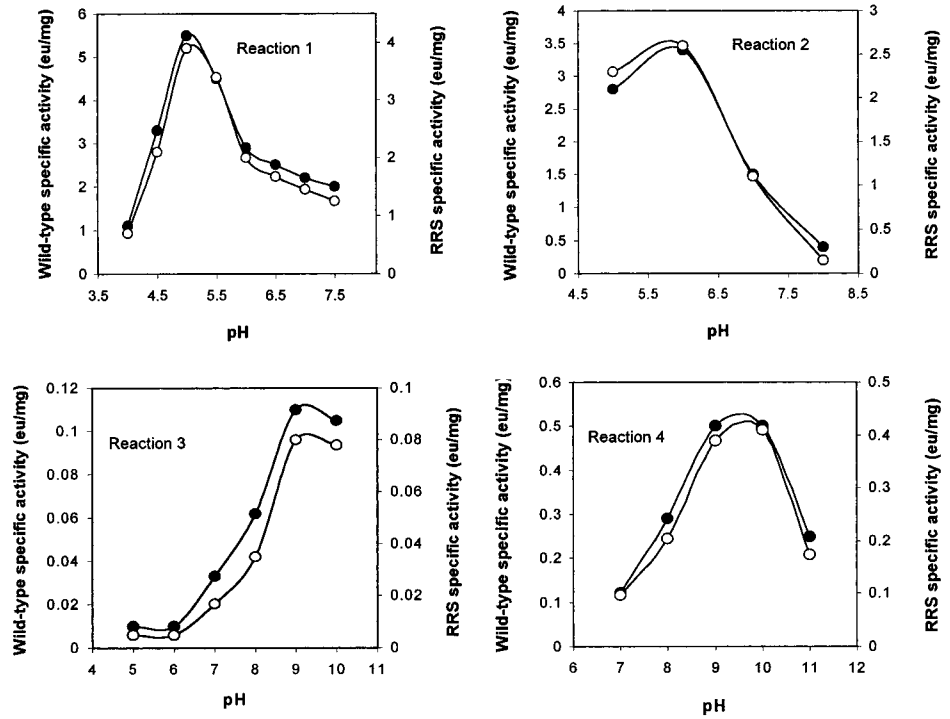


FIGURE 8: Effect of pH on activity. Catalysis of the indicated reaction by wild-type *S. solfataricus* HMG-CoA reductase (●) or enzyme L403R/G404R/A406S (○) was assayed at the indicated hydrogen ion concentrations under otherwise standard conditions.

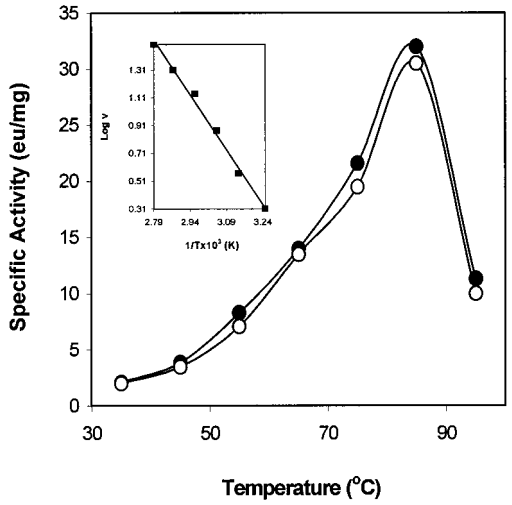


FIGURE 9: Effect of temperature on activity. Catalysis of reaction 1 by wild-type *S. solfataricus* HMG-CoA reductase (●) or enzyme L403R/G404R/A406S (○) was conducted at the indicated temperatures under otherwise standard conditions. Inset: Arrhenius plot of the activity of mutant enzyme L403R/G404R/A406S from 35 to 90 °C.

reductive deacylation of HMG-CoA (reaction 1) and of the reduction of mevaldehyde (reaction 2). Treatment of the phosphorylated mutant enzyme with a protein phosphatase restored activity to initial values. As previously noted for the hamster enzyme (*16*), addition of desthiocoenzyme A abolished the ability of phosphorylation to attenuate the activity of enzyme L403R/G404R/A406S for catalysis of mevaldehyde reduction. At this time, we are unable to provide a convincing explanation for this observation, other than to note that desthiocoenzyme A may displace tightly bound coenzyme A thioanion from the active site.

We previously constructed a mutant of the class II HMG-CoA reductase of *P. mevalonii* whose activity is regulated by phosphorylation (*24*). The comparable regulatory responses of the wild-type hamster enzyme and of the engineered HMG-CoA reductases of *S. solfataricus* and *P. mevalonii* provide a convincing case for a common mechanism of regulation of activity. On the basis of the strictly limited sequence identity between the hamster enzyme and both *P. mevalonii* HMG-CoA reductase (22%) and of *S. solfataricus* HMG-CoA reductase (43%), we also infer that residues outside the phosphorylation site probably play no role in phosphorylation-mediated down-regulation of activity.

In addition to their role in regulation of activity, comparison of residues 400−409 of *S. solfataricus* HMG-CoA reductase with their cognate residues 381−390 of the first helix of the "flap" domain of *P. mevalonii* HMG-CoA reductase (*5*) can provide potential structural insights. While the structure of the corresponding region of wild-type *S. solfataricus* HMG-CoA reductase is unknown, the residues that are underlined in Figure 4 could form the polar face of a short amphipathic α-helix that may serve to close the active site when substrates bind.

Mutant enzyme L403R/G404R/A406S represents a class I HMG-CoA reductase whose stability characteristics recommend it as a good candidate for crystallization and subsequent solution of the three-dimensional structures of its phospho and dephospho forms. When solved, these structures should reveal additional structural features critical for the phosphorylation-mediated regulation of the activity of class I HMG-CoA reductases.

ACKNOWLEDGMENT

We thank our colleagues John Burgner, Matija Hedl, Kenneth Li, and Autumn Sutherlin for helpful suggestions.

REFERENCES

1. Bochar, D. A., Friesen, J. A., Stauffacher, C. V., and Rodwell, V. W. (1999) Biosynthesis of mevalonic acid from acetyl-

CoA, Chapter 2 in Isoprenoids Including Carotenoids and Steroids (Cane, D., Ed.), Vol. 2 of *Comprehensive Natural Products Chemistry*, Pergamon Press, New York.
2. Bochar, D. A., Stauffacher, C. V., and Rodwell, V. W. (1999) *Mol. Genet. Metab. 66*, 122−127.
3. Doolittle, W. F., and Logston, J. M., Jr. (1998) *Curr. Biol. 8*, R209−R211.
4. Lawrence, C. M., Rodwell, V. W., and Stauffacher, C. V. (1995) *Science 268*, 1758−1762.
5. Tabernero, L., Bochar, D. A., Rodwell, V. W., and Stauffacher, C. V. (1998) *Proc. Natl. Acad. Sci. U.S.A. 96*, 7167−7171.
6. Wang, Y., Darnay, B. G., and Rodwell, V. W. (1990) *J. Biol. Chem. 265*, 21634−21641.
7. Frimpong, K., and Rodwell, V. W. (1994) *J. Biol. Chem. 269*, 11478−11483.
8. Darnay, B. G., and Rodwell, V. W. (1993) *J. Biol. Chem. 268*, 8429−8435.
9. Darnay, B. G., Wang, Y., and Rodwell, V. W. (1992) *J. Biol. Chem. 267*, 15064−15070.
10. Bochar, D. A., Tabernero, L., Stauffacher, C. V., and Rodwell, V. W. (1999) *Biochemistry 38*, 8879−8883.
11. Bochar, D. A., Stauffacher, C. V., and Rodwell, V. W. (1999) *Biochemistry 38*, 15848−15852.
12. Hunter, C. F., and Rodwell, V. W. (1980) *J. Lipid Res. 21*, 399−405.
13. Dale, S. Arro, M., Becerra, B., Morrice, N. G., Boronat, A., Hardie, D. G., and Ferrer, A. (1995) *Eur. J. Biochem. 233*, 506−513.
14. Clarke, P. R., and Hardie, D. G. (1990) *EMBO J. 9*, 2439−2446.
15. Omkumar, R. V., Darnay, B. G., and Rodwell, V. W. (1994) *J. Biol. Chem. 269*, 6810−6814.
16. Omkumar, R. V., and Rodwell, V. W. (1994) *J. Biol. Chem. 269*, 16862−16866.
17. Cobb, C. E., and Corbin, J. D. (1988) *Methods Enzymol. 159*, 202−208.
18. Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
19. Bochar, D. A., Brown, J. R., Doolittle, W. F., Klenk, H.-P., Lam, W., Schenk, M. E., Stauffacher, C. V., and Rodwell, V. W. (1997) *J. Bacteriol. 179*, 3632−3638.
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463−5467.
22. Kim, D.-Y., Bochar, D. A., Stauffacher, C. V., and Rodwell, V. W. (1999) *Protein Expression Purif. 17*, 435−442.
23. Sato, R., Goldstein, J. L., and Brown, M. S. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 9261−9265.
24. Friesen, J. A., and Rodwell, V. W. (1997) *Biochemistry 36*, 1157−1162.
25. Devereux, J., Haeberli, P., and Smithies, O. (1984) *Nucleic Acids Res. 12*, 387−395.

BI991749T