Utility of Acetyldithio-CoA in Detecting the Influence of Active Site Residues on Substrate Enolization by 3-Hydroxyl-3-methylglutaryl-CoA Synthase*

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Hydroxymethylglutaryl-CoA synthase-catalyzed condensation of acetyl-CoA with acetoacetyl-CoA requires enolization/carbanion formation from the acetyl C-2 methyl group prior to formation of a new carbon-carbon bond. Acetyldithio-CoA, a readily enolizable analog of acetyl-CoA, was an effective competitive inhibitor of avian hydroxymethylglutaryl-CoA synthase (K_i = 28 μM). In the absence of cosubstrate, enzyme catalyzed the enolization/proton exchange from the C-2 methyl group of acetyldithio-CoA. Mutant enzymes that exhibited impaired formation of the covalent acetyl-S-enzyme reaction intermediate exhibited diminished (D159A and D203A) or undetectable (C129S) rates of enolization of acetyldithio-CoA. The results suggest that covalent thioacetylation of protein, which has not been detected previously for other enzymes that enolize this analog, occurs with hydroxymethylglutaryl-CoA synthase. Enzyme catalyzed the transfer of the thioacetyl group of this analog to 3'-dephospho-CoA suggesting the intermediacy of a covalent thioacetyl-S-enzyme species, which appears to be important for proton abstraction from C-2 of the thioacetyl group. Avian enzyme glutamate 95 is crucial to substrate condensation to form a new carbon-carbon bond. Mutations of this invariant residue (avian enzyme E95A and E95Q; Staphylococcus aureus enzyme E79Q) correlated with diminished ability to catalyze enolization of acetyldithio-CoA. Enolization by E95Q was not stimulated in the presence of acetoacetyl-CoA. These observations suggest either a direct (proton abstraction) or indirect (solvent polarization) role for this active site glutamate.

EXPERIMENTAL PROCEDURES

Materials—Escherichia coli BL21(DE3) and the expression vectors pET-3d and pET-23d were purchased from Novagen (Madison, WI). Deoxynucleotides were purchased from Operon Technologies (Alameda, CA). QuikChange site-directed mutagenesis kits were obtained from Stratagene (La Jolla, CA). Qiagen (Chatsworth, CA) plasmid kits were used to isolate plasmid DNA from bacterial cultures. Restriction enzymes were purchased from New England Biolabs (Beverly, MA) and Amersham Biosciences. DNA sequencing was performed on an ABI 3100 Genetic Analyzer at the Protein/Nucleic Acid Facility of the Medical College of Wisconsin. Ampicillin and isopropylthigalactoside were purchased from United States Biochemical (Cleveland, OH). [1-14C]Acetyl-CoA is a product of Moravek Biochemicals (Brea, CA). All other reagents were purchased from Sigma, Aldrich, or Amersham Biosciences.

Construction of Mutant HMG-CoA Synthase—Mutagenesis to produce the E95Q form of avian enzyme and the E79Q form of the Staphylococcus aureus enzyme was performed using the Stratagene QuikChange mutagenesis kit and pairs of appropriate complementary mutagenic primers. Mutant plasmids were used to transform competent XL1-Blue cells. Mutagenic plasmid DNA was isolated from selected transformants and analyzed by restriction mapping and DNA sequencing. Mutant clones that were determined to be free from any PCR artifacts and confirmed to contain the desired substitution were transformed into competent BL21(DE3) cells for expression and isolation as described below.

Isolation and Assay of HMG-CoA Synthase Proteins—The procedure developed for purification of the avian wild-type enzyme (10) was followed for isolation of all mutant avian enzymes (C129S, D159A, D203A, E95A, and E95Q) from isopropylthigalactoside-induced bacterial cul-
tories. Wild-type and E79Q S. aureus HMG-CoA synthases have been engineered from genomic S. aureus DNA to incorporate C-terminal histidine tags (procedure to be described elsewhere) and were isolated from a nickel affinity resin column (1.0 × 15 cm) that had been exhaustively washed with equilibration buffer (50 mM sodium phosphate, pH 7.0, containing 300 mM NaCl, 20 mM imidazole, and 5 mM mercapto-ethanol) prior to elution of homogeneous protein using an imidazole gradient (20-300 mM, 100-ml volume). Protein content of the purified enzyme was estimated by the Bradford assay (11) using bovine serum albumin as the standard. All wild-type and mutant proteins are characterized by a high degree of homogeneity as assessed by SDS-polyacrylamide gel electrophoresis.

Either the standard spectrophotometric assay (2) or the more sensitive radioisotopic assay (2) was used to measure activity. In the spectrophotometric assay, the reaction mixture included 100 mM Tris-Cl, pH 8.2, 100 μM EDTA, appropriate amounts of HMG-CoA synthase (~6 μg for wild-type enzyme), 50 μM acetoacetyl-CoA, and 200 μM acetyl-CoA. The reaction was performed at 30 °C, and acetyl-CoA-dependent loss of acetoacetyl-CoA was measured as a decrease in 300 nm absorbance using a millimolar extinction coefficient of 3.6. For improved sensitivity, the spectrophotometric assay can be performed in the presence of 40 mM Tris-HCl, pH 8.2, 100 μM EDTA, appropriate amounts of HMG-CoA synthase, 50 μM acetoacetyl-CoA, and 200 μM acetyl-CoA.

For the radioisotopic assay, the reaction mixture included 100 mM Tris-Cl, pH 8.2, 100 μM EDTA, 50 μM acetoacetyl-CoA, 200 μM [1-14C]acetyl-CoA (8,600 cpm/nmol), and appropriate amounts of wild-type or mutant HMG-CoA synthase. The reaction was initiated by addition of radiolabeled acetyl-CoA to the assay mixture containing the rest of the components at 30 °C. At specified time intervals, 40-μl aliquots were removed from the incubation mixture and acidified with 6 M HCl. The mixture was heated to dryness, and acid-stable radioactivity after its conversion to citrate with excess citrate buffer, pH 6.7 at ambient temperature (22 °C). The final concentrations of HMG-CoA synthase used were 0 (as control), 15, 30, and 60 μM. After incubating enzyme in buffer solution at ambient temperature for 15 min, dithioacetyl-CoA and 3'-dephospho-CoA were added. 150-μl aliquots were taken at 0.4, 2, 4, 6, 8, and 10 min. To each aliquot, 5 μl of acetic acid was added to quench the reaction exchange, and 45 μl of 2-propanol (HPLC grade) were added to precipitate protein; the mixtures were then immediately frozen on dry ice. For further analysis, these mixtures (200 μl) were thawed and spin-filtered to remove denatured protein. 138 μl of the filtrates were injected for HPLC analysis. The HPLC column was a Spherical RP18 (250 × 4.6 mm, 5 μm), and the mobile phase was 50 mM NaP buffer, pH 4.5, MeOH (77:23). The column was eluted at flow rate of 0.8 ml/min. The UV detection was at 260 nm (absorption of CoA nucleotide). Under these conditions, the retention time of CoA was 2.8 min, 3'-dephospho-CoA was 4.1 min, dithioacetyl-CoA was 9.2 min, and dithioacetyl-diphospho-CoA was 16.6 min. The dithioacetyl-diphospho-CoA peak was further confirmed by its UV spectrum (A260 nm/3′A260 nm = 1.36) obtained from an aliquot of 50 μg protein/ml (data not shown).

The ability of HMG-CoA synthase to catalyze solvent deuterium exchange with methyl protons of acetyl-CoA was monitored by 1H NMR. HMG-CoA synthase proteins were subjected to three cycles of concentration in an Amicon ultrafiltration device to 250 μl followed by resuspension in 3 ml of a D2O solution of 20 mM potassium phosphate buffer, pH 7.8. Enzyme and acetyl-CoA solutions were both chelated separately. Exchange reactions containing HMG-CoA synthases were measured using samples that included 9 μM acetyl-CoA, 0.2 mM EDTA in 50 mM potassium phosphate buffer, pH 7.8, and various concentrations of enzyme. For selected enzymes, experiments using 5 μM acetyl-CoA were also performed; the exchange rate was unchanged at this substrate analog concentration (data not shown), suggesting that enzyme was saturated at 9 μM acetyl-CoA.

The nonenzymatic exchange rates were measured by omitting HMG-CoA synthase from the reaction mixture. 1H NMR experiments were performed on a Bruker AC-300 instrument operating at 300 MHz for 1H. After adding enzyme to the reaction mixture, sample was loaded into a 5-mm NMR tube, and spectra were recorded at 22 °C. 1H NMR spectra of the reaction mixture were recorded every 5 min over a period of 3 h. Each spectrum is comprised of a total of 64 acquisitions.

Proton exchange was estimated (7, 13) from the time-dependent change of the ratio of the 2.8 ppm α-hydrogen methyl resonance to the 0.75 ppm pantetheine methyl resonance (which is unaffected by the exchange process). kexch was calculated from Equation 1,

\[
\frac{k_{\text{obs}} - k_{\text{enzyme}}}{k_{\text{control}}} = \left[\frac{k_{\text{enzyme}}}{k_{\text{control}}} \right] (E) (2)
\]

where \(k_{\text{enzyme}}\) is calculated from the slope of the least squares fit of the data plotted as the log of the peak ratio versus time (SigmaPlot) for exchange reactions containing enzyme, while \(k_{\text{control}}\) is calculated from the slope measured in background controls performed without enzyme.

The exchange rate \(k_{\text{exch}}\), was calculated using Equation 2:

\[
k_{\text{exch}} = 3[A\text{cetyl-CoA}]_{0}/[\text{Protein}]
\]

**RESULTS**

Inhibition of HMG-CoA Synthase by Acetylthio-CoA—The structural homology between acetyl-CoA and acetylthio-CoA suggested that the latter compound might be an inhibitor of the HMG-CoA synthase reaction. Steady state kinetic experiments indicated that acetylthio-CoA does not support substantial enzyme-catalyzed condensation with acetoacetyl-CoA during
D203A mutant enzymes using either 5 or 9 mM levels of acetyl-CoA (10-15, 16) of Ac-CoA saturation of the complex formation for wild-type and mutant enzymes based on an analog is adequate to optimize binary enzyme-acetyldithio-CoA/CoA, and 6.5 µg of HMG-CoA synthase. Enzyme activity was estimated at 30 °C using a spectrophotometric assay that measures acetoacetyl-CoA disappearance at 300 nm. The final concentrations of acetyldithio-CoA inhibitor are as follows: ○, 0 µM; ●, 25 µM; □, 50 µM; and ■, 100 µM. Analysis of the data (Grafit) indicates that acetyldithio-CoA is a competitive inhibitor with respect to acetyl-CoA; the Ki value of acetyldithio-CoA is 28.0 ± 2.6 µM.

the short time period (several minutes) required for standard spectrophotometric assays. Under such conditions, it was possible to straightforwardly demonstrate that this analog is a competitive inhibitor with respect to acetyl-CoA (Fig. 1). As a competitive inhibitor, acetyldithio-CoA exhibited relatively high affinity with an estimated Ki value of 28.0 ± 2.6 µM. Such a value is comparable to acetyl-CoA requirements (10–40 µM) for half-saturation of wild-type and mutant enzymes in formation of binary complexes (15, 16) involved in enzyme acylation or acetyl-CoA hydrolysis partial reactions. These results prompted a test of whether enzyme-catalyzed exchange of the C-2 methyl protons of the analog could be detected.

**Enzyme-catalyzed Proton Exchange from Acetyldithio-CoA**—Avian cytosolic HMG-CoA synthase was observed to catalyze proton exchange from the C-2 methyl group of acetyldithio-CoA (Fig. 2A). The 9 mM concentration used for NMR detection of proton exchange is large in comparison with the measured inhibitor constant (Ki = 28 ± 2 µM). This concentration of analog is adequate to optimize binary enzyme-acetyldithio-CoA complex formation for wild-type and mutant enzymes based on previous observations (10, 15, 16) of Ac-CoA saturation of the mutants utilized in these studies. This prediction was tested by performing proton exchange experiments with wild-type and D203A mutant enzymes using either 5 or 9 mM levels of acetyldithio-CoA. The change in analog concentration had no influence on the measured exchange rate.

Under reaction conditions selected to maintain modest background levels of nonenzymatic exchange, it was possible to demonstrate that enzyme-catalyzed exchange is dependent on protein concentration (Fig. 2B). In contrast with observations for wild-type enzyme, comparable levels of mutant C129S synthase did not stimulate proton exchange above background rates (Fig. 2A and Table I). Since Cys-129 normally supports formation of the acetyl-S-enzyme reaction intermediate and is saturated by acetyl-CoA at levels comparable to wild-type synthase, it seemed possible that enzyme acylation might influence the enolization/proton exchange reaction. For this reason, two other HMG-CoA synthase mutants, D159A and D203A, which do not exhibit substantially altered ability to form binary acyl-CoA complexes but which exhibit very slow kinetics of acylation by acetyl-CoA (15), were tested for their ability to enolize acetyldithio-CoA. Both mutant enzymes (Table I) exhibited diminished rates of proton exchange (6.5- and 4.4-fold, respectively), suggesting that the enolization process might involve an acyl-enzyme species rather than the original acyl-CoA derivatives.

**Acetyldithio-CoA-dependent Transacylation**—HMG-CoA synthase-catalyzed exchange of the α-hydrogens of acetyldithio-CoA. A depicts the time dependence of the exchange reaction measured using 9 mM acetyldithio-CoA in 50 mM potassium phosphate buffer, pH 7.8, containing 0.2 mM EDTA. Data are plotted as the log of the ratio (R) of the dithioacetyl methyl proton resonance (2.8 ppm) to the pantetheine methyl proton resonance (0.75 ppm) versus time. A control experiment (●) was performed using conditions outlined above, but no enzyme was included. In measurements on enzyme-catalyzed exchange, the identities and concentrations of HMG-CoA synthase enzymes are: ■ wild-type, 0.1 mM; ● wild-type, 0.2 mM; ▲ C129S, 0.2 mM; and ▼ C129S, 0.4 mM. In B, kobs (calculated by a least squares fit of the rate data with a correction for the nonenzymatic control exchange rate) is plotted as a function of concentration of wild-type avian HMG-CoA synthase used in the exchange reaction.
square fit of the log of the ratio of the first order rate constant determined from the slope of the least squares fit of the log of the ratio of the $\alpha$-hydrogen resonance at 2.8 ppm to the pantetheine methyl resonance at 0.75 ppm versus time. The control sample was identical to enzyme samples except enzyme was excluded from the reaction mixture.

$^{b}$Enzyme-catalyzed proton exchange of acetyldithio-CoA with HMG-CoA synthase proteins was conducted using 9 mM acetyldithio-CoA in 50 mM potassium phosphate buffer, pH 7.8, containing 0.2 mM EDTA.

reaction and is mediated by formation of a covalent acyl-S-enzyme intermediate.

$$\text{acyl-CoA} + \text{enzyme} \rightleftharpoons \text{acyl-enzyme} + \text{CoA}$$

**REACTION 2**

$$\text{acyl-enzyme} + 3'\text{-dephospho-CoA} \rightleftharpoons \text{acyl-3'-dephospho-CoA} + \text{enzyme}$$

**REACTION 3**

When acetyldithio-CoA was tested as an acyl donor in the transacylation reaction, it was possible to measure the progress of the reaction at various time intervals by quenching, deproteinizing, and freezing aliquots of the reaction mixture. Subsequent estimation of acetyldithio-3'-dephospho-CoA formation was accomplished by HPLC separation and quantitation of the peak due to this product ($A_{260 \text{ nm}}$ detection; retention time, 16.6 min), which was well resolved from chromatogram peaks attributable to CoA, 3'-dephospho-CoA, and acetyldithio-CoA (retention times of 2.8, 4.1, and 9.2 min, respectively; reverse phase C$_{18}$ chromatography performed using 50 mM sodium phosphate (pH 4.5), methanol (77:23) at a flow rate of 0.8 ml/min). Acetyldithio-3'-dephospho-CoA was characterized by an $A_{260 \text{ nm}}/A_{340 \text{ nm}}$ ratio of 1.3, which is comparable to that measured for acetyldithio-CoA. The transacylation reaction supported by acetyldithio-CoA exhibited the expected linearity with time (Fig. 3A) and enzyme concentration (Fig. 3B). The observed transacylation, together with the contrasts between the abilities of wild-type enzyme and C129S, D159A, and D203A mutants to support enolization of acetyldithio-CoA, suggests that HMG-CoA synthase forms a covalent acetyl-enzyme adduct to Cys-129. These results for HMG-CoA synthase contrast with the reported inability (9) of $\beta$ ketothiolase to utilize acetyldithio-CoA to form an acyl-enzyme adduct.

**Enolization of Acetyldithio-CoA by Glutamate 95 Mutants—**

Previous work (16) has demonstrated that the E95A mutant form of HMG-CoA synthase is not substantially impaired in early stages of the reaction (e.g. Michaelis complex or covalent acetyl-enzyme formation) but fails to catalyze condensation with the second substrate, acetacetyl-CoA, at detectable levels. The ability of E95A to support proton exchange from the C-2 methyl group of acetyldithio-CoA was tested (Fig. 4). The exchange rate was diminished (2.9-fold, Table II) in comparison with wild-type enzyme. An E95Q mutant was also constructed to minimize stereic differences between side chains. The E95Q protein was purified and partially characterized (Table III). Even with the more conservative side chain substitution, lack of the active site carboxyl group in E95Q resulted in a diminution in specific activity for the overall condensation reaction of almost 5 orders of magnitude. As in the case of E95A, the E95Q mutant seemed impaired in the latter chemical steps of the reaction since $V_m$ for the partial reaction involving hydrolysis of acetyl-CoA was reduced by only 5-fold. Also the $K_m$ for acetyl-CoA in this partial reaction was altered by only 2-fold (Table III), suggesting that interaction of E95Q with this substrate is not markedly different from that observed for wildtype enzyme. While the combined stoichiometry of trapped Michaelis complex with acetyl-CoA and covalent acetyl-enzyme adduct was diminished by 2-fold, $^{13}$C NMR measurements of $[1,2,13]C$ acetyl-enzyme indicated the same distinctive chemical shifts that characterize wild-type enzyme (Table III). These observations do not suggest any perturbation of the local dielectric environment or thioester carbonyl polarization (17) for the E95Q mutant in comparison with wild-type enzyme. Thus, any differences in enolization/proton exchange from acyl-en-

![Figure 3](http://www.jbc.org/Downloaded_from)
zyme species formed using E95Q are likely to primarily reflect the lack of a carboxyl side chain. E95Q exhibited a slightly larger diminution in exchange rate (4.1-fold, Fig. 4 and Table II) than that observed for E95A. The observations for E95A and E95Q are also supported by preliminary results generated using a mutant of *S. aureus* HMG-CoA synthase in which the corresponding active site glutamate is replaced by glutamine (E79Q); a 6.9-fold diminution in exchange rate (*versus* wild-type *S. aureus* enzyme) was observed. While these rates were not reduced to the background levels measured for C129S synthase, the substantial effects implicate the glutamate 95 of avian HMG-CoA synthase in the enolization of the acyl-enzyme intermediate that precedes condensation with the second substrate, acetoacetyl-CoA.

Enolization of acetyl-CoA by several Claisen condensation enzymes (*e.g.* malate synthase and citrate synthase) is enhanced in the presence of an analog of the cosubstrate (4, 5). The inability of glutamate 95 mutants of HMG-CoA synthase to catalyze the condensation reaction with the second substrate, acetoacetyl-CoA, allowed a test of the influence of second substrate site occupancy on the efficiency of enolization. Interestingly inclusion of a 2-fold excess of acetoacetoy-CoA, a high affinity substrate (*K_m* ≈ 1 μM), with E95Q enzyme did not stimulate but instead diminished the rate of proton exchange from acetyldithio-CoA (Fig. 4). This observation for HMG-CoA synthase, which appears to enolize the covalent acyl-enzyme adduct, contrasts with the substrate analog stimulation of proton exchange by malate synthase (5) and citrate synthase (4).

However, those enzymes do not form covalent enzyme adducts from acyl-CoA substrates.

**DISCUSSION**

C-C bond-forming enzymes such as malate synthase, citrate synthase, and β ketothiolase share with HMG-CoA synthase the requirement to deprotonate/enolize their common substrate, acetyl-CoA, prior to condensation with their respective cosubstrate. While proton exchange from the acyl-CoA substrate is not facile for these enzymes, such exchange is accelerated for malate synthase and citrate synthase when analogs of cosubstrate (*e.g.* pyruvate and S-malate, respectively) are present. In the case of HMG-CoA synthase some contrasts are apparent. When acetyldithio-CoA was utilized as a substrate analog, substantial proton exchange/enolization was catalyzed in a time- and enzyme concentration-dependent reaction. No cosubstrate was required to support the observed exchange reaction. The availability of a mutant HMG-CoA synthase (avian E95Q) that does not catalyze the overall C-C bond-forming reaction at any substantial rate allowed the use of the actual cosubstrate, acetyl-CoA, to test the impact on acetyldithio-CoA enolization. In contrast to results for malate synthase or citrate synthase, no stimulation of HMG-CoA synthase E95Q-catalyzed exchange by acetyl-CoA was observed. Instead a slight decrease in proton exchange rate was measured. While such a decrease could be attributable to subtle changes in the E95Q active site, they may simply reflect hindered solvent access.

Another significant difference between C-C bond-forming enzymes that can utilize acetyl-CoA and/or acetyldithio-CoA is illustrated by the inability of β ketothiolase to form a thioacetyl adduct to enzyme (9). HMG-CoA synthase utilizes such an intermediate in catalyzing exchange of the acetyldithio moiety between CoA and 3′-dephospho-CoA groups. In other respects, these enzymes catalyze more homologous chemical events. Thiолase enolizes noncovalently bound acetyldithio-CoA prior to condensation with an acetyl-S-thiolase intermediate. HMG-CoA synthase enolized covalent thioacetyl-S-enzyme even in the absence of the cosubstrate that would be attacked by the C-2 carbannion of thioacetyl-S-enzyme. This observation raises a question over whether acetyldithio-CoA is actually a slow alternate substrate, rather than merely a substrate analog, for HMG-CoA synthase. HPLC monitoring of acyl-CoA derivatives produced from a mixture containing HMG-CoA synthase, acetyldithio-CoA, and acetoacetyl-CoA suggests that there is indeed a slow formation of a condensation product. However, after desalting of the putative product HPLC peak (C18 Sep-Pak), a methanol solution of the CoA derivative was subjected to matrix-assisted laser desorption ionization analysis, which indicated a molecular mass equivalent to HMG-CoA. Thus, using this mass spectroscopy approach, it was not possible to detect significant sulfur substitution in the C-5 carboxyl of HMG-CoA. Model organic studies (18, 19) suggest that nonenzymatic sulfur/oxygen exchange of similar compounds occurs and may account for lack of detection of a sulfur-substituted C-5 carboxyl in HMG-CoA. Alternatively reversible enzy-

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*H. M. Mizziorko, * unpublished structural observations.*

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Table II

| Enzyme         | k<sub>exchange</sub>*<sup>a</sup> | k<sub>exchange control</sub>*<sup>b</sup> |
|----------------|-------------------------------|---------------------------------|
| HMG-CoA synthase*<sup>c</sup> (wild type) | 4.5 ± 0.23 | |
| E95A           | 1.55 ± 0.19                   | 2.9                             |
| E95Q           | 1.10 ± 0.03                   | 4.1                             |
| E95Q + acetoacetyl-CoA | 0.33 ± 0.07 | 13.6                            |

*<sup>a</sup> *k<sub>exchange</sub>* was calculated from the equation 3[(Ac-dithio-CoA]<sub>enzyme</sub>]/[Enzyme]). *<sup>b</sup> *k<sub>exchange control</sub>* was calculated using the equation *k<sub>enzyme</sub> = k<sub>control</sub>* where *k* is the first order rate constant determined from the slope of the least squares fit of the log of the log of the α-hydrogen resonance at 2.8 ppm relative to the pantetheine methyl resonance at 0.75 ppm versus time. The control sample was identical to enzyme samples except enzyme was included from the reaction mixture.

*<sup>c</sup> *Enzyme-catalyzed proton exchange of acetyldithio-CoA by wild-type HMG-CoA synthase and active site glutamate mutants.*

**FIG. 4.** HMG-CoA synthase glutamate 95 mutant catalysis of proton exchange from acetyldithio-CoA. The time dependence of the exchange reaction was measured in samples containing 9 mM acetyldithio-CoA in 50 mM potassium phosphate buffer, pH 7.8, containing 0.2 mM EDTA. Data are plotted as the log of the ratio (R) of the dithioacetyl methyl proton resonance (2.8 ppm) to the pantetheine methyl proton resonance (0.75 ppm) *versus* time. A control experiment (*) was performed using conditions outlined above, and no enzyme was included. In measurements on glutamate 95 mutant enzyme-catalyzed exchange, the following enzymes were used: ▼, E95A, 0.2 mM; ▶, E95Q, 0.2 mM; and ▲, E95Q, 0.2 mM in the presence of 0.4 mM acetoacetyl-CoA.
catalyzed addition of solvent water across a dithioester linkage in either a thioacetyl-S-enzyme or an enzyme-dithio-HMG-CoA adduct is quite possible. Collapse of the tetrahedral intermediate formed after solvent attack will reform the covalent adducts, leaving group (20). Such sulfur/oxygen exchange from an acetyl-enzyme or thioacetyl-enzyme adduct may result in loss of sulfur since this would be a good direct (general base catalyst) or indirect (solvent polarization) role for the glutamate C-5 carboxyl in deprotonation of acetyl-enzyme.

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