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Expression, Purification, and Crystallization of the *Escherichia coli* Selenomethionyl β-Ketoacyl–Acyl Carrier Protein Synthase III

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Bacterial β-ketoacyl–acyl carrier protein (ACP) synthase III (KAS III, also called FabH) catalyzes the condensation and transacylation of acetyl-CoA with malonyl-ACP. In order to understand the mode of enzyme/substrate interaction and design small molecule inhibitors, we have expressed, purified, and crystallized a selenomethionyl-derivative of *E. coli* KAS III. Several lines of evidence confirmed that purified selenomethionyl KAS III was homogenous, stably folded, and enzymatically active. Dynamic light scattering, size exclusion chromatography, and mass spectrometry results indicated that selenomethionyl KAS III is a noncovalent homodimer. Diffraction quality crystals of selenomethionyl KAS III/acetyl-CoA complex, which grew overnight to a size of 0.2 mm^3, belonged to the tetragonal space group $P4_12_12$.

The pathway for the biosynthesis of saturated fatty acids is very similar in prokaryotes and eukaryotes. However, the organization of the biosynthetic apparatus is very different. Multicellular organisms possess a type I fatty acid synthase (FAS) in which all of the enzymatic activities are carried out by one multifunctional polypeptide, the mature protein being a homodimer (1). The acyl carrier protein (ACP) is an integral part of the protein complex. In contrast, in most bacterial and plant FASs (type II) each of the reactions is catalyzed by distinct monofunctional enzymes and the ACP is a discrete protein (for reviews see 2, 3). Because of this difference, there appears to be considerable potential for selective inhibition of the bacterial systems by broad-spectrum antibacterial agents.

In the biosynthetic cycle, malonyl-ACP is synthesized from ACP and malonyl-CoA by malonyl CoA:ACP transacylase (also called FabD) (4). Malonyl-ACP is then condensed with acetyl-CoA by β-ketoacyl-ACP synthase III (KAS III, also called FabH) to form acetoacetyl-ACP (5, 6). The next step in the elongation cycle is ketoester reduction by β-ketoacyl-ACP reductase (also called FabG) (7). Subsequent dehydration by β-hydroxyacyl-ACP dehydrase leads to trans-2-enoyl-ACP (8) which is in turn converted to acyl-ACP by enoyl-ACP reductase (also called FabI) (9). In subsequent rounds malonyl-ACP is condensed with the growing-chain acyl-ACP by KAS I (also called FabB) and KAS II (also called FabF) (6, 10). Further rounds of this cycle, adding two carbon atoms per cycle, eventually lead to the formation of mature acyl-ACPs whereupon the cycle is stopped due to feedback inhibition of KAS III and KAS I (11).

Several lines of evidence have indicated that the KAS enzymes are central to the initiation and elongation steps in bacterial fatty acid synthesis and play a pivotal role in the regulation of the entire pathway (6, 10, 12–15). Among these, KAS III is unique in that it utilizes acetyl-CoA as an acyl-group donor whilst KAS I and II both utilize acyl-ACPs as primers. KAS III also possesses acetyl-CoA:ACP transacylase activity *in vitro,* although the physiological role of this reaction is unknown. Both KAS I and II are capable of initiating fatty acid synthesis *via* decarboxylation of malonyl-ACP to produce acetyl-ACP, although this reaction cannot substitute for KAS III. Unlike KAS I and II, which are sensitive to both antibiotic cerulinin (16, 17) and thiolactomycin (6, 18, 19), KAS III is only sensitive

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to thiolactomycin (5). Extensive work with these inhibitors has proven that the fatty acid biosynthetic pathway is essential for bacterial viability. Additionally it has been shown genetically in *S. pneumoniae* that there are no mechanisms for compensating for the loss of KAS III or KAS I activity (J. Lonsdale, unpublished results). Thus, all lines of evidence indicate that KAS III plays a critical role in the fatty acid biosynthesis pathway and provides a valid and unexploited target for drug discovery.

In order to understand further the mechanism of substrate and inhibitor binding and to develop potent and specific inhibitors in a rational manner, we sought to determine the three-dimensional structure of *E. coli* KAS III in the presence of acetyl-CoA (20). In this paper, we describe in detail the expression, purification and biophysical characterization of selenomethionyl KAS III and its crystallization in the presence of acetyl-CoA.

MATERIALS AND METHODS

Cloning and expression of KAS III gene in E. coli. The *E. coli KAS III* gene (6) was amplified from genomic DNA by polymerase chain reaction (PCR) using the primers: (5′-TATACATATGTATACGAAGATTATTGGT-3′; SEQ ID AKK2) and (5′-ATATGGATCCCTAGAAACGAACCAGCGCGG-3′; SEQ ID AKK3). *NdeI* and *BamHI* restriction sites were incorporated at the 5′ and 3′ ends respectively of each primer to facilitate ligation of the amplified DNA into the appropriate vectors. PCR was performed using the GeneAmp, PCR System 2400 (Perkin Elmer Cetus Co). PCR-amplified DNA fragments were purified and ligated overnight to the *NdeI and BamHI* sites of already digested vector pET29. The ligation mixture was used to transform *E. coli* DH10B competent cells. The construction of pET29 was confirmed by restriction and sequence analysis.

To obtain soluble selenomethionyl KAS III for purification and crystallization studies, the methionine pathway inhibition protocol was followed (21, 22). *E. coli* strain BL21 (DE3) was transformed with pET29 containing the *KAS III* gene. 50 μl of the seed culture expressing the KAS III gene product was inoculated into 100 ml of Luria broth, containing kanamycin (50 μg/ml) and D-glucose (0.6%). On reaching $OD_{600} = 2$, the cells from the seed culture were isolated by centrifugation (10 min at 15,000*g*), resuspended in 100 ml of M9 minimal medium containing 1 mM $CaCl_2$, 1 mM $MgSO_4$, kanamycin (50 μg/ml) and glucose (0.6% w/v). The resuspended pellets were then added to 900 ml of the same medium and the cells were grown at 37°C to mid-log phase (A600 = 1.5), at which point lysine, phenylalanine, threonine at 100 mg/l each, and selenomethionine, isoleucine, leucine, and valine (all from Sigma), at 50 mg/l were added. The culture was shaken for 15 min, and then induced with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG). The culture was grown for 4 h, and harvested by centrifugation (30 min at 15,000*g*). 5-ml aliquots were taken prior to and during induction to monitor the expression of selenomethionyl KAS III. 12 g of cell paste (wet wt) was recovered from 5 liter culture. In addition, the natural KAS III was expressed under identical conditions except for the use of casamino acid mixture (DIFCO) instead of the specific amino acids mentioned above.

Purification of selenomethionyl KAS III. All lysis and purification steps were carried out using degassed buffers in a cold room or on ice. 4.5 g of *E. coli* cells overexpressing KAS III were resuspended in 50 ml of lysis buffer (buffer A) containing 20 mM Tris-HCl (pH 8.0), 50 mM sodium chloride, 10% glycerol, 0.2 mM PMSF, and 2 mM DTT. Cells were lysed twice at 10,000 psi using Microfluidizer (Microfluidics Corporation). Cell debris was removed by centrifugation (Sorvall RC-5B) at 35,000*g* for 30 min. The supernatant was applied to a 2.6 × 4 cm Source Q column (Pharmacia) equilibrated in buffer A. The column was washed with 10 column volumes of buffer A, and eluted with a 10 column volume linear gradient of 0–1.0 M NaCl in buffer A. Fractions containing KAS III, eluted at 0.2–0.25 M NaCl, were pooled and applied to a hydroxyapatite column (2.6 × 6 cm, Type I, 40 micron) (Bio-Rad) equilibrated in buffer A. The column was eluted with a 30 column volume linear gradient of 0–400 mM potassium phosphate in buffer A. KAS III, which eluted at 80–180 mM potassium phosphate, was diluted 1:2 with 50 mM Tris (pH 7.5), 200 mM NaCl, 10% glycerol, and 2 mM DTT (buffer B) and applied to a blue Sepharose column (1.6 × 7.5 cm, Pharmacia) equilibrated in buffer B. The column was eluted with 50 mM Tris (pH 7.5), 10% glycerol, 2 mM DTT, and 1 M NaCl. Blue Sepharose eluted KAS III fractions were next applied to a Superdex 200 size exclusion column (2.6 × 60 cm, Pharmacia) equilibrated in 20 mM Tris (pH 7.5), 50 mM NaCl, and 2 mM DTT. Purified protein was concentrated to 13 mg/ml using an Amicon 3 (Waters) filtration device.

Protein analysis. SDS-PAGE was performed as described (23). Protein bands were visualized using Coomassie brilliant blue 250. Native isoelectric focusing (IEF) analyses were performed on Nu-PAGE gels essentially as recommended by the manufacturer (Novex, San Diego, CA). N-terminal sequence analyses were performed on the protein blotted on PVDF membranes (24). Sequencing was performed on a Hewlett-Packard G1000A N-terminal Protein Sequencer with on-line phenylthiohydantoin (PTH) identification using an HP1100 HPLC. Samples were applied directly to biphasic sequencing cartridges and standard 3.1 sequencing method cycles were used. Amino acid composition analyses were performed using a Beckman System 6300 amino acid analyzer after hydrolysis with 6 N HCl at 110°C for 24 h.

Mass spectrometry analysis. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) data were obtained from a PerSeptive Biosystems Voyager RP (Perkin Elmer-PerSeptive Biosystems) laser desorption time-of-flight mass spectrometer. Protein samples were prepared for analysis by diluting 1:5 with 3,5-dimethoxy-4-hydroxycinnamic acid (10 mg/ml in 2:1 0.1% trifluoroacetic acid/acetonitrile) for a final concentration of 1–10 pmol/μl. Bovine α-lactoglobulin A (Sigma) was included as an internal calibrant (MH^+ 18364 Da). Desorption/ionization was accomplished using photon irradiation from a 337-nm pulsed nitrogen laser and 30-keV accelerating energy. Spectra were averaged over 100 laser scans.

Enzyme assay. KAS III activity was measured by following the formation of acetoacetyl-ACP using [^{3}H]-acetyl-CoA and malonyl-ACP. The substrate [^{3}H]-acetyl-CoA is soluble in 10% TCA while the resulting [^{3}H]-acetoacetyl-ACP is insoluble. Typically, final reaction conditions contained 34 μM acetyl-CoA, 0.15 μM [^{3}H]-acetyl-CoA (specific activity 25 Ci/mmol), 7 μM malonyl-ACP in 100 mM sodium phosphate buffer (pH 7.0) and 2 mM DTT. The reaction was initiated with the addition of KAS III and the reaction mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 10% TCA, and 2 mg/ml of BSA was added as a carrier. The contents of the reaction mixtures were filtered and washed 2 times with 10% TCA and 2 mg/ml BSA on Wallac GF/A filtermats using a TomTec harvester. The filtermats were dried completely at 60°C and the radioactivity was quantified using Wallac Betaplate scintillation cocktail and a Wallac Microbeta 1450 liquid scintillation. Specific activity (after normalization for substrate concentration) was determined as μmoles of acetoacetyl-ACP formed/min/mg of protein.

Dynamic light scattering analysis. Dynamic light scattering analysis was performed using a DynaPro-MSTC instrument (Protein Solutions, Inc.). The purified protein (20 μl) was injected through a

0.02 μm syringe filter kit into a cuvette provided by the manufacturer at 20°C. The protein concentration was 7 or 13 mg/ml. Data analyses were performed with Protein Solutions Dynamics V4.0 software.

Circular dichroism (CD) spectroscopy. CD analysis was performed on purified KAS III (0.15 mg/ml in 10 mM Tris-HCl (pH 7.4), 25 mM NaCl, and 1 mM DTT) at 20°C. Far-UV CD spectra were recorded on a Jasco J-710 CD instrument using a 0.1-cm-path-length cell. Data (10 accumulations) were collected using a time constant of 2 s with a 1 nM constant spectral band width at 50 nm/min. The CD data were analyzed for secondary structure prediction by an algorithm (Softsec for Windows Version 1.2) provided by the manufacturer (Jasco). Thermal stability of selenomethionyl KAS III was measured by scanning temperature at 1°C/min while monitoring the CD signal at 220 nm.

Crystallization. Initially, sparse matrix screening (25) was used with natural KAS III to identify crystallization conditions which were then modified slightly to grow crystals of the selenomethionyl FabH in complex with one of its substrates, acetyl-CoA (Sigma). Sitting drop vapor diffusion experiments were set up with equal volumes of the protein solution and the reservoir. All solutions used in reservoirs were degassed prior to setting up crystallization experiments.

RESULTS

Expression of Selenomethionyl KAS III in E. coli

To express the selenomethionyl derivative of *E. coli* KAS III, the *E.coli* strain BL21 (DE3) harboring pET29 plasmid was grown in the presence of selenomethionine as described under Materials and Methods. To confirm and monitor the incorporation of selenomethionine in expressed KAS III, natural *E. coli* KAS III was also produced. As shown in Fig. 1A, the expression level of the selenomethionyl KAS III (33 kDa species) was comparable to that of the natural KAS III implying that under the experimental growth conditions selenomethionine was not toxic to cells.

Consistent with the results of Tsay and co-workers (6), our earlier N-terminal sequence analysis of the purified natural form of KAS III confirmed the presence of methionine at its N-terminus. We therefore used N-terminal sequence analysis as a quick way to monitor and evaluate the efficiency of selenomethionine incorporation during the expression of KAS III. Samples from natural and selenomethionyl KAS III, each following four hours after induction with IPTG, were subjected to SDS-PAGE, and blotted and sequenced as described under Materials and Methods. N-terminal sequence analysis of the natural KAS III showed the presence of the PTH derivative of N-terminal methionine at cycle 1 (Fig. 1B, top). The KAS III protein expressed in the presence of selenomethionine, however, did not show a peak at the corresponding position, indicating the absence of methionine at cycle 1 (Fig. 1B, bottom). Instead, a unique peak, corresponding to the PTH derivative of selenomethionine was observed, which eluted 1.6 min later than the natural phenylthiohydantoin (PTH)-methionine (Fig. 1B, bottom). The delayed elution is consistent with the increase in hydrophobicity of the PTH-selenomethionyl amino acid derivative compared to the natural PTH methionine (26).

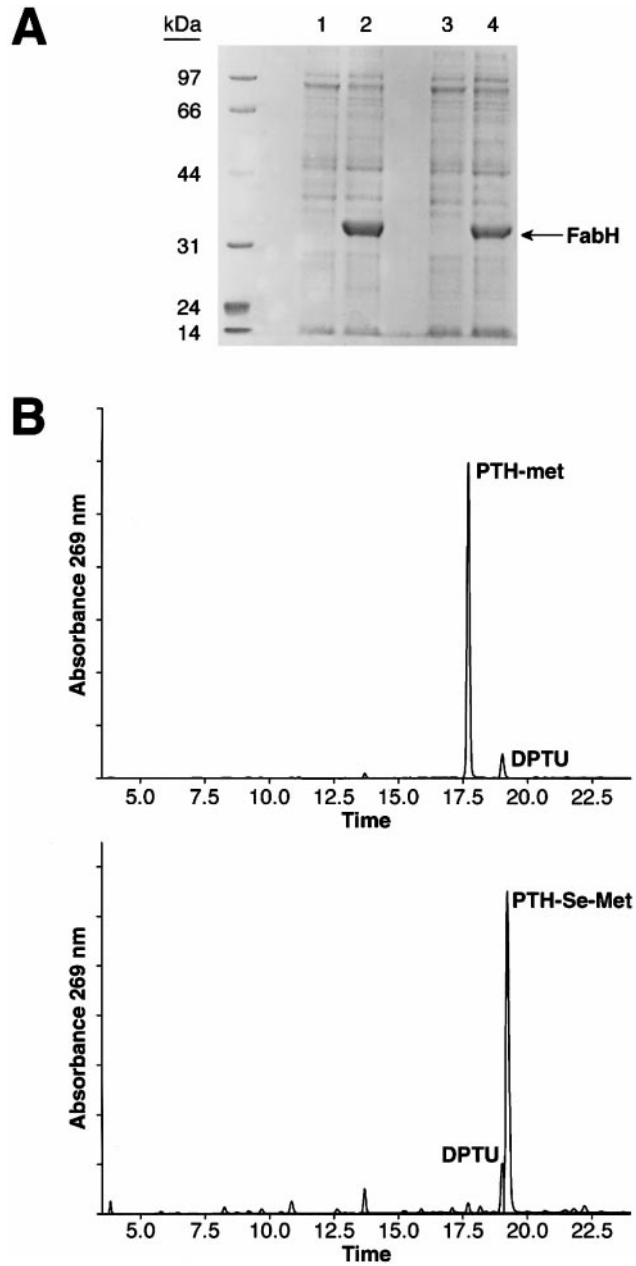

FIG. 1. Expression of KAS III. (A) SDS-PAGE analysis of natural and selenomethionyl KAS III. Cell pellets obtained from 100 μl of cell culture either uninduced (Lanes 1 and 3) or 4 hours post induction (Lanes 2 and 4) expressing natural (Lanes 1 and 2) or selenomethionyl KAS III (Lanes 3 and 4) were solubilized and analyzed by SDS-PAGE under reducing conditions. The arrow marks the unique induced band (FabH, also called KASIII) at about 33 kDa. (B) N-terminal sequence analysis of natural and selenomethionyl KAS III. Samples (Lanes 2 and 4) were blotted onto Problot membranes and N-terminal sequences of natural (top) and selenomethionyl KAS III (bottom) were determined as described under Materials and Methods. PTH-derivative of methionine (Met) or selenomethionine (Se-Met) present in cycle 1 of natural and selenomethionyl KAS III are shown. DPTU is a reference standard.

The second residue at the N-terminus is tyrosine, which eluted in equimolar amounts at identical positions in both natural and selenomethionyl KAS III (results not shown). Based on the elution profiles of the first two residues, we calculated that >96% selenomethionine was incorporated in the selenomethionine KAS III. This was further confirmed with the purified preparations of the protein (see below).

Purification and Characterization of Selenomethionyl KAS III

Selenomethionyl KAS III was purified from *E. coli* cell lysate using a combination of anion exchange, hydroxyapatite and blue sepharose dye binding chromatography. To avoid oxidation of selenomethionine, all buffers were degassed, and DTT (2 mM) was included throughout the purification. The blue sepharose eluted selenomethionyl KAS III was further purified and buffer exchanged over a Superdex 200 size exclusion column, which resulted essentially in a single peak. A similar protocol was used for the purification of natural *E. coli* KAS III. The overall yield of purified selenomethionyl KAS III was ~5 mg/g (wet wt) of *E. coli* cells.

Purified selenomethionyl KAS III was nearly homogeneous as judged by analytical Superdex 200 size exclusion chromatography (Fig. 2A) and SDS-PAGE (Fig. 2B). To confirm isoelectric homogeneity and to determine its isoelectric point (pI), we subjected purified selenomethionyl KAS III to IEF analysis under native conditions (Fig. 2C). Purified selenomethionyl KAS III migrated predominantly with a pI of 5.1, which is in close agreement to its theoretical pI of 5.01 calculated from its amino acid sequence. As expected, natural KAS III also migrated with a pI of 5.1 under identical conditions (not shown). Together, the SDS-PAGE, size exclusion chromatography and native IEF results indicated that the purified selenomethionyl KAS III was homogeneous and therefore suitable for crystallization studies.

The N-terminal sequence determined for the purified protein product (SeM-YTKIIGTGS) was as expected, and also further confirmed the presence of selenomethionine (Se) at the N-terminus of the purified protein. Since, unlike methionine, selenomethionine is decomposed during acid hydrolysis (26, 27), we further confirmed the presence of selenomethionine by following its absence in the purified selenomethionyl KAS III using amino acid analysis. Results (not shown) confirmed that all eight methionines present in natural KAS III were substituted with selenomethionine in selenomethionyl KAS III.

The molecular mass of selenomethionyl KAS III was determined by several methods. The predicted molecular mass for native KAS III based on its primary amino acid sequence is 33,516 Da. MALDI-MS analysis of the selenomethionyl incorporated KAS III protein construct provided a mass of 33,889 Da (Figs. 3A and 3B for natural and selenomethionyl KAS III, respectively). This is in close agreement with the predicted +375 Da shift in mass (33,891 Da theoretical, based on the primary amino acid sequence) expected for the sulfur to selenium side-chain substitution of eight methionine residues within the protein. Together, the N-terminal sequence, amino acid composition and MALDI-MS analyses confirmed the homogeneous incorporation of selenomethionine in expressed KAS III molecule.

To determine the molecular weight and association status of purified selenomethionyl KAS III in solution, we subjected it first to size exclusion chromatography. Molecular weight of selenomethionyl KAS III (1 mg/ml) by size exclusion chromatography performed under nondenaturing conditions was determined to be approximately 65 kDa, implying dimeric nature of the protein. Analogous results were obtained for the natural KAS III (not shown).

Since crystallization of selenomethionyl KAS III was carried out at higher (>5 mg/ml) concentration, we next examined its molecular weight at two different (7 mg/ml and 13 mg/ml) concentrations using a dynamic light scattering technique (28). This technique, which measures the translational diffusion coefficient of a macromolecule undergoing Brownian motion in solution, is also useful in determining the aggregation state and, therefore, crystallizability of proteins at high concentrations (29, 30). Purified selenomethionyl KAS III was monodisperse at both concentrations. Assuming the protein to be globular in solution, similar to that observed in the crystal form (20), molecular size estimation showed selenomethionyl KAS III to have a hydrodynamic radius of 3.47 nM. Using the hydrodynamic radius, the molecular weight of selenomethionyl KAS III was estimated to be 56 kDa. Based on primary amino acid sequence, and SDS-PAGE, SEC and dynamic light scattering analysis, purified selenomethionyl KAS III appeared to be a noncovalent homodimer.

Enzymatic activity of purified preparations of native and selenomethionyl KAS III was measured by following the formation of acetoacetyl-ACP using [^{3}H]-acetyl-CoA and malonyl-ACP. Specific activity of selenomethionine *E. coli* KAS III was 5.9 μmoles/min/mg, which is similar to that of natural KAS III (5.3 μmoles/min/mg), supporting the native-like properties of selenomethionyl KAS III.

We also evaluated the secondary structure and thermal stability of purified selenomethionyl KAS III by circular dichroism spectroscopy. The Far UV-visible CD spectrum of selenomethionyl KAS III, shown in Fig. 4, indicated that the purified protein is folded well. Analysis of the spectrum predicted the presence of both α-helix (~46%) and β-sheet (~24%) structural ele-

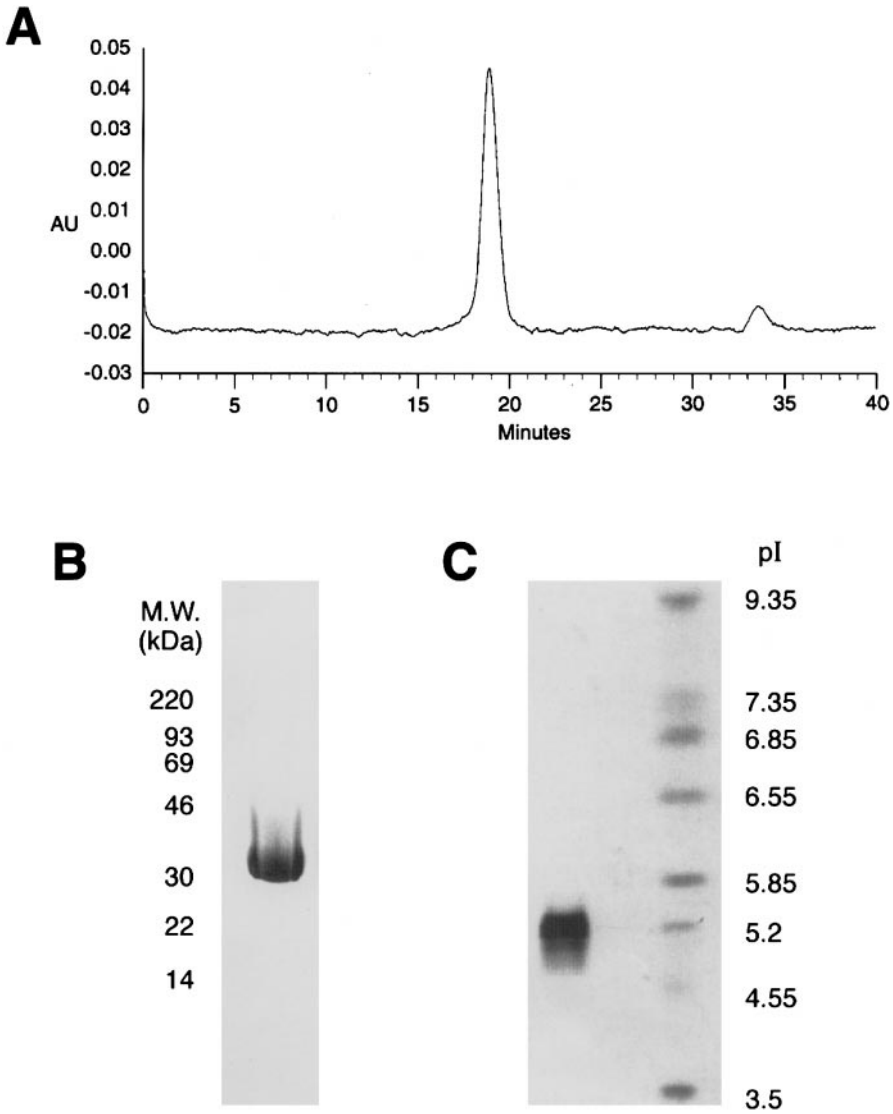

FIG. 2. Characterization of purified selenomethionyl KAS III. (A) Size exclusion chromatography. 50 μg of purified KAS III (1 mg/ml) was analyzed on a Superdex 200 column that was equilibrated with 20 mM sodium phosphate and 150 mM NaCl (pH 7.2) and calibrated with the following molecular mass standards (Pharmacia): thyroglobulin (666 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and vitamin B12 (1 kDa). (B) SDS-PAGE analysis. Purified selenomethionyl KAS III (20 μg) was subjected to 10% SDS-PAGE under reducing conditions. The band was visualized with Coomassie blue stain. (C) IEF analysis. Purified selenomethionyl KAS III (25 μg) was analyzed by IEF on a Novex (pH 3–10) gel at room temperature under native conditions. The protein bands were visualized using Coomassie blue stain. The known pI values for the marker proteins (Pharmacia) are shown.

ments. The inset to Fig. 4 shows the temperature dependence of selenomethionyl KAS III secondary structure. The results demonstrate that purified protein is stably folded and unfolding begins to occur above 50°C with an apparent T_m near 70°C.

Crystallization of Selenomethionyl KAS III

For crystallization studies, purified selenomethionyl FabH (13 mg/ml) in a buffer containing 20 mM Tris (pH 7.5), 50 mM NaCl, and 10 mM DTT was mixed with 4 mM acetyl-CoA. The reservoir solution used was 12–14% PEG4000, 40 mM Bis Tris propane (pH 7.0) and 80 mM magnesium acetate. Droplets contained 2 μl protein–substrate complex and 2 μl reservoir solution. Diamond-shaped crystals grew overnight at room temperature to a size of 0.2 mm^3. Diffraction data from frozen cocrystals were measured to 1.9 Å. The selenomethionyl FabH/acetyl CoA complex crystal belonged to the tetragonal space group $P4_12_12$. The eight selenomethionine molecules of FabH were correctly located by the program SOLVE and the structure was determined (20).

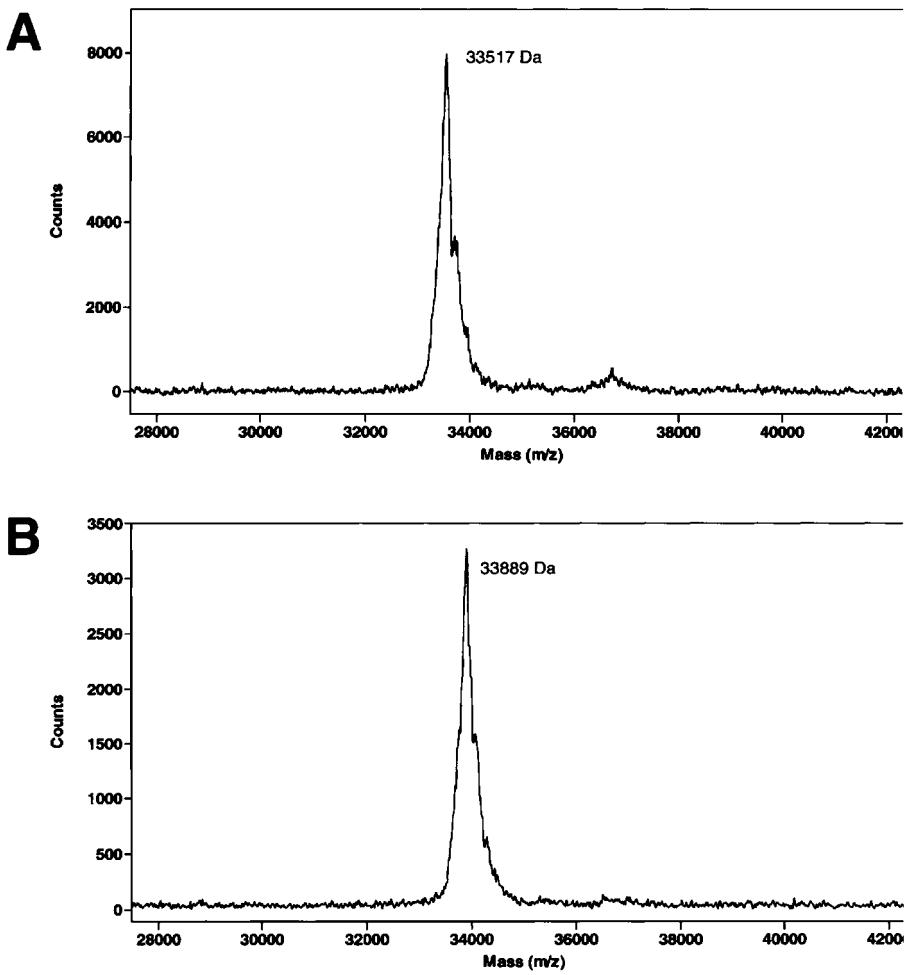

FIG. 3. Mass spectrometry analysis of KAS III. Purified (10 μg) natural (A) and selenomethionyl KAS III (B) were analyzed by MALDI-MS.

DISCUSSION

The β-ketoacyl-ACP synthase III (KAS III, also called FabH) of *E. coli* catalyzes the condensation of acetyl-CoA with malonyl-ACP and also possesses acetyl-CoA:ACP transacylase activity. Based on several lines of evidence, KAS III plays an important role in the bacterial fatty acid biosynthesis pathway and thus provides a valid and unexploited target for drug discovery.

The utility of selenomethionine for macromolecular phase determination via multiwavelength, anomalous dispersion (MAD) is proven in protein crystallography (26, 31). The MAD technique helps in model building by pinpointing the methionine residues and also allows all data to be collected from a single crystal. The *E. coli* KAS III contains eight methionine residues in its sequence. In order to solve its crystal structure, we therefore sought to express, purify and properly characterize a selenomethionyl variant of *E. coli* KAS III.

The selenomethionyl KAS III was expressed by following the methionine pathway inhibition protocol (21, 22). This procedure does not require use of a met$^-$ strain and also does not require the design of new expression vectors. The presence of N-terminal methionine in the expressed protein allowed us to effectively monitor and evaluate the selenomethionine incorporation simply by subjecting the cell lysates to western blotting followed by N-terminal sequence analysis. Full incorporation of selenomethionine in the expressed protein was achieved in four hours following induction with IPTG.

Properties of the purified selenomethionyl KAS III were virtually identical to those of natural KAS III indicating that selenomethionine can replace methionine residues without altering the properties of KAS III. The specific activity of selenomethionyl KAS III was, however, slightly higher than that of the natural KAS III. While methionines are not implicated in the active site of *E. coli* KAS III (6), Met207 is located in the substrate binding tunnel (20). The higher specific activity of selenomethionyl KAS III may be an effect of the selenium in Met207 on the enzymatic reaction,

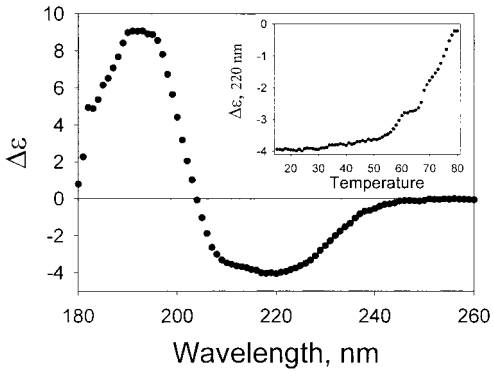

FIG. 4. Far UV-circular dichroism spectrum of purified selenomethionyl KAS III shown in units of difference molar extinction coefficient per mole of peptide bond. Analysis was performed on purified KAS III (0.15 mg/ml in 10 mM Tris, 25 mM NaCl, 1 mM DTT, pH 7.4) at 20°C. Inset: Thermal stability of selenomethionine KAS III monitored by circular dichroism signal at 220 nm. Half-unfolded T_m is approximately 70°C.

may be caused by differences in the hydrophobicity or differences in the van der Waals radius of the protein due to selenium (31) or a combination of these. Similar observations have been reported for interleukin-5 (32) and tryptophanyl-tRNA synthetase (33). Obviously, further studies, including site directed mutagenesis, are needed to elucidate the role of Met207 in the catalysis by KAS III.

Based on primary amino acid sequence, SDS-PAGE, SEC and dynamic light scattering analysis, purified selenomethionyl KAS III appeared to be a noncovalent homodimer. The homodimeric nature of the *E. coli* KAS III is the same as that reported for the *E. coli* KAS I (13) and KAS II (13, 14) proteins. KAS III of *Streptomyces glaucescens*, which contains a Type II FAS, has also been shown to be homodimeric in nature (34). All four enzymes participate in the chain elongation steps of the fatty acid biosynthesis pathway.

In agreement with the solution studies, the crystal structure of *E. coli* KAS III in the presence and absence of acetyl-CoA revealed a homodimer (20). Similarly, KAS II in the crystal was shown to be homodimeric in nature (14). Since the apo KAS III in solution and in the crystal was homodimeric, the presence of acetyl-CoA seems to have no effect on the aggregation status. Interestingly, the CoA molecule was bound to KAS III in the crystal indicating a high affinity for KAS III (20).

The CD results indicated that selenomethionyl KAS III was stably folded with an apparent Tm near 70°C. Deconvolution of the circular dichroism data predicted 46% α-helix and 24% β-sheet secondary structural elements. These values are in reasonable agreement with those determined from the crystal structure of FabH: 39% α-helix and 28% β-sheet (20). The crystal structure of *E. coli* FabH displayed a five-layered core structure, α-β-α-β-α, where each α comprises two α-helices and each β consists of a five-stranded, mixed β-sheet (20). Interestingly, the core structures of *E. coli* KAS II and KAS III despite 14% sequence identity, are similar (14), and a similar core structure has been reported for thiolase I of *Saccharomyces cerevisiae* which catalyzes the formation of acetyl-CoA from ketoacyl-CoA (35). Taken together, the overall core structure, association state, and amino acid sequence surrounding the active site cysteine appear to be the common features of the enzymes participating in the condensation reactions.

In conclusion, the availability of purified KAS III and its structure in the presence of acetyl-CoA will facilitate further understanding of the mode of enzyme/substrate interaction and may help in the identification of novel inhibitors of this important target using a structure-based approach.

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