The mevalonate-dependent pathway is used by many organisms to synthesize isopentenyl pyrophosphate, the building block for the biosynthesis of many biologically important compounds, including farnesyl pyrophosphate, dolichol, and many sterols. Mevalonate kinase (MVK) catalyzes a critical phosphoryl transfer step, producing mevalonate 5′-phosphate. The crystal structure of thermostable MVK from *Methanococcus jannaschii* has been determined at 2.4 Å, revealing an overall fold similar to the homoserine kinase from *M. jannaschii*. In addition, the enzyme shows structural similarity with mevalonate 5-diphosphate decarboxylase and domain IV of elongation factor G. The active site of MVK is in the cleft between its N- and C-terminal domains. Several structural motifs conserved among species, including a phosphate-binding loop, have been found in this cavity. Asp258, an invariant residue among MVK sequences, is located close to the putative phosphate-binding site and has been assumed to play the catalytic role. Analysis of the MVK model in the context of the other members of the GHMP kinase family offers the opportunity to understand both the mechanism of these enzymes and the structural details that may lead to the design of novel drugs.

The ability to make isopentenyl pyrophosphate is essential for all organisms, because this molecule is the building block for synthesizing many biologically important compounds, such as ubiquinone, cholesterol, dolichol, steroid hormones, and some vitamins (1). Two metabolic pathways, either mevalonate-dependent or –independent, are available for the production of isopentenyl pyrophosphate. For mevalonate-dependent isoprenoid synthesis, assembly begins with the condensation of three acetyl-CoAs to generate a β-hydroxy-β-methylglutaryl-CoA molecule, which is reduced to mevalonate. Mevalonate kinase transfers the γ-phosphoryl group from ATP to the 5-hydroxyl oxygen in mevalonate to produce phosphomevalonate (Fig. 1). A second phosphoryl group is then added by phosphomevalonate kinase and the product, 5-pyrophosphomevalonate, is decarboxylated to isopentenyl pyrophosphate (1, 2). Despite the existence of a mevalonate-independent pathway in some microorganisms, the process that generates isopentenyl pyrophosphate via mevalonate is critical in most eukaryotes and some microorganisms.

Kinesins have been classified mainly into three distinct families according to their sequence similarities and structural folds (3). The classical P-loop-containing kinesins share a common loop between a β-strand and an α-helix, with the consensus sequence Gly-Xaa1-Gly-Xaa2-Gly-lys (3, 4). A second group, composed primarily of protein kinasen (4), has a conserved loop connecting two antiparallel β-strands that are involved in phosphate binding. The third group, which includes actin, Hsp70, and sugar kinasen (4, 6), uses two β-hairpins to bind the phosphate in ATP.

Mevalonate kinase is a member of the distinct GHMP superfamily, which includes galactokinase (G), homoserine kinase (H), mevalonate kinase (M), and phosphomevalonate kinase (P) (4, 7). 4-Diphosphocytidyl-2C-methylerythritol kinase, mevalonate 5′-diphosphate decarboxylase, and archaeal shikimate kinase have also been assigned as members of this family (4, 8). It is important to note that shikimate kinases classified in yeast and prokaryotes have the P-loop kinase fold (8, 9). These proteins share several structural features. A common motif, Pro-Xaa1-Gly-Leu-Gly-Ser-Ser-Ala-Ala, is highly conserved and is thought to be involved in the binding of ATP, functioning as a phosphate-binding loop. The crystal structure of the homoserine kinase, which shares about 20% sequence identity with the mevalonate kinase, has recently been solved, both as the apoprotein and as complexed forms (4, 10). Its structure shows a novel left-handed βαββ fold similar to domain IV of elongation factor G (4). Similar to classic P-loop kinasen, the phosphate binding site is composed of a conserved loop between a β-strand and an α-helix, with the consensus sequence Pro-Xaa3-Gly-Leu-Gly-Ser-Ser-Ala-Ala. Unexpectedly, the HSJK-substrate complex structures suggest that the orientation of the bound ATP to HSK is different from classical P-loop kinasen, with the purine base located at what would be the phosphoryl acceptor substance binding site in a classic P-loop kinase (3–5, 10). Moreover, ATP seems to adopt an energeti...
EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The Methanococcus jannaschii MVK sequence was initially cloned into pET28a vector with an N-terminal His tag and a T7 promoter, and the construct was used to transform BL21(DE3) Escherichia coli strain (2). Cells were grown in LB medium containing 100 μg/ml kanamycin at 37 °C until A600 was around 0.8. Cells were harvested, washed with M9 minimal medium (with 100 μg/ml kanamycin and 50 μg/ml 19 amino acids except Met), and then grown in this medium for 1 h at 37 °C. Selenomethionine was added to 50 μg/ml and isopropyl β-D-thiogalactopyranoside to 1 mM concentration. Cells were grown at 18 °C for 20 h and then harvested and frozen at −20 °C. Cells were thawed, re-suspended in 20 mM Tris, 10 mM dithiothreitol (pH 8.0), and then lysed by French press and sonication. The extract was heated to 70 °C for 15 min to precipitate most E. coli proteins. The enzyme was purified to homogeneity, as judged by SDS-PAGE, on a nickel affinity column (AP Biotech) following the instructions of the supplier.

Crystallization and Data Collection—Crystallization was accomplished by the hanging drop vapor diffusion method. The MVK (10 to 15 mg/ml) in 5 mM Tris, 10 mM diithiothreitol (pH 8.0) was mixed with an equal volume of the reservoir solution (28% dioxane and 10 mM diithiothreitol) and equilibrated against 100–200 μl of reservoir solution in which components were the same as the precipitation solution. Single crystals grew overnight at 16°C but were generally very small. Well formed crystals were picked and transferred to newly set protein drops in the same precipitation solution except with the addition of 10 mM MgCl₂. Larger crystals grew to a size of about 0.2–0.3 mm in three dimensions. All crystals belong to the space group P2₁, with cell dimensions a = 66.13 Å, b = 44.02 Å, c = 64.84 Å, α = 90°, β = 102.71°, γ = 90°, with one molecule in each asymmetric unit.

Data were collected at 121 K using a cryoprotection solution containing the original precipitant with additional 20% 2-methyl-2,4-pentanediol. Data were collected at beamline 14-BM-D at the Advanced Photon Source in Argonne National Laboratory. A four-wavelength (peak, edge, high energy remote, and low energy remote) MAD data set was collected from a single crystal (Table I). 1° oscillation widths were collected through a range of 360° for each wavelength. Data were integrated and reduced using DENZO (11) and SCALEPACK (11).

Phasing and Refinement—The structure was solved by the multiple wavelength anomalous dispersion (MAD) method. SOLVE (12–17) was used to determine the heavy atom position and calculate the initial phases. All six selenium sites were found by this method. Phases were improved by using density modification with RESOLVE (18, 19). The map quality was sufficiently good to enable the complete model to be built in the program SPOCK (20). CCP4 programs, including FFTBIG and PROCHECK, have been used to generate the density map and check the final structure quality (21). Refinement was carried out with CNS (crystallographic + NMR systems) (22). The current refinement statistics are listed in Table II.

Structure Analysis—DALI (23) was used to search the Protein Data Bank for proteins having folds similar to the MVK N- and C-domains. SwissPDB Viewer (24) was used to make structural alignments. The mevalonate molecule was generated and docked into the MVK cavity.

Crystal Structure of the Mevalonate Kinase

![Fig. 1. The reaction catalyzed by MVK.](http://www.jbc.org/)

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**FIG. 1. The reaction catalyzed by MVK.**

**FIG. 2. Sequence alignment of mevalonate kinase and homoserine kinase.** The mevalonate kinase sequences from *Homo sapiens* (Hs), *Rattus norvegicus* (Rn), *Arabidopsis thaliana* (At), *Schizosaccharomyces pombe* (Sp), *Saccharomyces cerevisiae* (Sc), *Methanobacterium thermoautotrophicum* (Mt), and *Methanococcus jannaschii* (Mj) and the homoserine kinase (HK) sequence from *M. jannaschii* (Mj) are aligned. Green, the completely conserved residues; yellow, identical residues; cyan, similar residues. Three conserved motifs are highlighted. The residues labeled by asterisks are the equivalents of those indicated as important by mutagenesis studies in human or rat MVK.

**TABLE I**

| Wavelength   | Resolution | Completeness overall/outer shell | Rmerge overall/outer shell |
|--------------|------------|----------------------------------|---------------------------|
| A            | A          | %                                | %                         |
| 0.9794 (edge) | 99.00–2.40 | 87.1/47.8                        | 6.8/12.2                  |
| 0.9792 (peak) | 99.00–2.40 | 94.5/72.1                        | 6.3/12.6                  |
| 0.9611 (high energy remote) | 99.00–2.40 | 96.2/79.4                        | 6.1/12.6                  |
| 0.9919 (low energy remote) | 99.00–2.40 | 94.7/69.2                        | 6.2/11.1                  |
| F.O.M.       |            | 0.646                            |                           |

**TABLE II**

| Resolution (Å) | R (%) | Rmerge (%) | Rfree (%) |
|----------------|-------|------------|-----------|
| 19.9–2.4       | 20    | 28         | 28        |
| 20             | 28    | 28         | 28        |
| 19.9–2.4       | 20    | 28         | 28        |
| 19.9–2.4       | 20    | 28         | 28        |

**Note:** Rmerge is the mean intensity of reflection. F.O.M. (figure of merit) is the mean value of the cosine of the error in phase angles: (cos(α − α_{ideal})).
Crystal Structure of the Mevalonate Kinase

RESULTS AND DISCUSSION

Overall structure of MVK—Three very conserved motifs, designated motif I, II, and III, have been identified in GHMP proteins (4). The multiple sequence alignment of MVK from different species and the M. jannaschii HSK is shown in Fig. 2. Motif I is close to the N terminus of the protein, including residues corresponding to Lys8 and Glu14 of the M. jannaschii MVK. Motif II corresponds to the region between Pro104 and Ser114 of the M. jannaschii MVK, with an internal Gly-Leu-Gly-Ser-Ser-Ala sequence that is invariant, and forms the phosphate-binding loop. Motif III is characterized by Lys272-Cys281 of the Methanococcus jannaschii MVK and has an invariant sequence Thr-Gly-Ala-Gly-Gly-Gly-Gly among various mevalonate kinases. In HSK, this region corresponds to the sequence Thr-Ile-Ser-Gly-Ser-Gly-Pro, a loop in close contact with the phosphate-binding loop, which is presumed to stabilize the conformation of the latter (4).

The structure of MVK contains all 312 residues of the wild-type MVK as well as five residues from the N-terminal histidine tag fusion. It is a monomer in crystal, although the dimer was reported in the aqueous solution (2). The R-factor has been refined to 0.20 with an accompanying Rfree value of 0.28 (Table II). Nearly 88% of the non-glycine residues are in the most favorable conformation.

The Cα of MVK is shown in Fig. 3a. Similar to HSK, the MVK monomer contains two structural domains. At the domain interface there is a deep cavity presumed to be the active site (Fig. 3, a and b). Two molecules of dioxane, used as the crystallization precipitant, have been found to occupy this cavity (Fig. 5b). The structure of MVK is readily superimposable with that of HSK (Fig. 3c), indicating the structural conservation among the GHMP kinases. The root mean square deviation is 1.6 Å between the 155 superimposed Cα atoms.

The Structure of the N-terminal Domain and the Fold Comparison—The N-terminal domain of MVK comprises a large mixed five-stranded β-sheet (formed by strand a, b, c, d, and e) and a smaller anti-parallel four-stranded β-sheet (formed by strand a, b, f, and g), which are packed with four long α-helices (Fig. 4a).

The coordinates for the N-terminal domain (defined as residues 1–176) were used to search for similar structures by DALI (23). The best match was with 1fwk, the structure of HSK (4), with a Z-score of 16.6. The second highest hit was 1f4, the mevalonate 5-diphosphate decarboxylase (MDD) from Saccharomyces cerevisiae (27), with a Z-score of 9.7. Other top hits include 1dar (elongation factor G, Z-score 5.4); 1b63, the Mutl protein (Z-score 5.0); and 1e3h, the guanosine pentaphosphate synthetase (Z-score 5.0). Two molecules of dioxane, used as the crys-

Overall structure of MVK, a, stereoscopic view of Cα of MVK labeled every 10 residues. Residue 1 is Met1, before which is a part of the His tag (five residues). b, MVK structure and its secondary structures. The N-domain is colored cyan and the C-domain green. The residues from the His tag are not shown. The helices are represented by capital letters and the strands by lowercase letters. c, stereoscopic view of Cα for the aligned structure of MVK (gray) and HSK (green; PDB code 1fwk). There is an ADP (cyan) molecule complexed with HSK. The His tag is not shown.
cleoprotein, Z-score 4.0), and many others.

The C-terminal domains of MVK, HSK, and MDD are shown in Fig. 4, d-f. Each possesses a similar core unit with several insertions composed mainly of α-helices. Compared with those in HSK and MDD, the C-terminal tail in MVK is truncated and lacks the C-terminal β-strand (strands l and n in HSK and MDD, respectively) that folds toward the N-terminal domain and joins the large mixed β-sheet.

Homology Model of the Active Site of MVK—Mutations such as E19D, S145A, S146A, E193Q, D204A, and A334T in human MVK and K13M in rat MVK have been shown to decrease the activity of the enzymes (28–31). In M. jannaschii MVK, Glu14, Ser111, Ser112, Glu144, Asp155, Ala276, and Lys8 are identical to the equivalent residues of the human and rat protein, respectively (Fig. 2). Among the mutations studied, D204A elicits the most pronounced effects on catalysis by decreasing $V_{\text{m}}$ by ~10,000-fold (28). These results helped to establish the probable mechanism of the enzyme and facilitated our analysis of the active cavity.

All of these conserved residues are located in a region that defines the active site cavity (Fig. 5a). For example, the residues corresponding to motif III form a Gly-rich loop very close to the phosphate-binding loop corresponding to motif II. Cys281 in the Gly-rich loop is ~2.0 Å from Cys197 in the phosphate-binding loop. The relative orientation of these two residues as well as a continuous electron density connecting their side chains suggest a disulfide bond, despite the existence of dithiothreitol in the crystallization condition. Formation of the disulfide linkage may stabilize the conformation of the putative phosphate-binding loop. In addition, Lys272 in the Gly-rich loop is 3.1 Å away from Glu14, a motif I residue, which indicates electrostatic interactions.

The positions of the phosphate-binding loops of MVK and HSK (Fig. 5, b and c) are nearly identical when the proteins are superimposed. A dioxane molecule is bound about 3.0 Å from the main chain amides of Ser111 and Ser112 (which are both invariant residues on the phosphate-binding loop) and probably forms hydrogen bonds with these two amino acids. In various HSK-substrate complex structures, this region is occupied by phosphate groups and hydrogen bonds with the main chain amides on the phosphate-binding loop are present. For example, one HSK-ADP structure shows that the β-phosphate group occupies this position and forms hydrogen bonds with the main chain amides of Ser97 and Ser98 (equivalents of Ser111 and Ser112). Close to the phosphate-binding loop is the invariant residue Glu144, which aligns with Glu130 in HSK, a residue that coordinates the Mg$^{2+}$ ion (10). These results reveal roughly the region in MVK where the phosphate tail of ATP may bind. In HSK, the purine ring is bound in a pocket close to the phosphate-binding site. A similar pocket is apparent in MVK that likely serves the same function. The backbone of Lys74–Cys76 of MVK can be superimposed to the backbone of Asp62–Ala64 of HSK. In HSK, these residues form van der Waals contacts with the purine ring (within 5 Å), and the main chain amide of Val63 forms a hydrogen bond with N7 in the purine. Although the exact amino acids are somewhat different, the similarity of the molecular surfaces and the constellation of protein atoms in this region suggest that it is likely that in MVK these corresponding residues may form similar interactions with the purine ring.

Based on the alignment of the structures of apo-MVK and HSK-AMPPNP-homoserine, an ATP molecule and a mevalonate molecule were docked into the cavity (Fig. 5d). The phosphate tail of the docked ATP molecule closely interacts with the phosphate-binding loop (within 5 Å) and forms hydrogen bonds with several main chain amides, including that of Ser112. The purine ring fits well into the putative purine-binding pocket, consistent with the analysis described above. The carboxyl group of the docked mevalonate molecule is near a large positively charged patch in the C-domain part of the cavity and forms hydrogen bonds with the main chain amide of Ala276 and the side chain amide of Arg196. The 3′-methyl group of the mevalonate fits into a hydrophobic patch at the N-domain side of the cavity. In the model, the 3′-OH group is within hydrogen bond distance of the side chain of Lys8, a conserved residue in motif I. The 5′-OH group in the docked mevalonate is less than 3.0 Å from the γ-phosphorus of the docked ATP molecule. The 5′-OH group of the modeled mevalonate is only about 4.0 Å from Asp155. The model indicates a possible reaction mechanism that is consistent with previous enzymological studies on MVK.

Possible Reaction Mechanism—The reaction is likely to be initiated by the nucleophilic attack to the γ-phosphorus of ATP by the 5′-OH of mevalonate (Fig. 6). Based on the analysis of the active cavity, the best candidate for the catalytic residue is Asp155 (located about 4.0 Å from the 5′-OH of the docked mevalonate), which is consistent with the mutation data available for human MVK (28). It is likely that Asp155 functions as a general base with its side carboxyl group in position to abstract a proton from the 5′-OH of mevalonate, thereby facilitating the nucleophilic attack. Notably, this residue is struc-
turally aligned with Asn141 in HSK, which has been shown to interact directly with the $\delta$-OH of the homoserine (10). Lys8, Ala276, and Arg196 appear to be key to the binding of mevalonate (Fig. 6). The phosphate-binding loop might be the main force to bind and stabilize the phosphate tail of ATP. The N terminus of $\alpha$-helix E is pointing toward the phosphate groups and may offer some stabilizing effect during phosphoryl transfer, as is the case in many other kinases (3,4). The Mg$^{2+}$ that might be coordinated by Glu144 is likely to activate the $\gamma$-phosphate. This mechanism is consistent with the model proposed by the Miziorko laboratory (29).

In conclusion, our work has defined the structure of M. jannaschii mevalonate kinase and revealed the structural conservation among GHMP proteins. As more GHMP protein structures are solved, the detailed comparison and analysis of these proteins with substrates and inhibitors will further our understanding of the enzymatic mechanisms and offer us opportunities to design potential drugs against various diseases.

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Structure of the *Methanococcus jannaschii* Mevalonate Kinase, a Member of the GHMP Kinase Superfamily

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