Pantothenate kinase catalyzes the first step in the biosynthesis of coenzyme A, the major acyl group carrier in biology. In bacteria, regulation of pantothenate kinase activity is a major factor in controlling intracellular coenzyme A levels, and pantothenate analogs are growth-inhibiting antimetabolites. We have extended the structural information on *Escherichia coli* pantothenate kinase by determining the structure of the enzyme-ADP-pantothenate ternary complex. Pantothenate binding induces a significant conformational change in amino acids 243–263, which form a "lid" that folds over the open pantothenate binding groove. The positioning of the substrates suggests the reaction proceeds by a concerted mechanism that involves a dissociative transition state, although the negative charge neutralization of the γ-phosphate by Arg-243, Lys-101, and Mg²⁺ coupled with hydrogen bonding of the C1 of pantothenate to Asp-127 suggests different interpretations of the phosphoryl transfer mechanism of pantothenate kinase. N-alkylpantothenamides are substrates for pantothenate kinase. Modeling these antimetabolites into the pantothenate active site predicts that they bind in the same orientation as pantothenate with their alkyl chains interacting with the hydrophobic dome over the pantothenate pocket, which is also accessed by the β-mercaptoethyamine moiety of the allostERIC regulator, coenzyme A. These structural/biochemical studies illustrate the intimate relationship between the substrate, allostERIC regulator, and antimetabolite binding sites on pantothenate kinase and provide a framework for studies of its catalysis and feedback regulation.

Coenzyme A (CoA) is the major acyl group carrier in living systems and is synthesized by a universal series of enzymatic steps beginning with vitamin B₃ (1, 2). *Escherichia coli* is capable of de novo pantothenate biosynthesis (1) but can also import pantothenate from its environment via a sodium-dependent symport process (3, 4). All of the genes and enzymes involved in the biosynthetic pathway have been identified in *E. coli* (1, 2). The pathway is initiated by pantothenate kinase (PanK) (ATP: D-pantothenate 4'-phosphotransferase, EC 2.7.1.33), the product of the coaA gene (5). Cysteine is next added to the phosphopantothenate and is rapidly decarboxylated to form 4'-phosphopantetheine by a bifunctional polypeptide (Dfp, renamed CoaBC) (6). The adenine group is added by 4'-phosphopantetheine adenylyltransferase (CoaD) (7, 8), and the 3'-ribose phosphate is added by the dephospho-CoA kinase (CoaE) (9).

PanK is a key regulatory enzyme of CoA biosynthesis. *E. coli* PanK is a homodimer of 36 kDa subunits, and the amino acid sequence contains an A-type ATP-binding consensus sequence, GXXXGKS. PanK exhibits highly positive cooperative ATP binding and mediates a sequential ordered mechanism with ATP as the leading substrate (10). PanK activity is inhibited by non-esterified CoA and to a lesser extent by its thioesters, which competitively interfere with ATP binding (10, 11). Recently, the crystal structure of PanK was determined in complex with either ATP or CoA (12). These structures revealed that ATP and CoA bound to the enzyme in distinctly different ways; however, their phosphate binding sites overlapped at Lys-101, explaining the kinetic competition between the CoA regulator and the ATP substrate. There is no structural information on the nature of the pantothenate binding site on the enzyme and its relationship to the cavities occupied by ATP and the CoA allostERIC regulator.

All of the steps in CoA biosynthesis are encoded by widely expressed, essential genes, making them attractive targets for antibacterial drug discovery (13). *E. coli* PanK belongs to a family of bacterial enzymes that is distinct from the eukaryotic counterparts that carry out the same reaction (14–16). Although these data suggest that targeting PanK may be a viable strategy to attack a subset of bacterial species, one potential drawback to this approach is that such inhibitors may not be broad spectrum, because there are different, uncharacterized PanK isofoms in important pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (17). Several CoA analogs

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The atomic coordinates and structure factors (code 1SQ5) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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1 The abbreviations used are: CoA, coenzyme A; PanK, pantothenate kinase; N5-Pan, N-pentylpantethanamide; N7-Pan, N-heptylpantethanamide; HoPan, hopantenate.
plasmid with an N-terminal His tag and purified with nickel-nitri
diol-agarose and gel filtration chromatography as described pre-
viously (12). Protein concentrations were determined by the method
of Bradford (24) using γ-globulin as a standard.

**Crystallization**—Co-crystals of PanK, ADP, and pantothenate
were grown by the hanging drop vapor diffusion method. Prior to crystal-
lization, PanK was incubated overnight with 30 mM ADP, 30 mM
pantothenate, and 30 mM magnesium nitrate. Harvesting equal volumes of
the protein and reservoir solutions were equil-
ibrated at 18 °C against the reservoir solution (11% polyethylene glycol 3,350 and
200 mM sodium citrate, pH 8.2). After several days, monocrystalline crystals appeared and were allowed to grow for 1 week.

The crystals were transferred to 50/50 Paratone-N/mineral oil for
cryopreservation.

**Data Collection and Structure Determination of the PanKADP-
Pantothenate Ternary Complex**—Single wavelength native data
were measured from a single crystal of the ternary complex with a MAR165
CCD detector operating at 100 K at the SER-CAT beamline 22-ID at the
Advanced Photon Source of the Argonne National Laboratory. The data
set was indexed, integrated, and scaled with the programs DENZO
and SCALEPACK (26). The crystals belong to space group C2 with
unit cell dimensions a = 181.2 Å, b = 181.7 Å, c = 47.7 Å, and β =
104.8°. The data collection statistics are summarized in Table I.

The structure of the ternary complex was determined by the molec-
ular replacement method using one subunit of a dimer of the
PanK-AMP-PNP binary complex (12) as a search model. The cross-
rotation and translation functions and Patterson correlation refinement
were calculated using the program CCP4 (27). Data between 10.0 and
4.0 Å were used with the rotation function and generated four out-
standing solutions corresponding to four PanK molecules in the asymmetic
unit. Multiple iterations of applying the translation function to each
solution allowed determination of the orientations and positions of
the other three molecules. Each of the four PanK molecules forms a biolog-
ically functional dimer with a symmetrically related molecule.
The model of all four molecules was subjected to rigid body refinement using
data between 6.0 and 4.0 Å in the program CNS (28). The high resolu-
tion restriction was set at 4.0 Å to allow for a possible large positional
shift in structure. The low resolution limit was set at 6.0 Å because
test intensities of the reflections below this resolution are severely affected
by the diffraction of solvent molecules in the crystal. The R-factor
in this stage was high (49%), indicating that the conformation of
the PanKADP-pantothenate ternary complex was significantly different
from that of the search model, the AMP-PNP-bound protein. Solvent
contributions to low resolution intensities were considered during the
first refinement. After subsequent simulated annealing (28) using data
between 20.0 and 2.2 Å resolution, the quality of the structure was
significantly improved: the working R-factor was 34.8%; the free R-
factor, 36.7%. The Fc – Fd difference map showed continuous densities
for both ligands, ADP and pantothenate, that were absent from the
search model, confirming the correct molecular replacement solution.
Several iterations of model building in the program O (29) and refine-
ment in the program CNS (28) further lowered both R-factors.
The electron density map of the ADP and pantothenate ligands is shown
in Fig. 2A. The final refinement statistics are shown in Table I. Because of
slight differences between the four subunits, non-crystallographic sym-
metry restraints were applied to only 53% of the total residues for each
subunit. In the final model, insufficient electron density led to sub-
stitution of several side chains with alanine: in the first molecule, residues
Glu-33, Glu-44, Arg-71, Asn-83, Arg-86, Arg-119, Leu-178, Asp-185,
Lys-246, Lys-264, and Glu-268; in the second molecule, residues Lys-40,
Asn-83, Gln-85, Arg-86, Arg-119, Arg-120, Leu-178, Asp-185, Lys-246,
and Glu-268; in the third molecule, residues Glu-253, Lys-254, Arg-86,
Arg-119, Lys-137, and Leu-178; in the fourth molecule, residues Glu-33,
Lys-40, Gln-85, Arg-86, Arg-119, Arg-120, Lys-137, Asp-185,
Asp-256, Lys-264, and Glu-268. Also, several residue backbones could
not be properly fit into the calculated maps: in the first molecule, residues
Ser-26-Pro-28, Thr-50, Gly-84, and Gln-192; in the second molecule, residues Asp-25-Asp-28; in the third molecule, residues Arg-25-Pro-
25-Pro-28, Gly-84, Glu-85, Ile-87, His-118, and Arg-120; in the fourth molecule, residues Ser-26-Pro-28 and Val-191.

**Assay of PanK**—The standard PanK assays contained
H11002 (45 mM; specific activity 55 Ci/mmol), ATP (100 mM), MgCl2 (10
mM), Tris-HCl (0.1 M; pH 7.5), and 100 ng of the purified PanK in a total
volume of 40 μl (10). The reaction mixture was incubated for 30 min.
The reaction was stopped by adding 4 μl of 10% (v/v) acetic acid to the
mix. Then 40 μl of the mixture was deposited on a Whatmann DE81
ion-exchange filter disk that was washed in three changes of 1% acetic
acid in 95% ethanol (25 ml/disk; 20-min wash) to remove unreacted

**EXPERIMENTAL PROCEDURES**

**Protein Preparation**—For crystallization experiments, the coaA gene
encoding *E. coli* PanK (5) minus the first eight amino acids (residues
9–316) was subcloned into pET21a and transformed into the *E. coli*
BL21 (DE3) strain. The cells were grown at 37 °C in Luria Bertani broth supplemented with 100 μg/ml ampicillin to an absorbance of 0.6
at 600 nm and then induced with 1 mM isopropyl-β-D-thiogalactopy-
ranoside for 3 h. The harvested cell pellets from 2 liters of bacterial
culture were resuspended in 150 ml of disruption buffer (30 mM
HEPES, pH 7.5, 30 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, and 1
mM phenylmethylsulfonyl fluoride) and lysed with a French Pressure
Age buffer (20 mM Tris-Cl, pH 8.0, 1 mM dithiothreitol, and 1 mM
The purified protein was concentrated to 30 mg/ml, dialyzed into stor-
age buffer (20 mM Tris-Cl, pH 8.0, 1 mM dithiothreitol, and 1 mM
EDTA), flash-frozen in liquid nitrogen, and stored at −80 °C. For ki-
netic experiments, *E. coli* PanK was overexpressed from a pET-15b

**FIG. 1.** Chemical structures of the PanK ligands used in this
study.

are inhibitors of key CoA-utilizing enzymes in *vitro* (18), and
one successful antibacterial strategy is the design of CoA anti-
metabolites based on pantothenate (19, 20). These compounds
are phosphorylated by PanK and converted by CoaD and CoaE
to ethyldethia-CoA, which is thought to interfere with cell
growth by blocking a group of enzymes that utilize CoA and its
thioesters (20). HoPan is another pantothenate antagonist that
has been used to treat mental disorders in humans (21–23), but
its effectiveness as an antibacterial agent has not been evalu-
ated. N5-Pan and N7-Pan are much larger molecules than
pantothenate (Fig. 1), and understanding the structural basis
for the ability of these bulky molecules to function as PanK
substrates is important for the design of other analogs.
The goal of this study was to characterize the structure of the
pantothenate binding site on PanK to determine its relation-
ship to the leading substrate, ATP, the allosteric regulator,
CoA, and the antimetabolites. Our data provide new insights
into the catalytic mechanism, CoA feedback regulation, and
design of pantothenate antimetabolites.
PanKADP: Pantothenate Ternary Complex

RESULTS

Nucleotide Binding Site—The three-dimensional fold of PanK in the PanKADP-pantothenate ternary complex is almost identical to that of the protein in previously published structures (Fig. 2D) (12). In the PanKADP-pantothenate structure, ADP is bound in a groove formed by residues from the P-loop and the loop connecting strands 10α/11 (Fig. 2C), similar to the location of the ATP analog in the PanK-AMP-PNP binary complex (Fig. 2D) (12). A comparison of the nucleotide binding site for the PanKADP-pantothenate and PanK-AMP-PNP structures shows that the significant differences are the interaction of ADP with the Arg-243 side chain and the presence/absence of a bound Mg$^{2+}$ ion. In the ternary complex, the side chain of Arg-243 is displaced relative to its position in the PanK-AMP-PNP structure in the direction of the nucleotide, and the guanidinium group of Arg-243 interacts with nonbridging oxygens of the ADP α- and β-phosphates (Fig. 2C). The same guanidinium group of Arg-243 in the AMP-PNP structure forms a salt-bridge interaction with nonbridging oxygen of γ-phosphate of AMP-PNP (12) (Fig. 2D). In the PanKAMP-PNP structure, Mg$^{2+}$ is coordinated by the nucleotide β- and γ-phosphates and the side chains of Ser-102 and Glu-199, suggesting the role of Mg$^{2+}$ in ATP binding (Fig. 2D). However, the PanKADP-pantothenate structure lacks Mg$^{2+}$ (Fig. 2C). A $F_o - F_c$ difference map of ADP bound at the nucleotide binding site shows full density of the bound ADP, indicating that ADP is stably bound with high occupancy (Fig. 2A). This finding suggests that Mg$^{2+}$, although necessary for ATP binding and the phosphoryl transfer reaction, is not a requirement for ADP binding.

Pantothenate Binding Site—The PanKADP-pantothenate structure offers the first view of the pantothenate substrate bound at the active site and identifies key residues conferring pantothenate substrate specificity. The pantothenate binding site is located at the distal end of a large surface groove starting...
Fig. 2. Structure of a monomer of the PanKADP-pantothenate ternary complex. A, stereoview of ADP and pantothenate (Pan) with electron density. Electron density map was calculated using the coefficients $F_o - F_c$ and contoured at 2.5σ. B, overall view of the ternary complex. The protein is shown in gray, and the ligands are colored by atom type: carbon, yellow; nitrogen, blue; oxygen, red; and phosphorus, pink. C, ADP interactions with PanK ADP are shown bound to PanK in the ternary complex with pantothenate. Side chains of residues Lys-101, Ser-102, Glu-199, and Arg-243 orient the nucleotide in the binding groove. Asp-127 and Tyr-240 are hydrogen bonded to Pan. D, AMP-PNP interactions with PanK (12) (Protein Data Bank code 1ESN). AMP-PNP is shown bound to PanK in a binary complex. A coordinated magnesium ion is shown in cyan. Side chains of residues Lys-101, Ser-102, Glu-199, and Arg-243 show the relative differences in orientation compared with the ternary complex structure, as does the shift in helix-H relative to the active site.
on one side of the protein, where the nucleotide binds (12), and continuing across the protein (Fig. 2C). Above the groove, the pantothenate binding site is bounded by α-helices H and I, which provide important contacts with the substrate. The pantothenate molecule is surrounded by hydrophilic residues, providing multiple hydrogen bonding opportunities (Fig. 3). The C1 hydroxyl group of pantothenate forms a hydrogen bond with the side chain carboxyl of Asp-127. This interaction illustrates the role of Asp-127 in deprotonation of the C1 hydroxyl group, which provides important contacts with the substrate. The pantothenate binding site is bounded by the side chains of residues Asp-127, Tyr-175, His-177, Tyr-240, and Asn-282 orient the substrate in the binding site. For visual clarity, an additional hydrogen bond with the backbone carbonyl of Lys-145 is not shown.

Induced Fit Binding of Pantothenate—The comparison of the PanKAMP-PNP structure to the PanKADP-pantothenate structure shows a large movement of the helix-H/loop region, corresponding to amino acids 243–263, which acts as a lid that closes over the pantothenate binding site (Fig. 4). The most significant conformational changes involve Glu-249 and Phe-259. In the PanKAMP-PNP structure, the carboxyl side chain of Glu-249 occupies the pantothenate binding groove in approximately the same location as will be occupied by the incoming pantothenate (12). The binding of the carboxyl group of pantothenate in the groove displaces the carboxyl side chain of Glu-249 to the surface of the molecule (Fig. 4A). Glu-249 and three adjacent residues (Arg-248, Gly-250, and Ala-251) become part of helix-H, extending its length by one turn longer compared to the PanKAMP-PNP structure. In the PanKAMP-PNP structure, the side chain of Phe-259 interacts with the aliphatic portion of the Glu-249 side chain (12). When Glu-249 moves to the surface of the molecule to become a part of helix-H, Phe-259 loses its interaction with Glu-249 and moves close to helix-H to form a hydrophobic interaction with the side chain of Phe-247 of helix-H (Fig. 4B). These structural rearrangements caused by pantothenate binding modify the active site, which is necessary to accommodate pantothenate and bring together aromatic residues Phe-244, Tyr-258, Phe-259, and Tyr-262 to form a hydrophobic pocket. This constellation of residues extends over the top of the carboxyl group of pantothenate but does not interact with pantothenate itself. A similar structural rearrangement of the helix-H/loop region with the assembly of aromatic residues that forms a hydrophobic pocket is observed in the PanK-CoA structure, where the thiol group of CoA was surrounded by aromatic residues in a hydrophobic pocket (12). The structural rearrangement of the helix-H/loop region in the PanK-CoA structure is facilitated by the phosphopantetheine moiety of CoA mimicking pantothenate binding to the enzyme.

Reactivity of N5-Pan and N7-Pan—Previous work established that N5-Pan in the presence of a mixture of PanK (CoaA), 4′-phosphopantetheine adenylyltransferase (CoaD), and deprophospho-CoA kinase (CoaE) was converted to the CoA analog, ethyldethia-CoA (20). Our experiments focused on examining the interactions between N5-Pan or N7-Pan (Fig. 5) and PanK. Both pantothenamides were effective inhibitors of PanK, exhibiting IC50 values of 60 μM under our standard assay conditions (Fig. 5A). These inhibitors were competitive with respect to pantothenate (not shown); therefore, we determined the experimental Kci values for these compounds as substrates for PanK. The pantothenate Kci was 41 μM, essentially the same as reported previously (10). Both N5-Pan and N7-Pan were also excellent substrates for PanK, exhibiting Kci values of 140 and 124 μM, respectively (Fig. 5B). Our Kci values are the same as...
reported previously using a direct radiochemical assay (10) but are higher than those reported using a two-enzyme-coupled assay (20). The reason for this discrepancy is not clear, but in all cases the apparent binding of the analogs to PanK was 3-fold less than that of the authentic substrate, illustrating that N5-Pan and N7-Pan are efficiently utilized by PanK.

**Relationship between the Binding Sites for Pantothenate, CoA, and N5-Pan**

Structural comparison of the PanK/ADP/pantothenate and PanK/CoA complexes shows that even though there are significant changes in the orientation of several structural elements, the space occupied by pantothenate in the ternary complex is essentially the same as that occupied by the pantothenate moiety of CoA in the PanK/CoA complex (Fig. 6). The structural basis for the utilization of the pantothenamides by PanK was explored using the Sybyl program to dock N5-Pan into the PanK/ADP/pantothenate structure. The fact that N5-Pan and N7-Pan are bulkier than pantothenate yet serve as efficient PanK substrates is unusual because most small molecule substrates are bound with high specificity. A superposition of the highest scoring docking solution of N5-Pan in the PanK/ADP/pantothenate structure shows that the analog’s hydroxyl group that accepts the phosphate from ATP resides in the same location as the pantothenate hydroxyl group, whereas the extended hydrophobic chain of N5-Pan interacts with the hydrophobic surface that extends over the carboxyl group of pantothenate that is lined by the aromatic residues of the helix-H/loop (Fig. 6, A and B). The same hydrophobic residues form the binding groove for the β-mercaptethylamine moiety of the CoA allosteric regulator (Fig. 6C). Thus, similar structural features of PanK form a flexible binding site that accommodates the relatively large bulk of the CoA molecule. For clarity, only the pantotheine moiety (PanSH) of CoA is shown.

**HoPan** was neither an inhibitor nor a substrate for *E. coli* PanK (Fig. 5A). Also, HoPan does not have any detectable antibacterial activity against *E. coli* (data not shown). These experiments indicate that HoPan does not form a productive

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**Fig. 5. Interaction of N5-Pan and N7-Pan with PanK.** A, inhibition of pantothenate kinase activity by N5-Pan (●), N7-Pan (○), and HoPan (□). Both N5-Pan and N7-Pan were effective competitive inhibitors of PanK, whereas HoPan was not an inhibitor. B, kinetic analysis of pantothenate (□), N5-Pan (●), and N7-Pan (○) as PanK substrates.

**Fig. 6. Interaction of ligands with the pantothenate binding pocket.** A, bound pantothenate (yellow) does not interact with the aromatic pocket formed by the side chains of residues Phe-244, Phe-247, Tyr-258, Phe-259, and Tyr-262 (all green) at the “top” of the pantothenate binding groove. B, N5-Pan (yellow) is shown docked into the pantothenate binding groove, and its binding is predicted to cause minimal perturbation of the aromatic pocket relative to pantothenate binding. C, bound CoA (yellow; Ref. 12; Protein Data Bank code 1ESM) interacts with several aromatic residues that rearrange to accommodate the relatively large bulk of the CoA molecule. For clarity, only the pantotheine moiety (PanSH) of CoA is shown.
complex with PanK. The extension of the carboxyl group of pantothenate by one carbon in HoPan results in the loss of hydrogen bond interactions with both Tyr-240 and Asn-282. The interaction with Tyr-240 on helix-H is important to the conformational change that reorganizes the structure for pantothenate binding, and the inability of HoPan to make this contact may prevent the substrate binding pocket from forming. Docking solutions using HoPan and the PanKADP-pantothenate structure with the pantothenate removed result in binding predictions that do not place the hydroxyl group in a position to be phosphorylated (data not shown). These structural considerations explain why this pantothenate analog does not interact with bacterial PanK.

DISCUSSION

The geometry of the ligands in the PanKADP-pantothenate ternary complex points to a concerted mechanism for phosphoryl transfer. An associative mechanism (an axial bond distance of 1.617 Å and, thus, the sum of the axial bond orders approaching 2.0) involves a pentavalent phosphorane intermediate, whereas the dissociative mechanism (an axial bond distance >3.3 Å, the sum of the axial bond orders of 0.0) proceeds via a trigonal planar metaphosphate intermediate (30). In practice, most enzymes fall between these two extremes, based on the reaction coordinate distance used to calculate the fractional bond order (31). In concerted mechanisms, one observes a dissociative transition state when the sum of the axial bond orders is less than unity, an SN2 mechanism when the sum of the axial bond orders in the transition state is close to unity, or an associative transition state when the sum of the axial bond orders is more than unity (30). In the structure of the PanKADP-pantothenate complex, the distance between the hydroxyl nucleophile of pantothenate (the incoming oxygen atom) and the β-phosphate oxygen atom of ADP (a leaving oxygen atom) is 5.1 Å. This distance translates into an average bond distance of transferred phosphorus to the entering and leaving oxygens of 2.55 Å. Using Pauling’s equation (31,32) and considering the value of 1.617 Å as the single covalent bond distance between phosphorus and oxygen (33), the calculated fractional bond order is 0.08 for the axial bonds between transferred phosphorus to the entering and leaving oxygens and thereby the sum of the axial bond orders of 0.16, suggesting a concerted mechanism with the dissociative transition state. A small torsional rotation alone of the pantothenate hydroxyl group would shorten the distance between the hydroxyl oxygen and the β-phosphate oxygen, thereby increasing the sum of the axial bond orders to 0.36, which is still typical for enzymatic reactions with the dissociative transition state (30). Taken together, the bond distance measurements suggest that PanK catalyzes phosphoryl transfer via a concerted mechanism that involves dissociative characteristics.

A metaphosphate transition state will have a charge of −1, whereas a phosphorane group in an associative transition state will have a charge of −3 (25). The structures of PanKADP-pantothenate and PanKAMP-PNP show that positively charged residues (Lys-101 and Arg-243) and a Mg2+ ion interact with the γ-phosphate, and the presence of these positive charges is consistent with a requirement to neutralize multiple negative charges that develop in the associative transition state. In addition, Asp-127 interacts with the C1 hydroxyl of pantothenate to increase its nucleophilicity, promoting an SN2-like attack on the γ-phosphate of ATP (Fig. 7). In our proposed mechanism, pantothenate is bound and oriented by hydrogen bonding interactions with Tyr-240, Asn-282, Tyr-175, and His-177. Asp-127 then activates the C1 hydroxyl oxygen of pantothenate, which attacks the γ-phosphate of ATP. The in-line transfer of the phosphate occurs with charge stabilization provided by Lys-101, Arg-243, and Mg2+. Therefore, charge neutralization of the γ-phosphate and the putative role of Asp-127 as a general base leave room for other interpretations of the phosphoryl transfer mechanism of PanK. Further experiments will be required to confirm that PanK catalyzes phosphoryl transfer via a concerted mechanism with a dissociative transition state.

The PanKADP-pantothenate ternary complex structure also offers an explanation for how the bulky pantothenamide antimetabolites are capable of being phosphorylated by PanK. N5-Pan and N7-Pan are effective inhibitors of bacterial cell growth and are thought to act through their conversion by PanK to phosphorylated intermediates that are subsequently utilized to produce inactive CoA analogs (see the Introduction). These findings, coupled with our observation that the affinity of PanK for the analogs is comparable with the authentic substrate pantothenate (Fig. 5), are unusual because enzymes that utilize small molecules are highly selective for their substrates. The flexibility of the pantothenate binding site may account for the ability of PanK to phosphorylate the pantothenamides. The pantothenate binding site is also part of the binding site for CoA, and despite the structural differences between pantothenate and the allosteric regulator, the binding of CoA induces a similar conformational change in PanK. Furthermore, the pantothenate moiety of bound CoA has the same location and orientation as the bound pantothenate substrate, whereas the β-mercaptoethylamine moiety of CoA extends into the hydrophobic dome over the pantothenate binding pocket (Fig. 6B). Therefore, pantothenamide binding is predicted to induce similar conformational changes in the pantothenate binding site. A modeling solution of N5-Pan with the PanKADP-pantothenate structure (with the pantothenate removed) supports the pro-

![Fig. 7. Proposed mechanism of phosphoryl transfer by PanK.](attachment:image.png)
posal that the hydroxyl of the pantothemamide is correctly oriented for phosphorylation by binding in the same conformation as the pantothenate substrate. The hydrophobic tail of the antimetabolite interacts with the hydrophobic domain (Fig. 6B), which binds the beta-mercaptoethyamine moiety of CoA (Fig. 6C) (12). This model suggests that the design of future pantothemamide derivatives with hydrophobic groups that more strongly interact with the aromatic residues of the hydrophobic roof of the pantothenate pocket will increase the affinity and specificity of the inhibitor/substrates.

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