Crystal Structure of UDP-\(N\)-acetylmuramoyl-\(L\)-alanoyl-\(D\)-glutamate: meso-Diaminopimelate Ligase from *Escherichia Coli*\*

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UDP-N-acetylmuramoyl-\(L\)-alanoyl-\(D\)-glutamate:meso-diaminopimelate ligase is a cytoplasmic enzyme that catalyzes the addition of meso-diaminopimelic acid to nucleotide precursor UDP-N-acetylmuramoyl-\(L\)-alanoyl-\(D\)-glutamate in the biosynthesis of bacterial cell-wall peptidoglycan. The crystal structure of the *Escherichia coli* enzyme in the presence of the final product of the enzymatic reaction, UDP-MurNAc-\(L\)-Ala-\(\gamma\)-D-Glu-meso-\(A_7\)pm, has been solved to 2.0 Å resolution. Phase information was obtained by multilambda anomalous dispersion using the K shell edge of selenium. The protein consists of three domains, two of which have a topology reminiscent of the equivalent domain found in the already established three-dimensional structure of the UDP-N-acetylmuramoyl-\(L\)-alanine: \(D\)-glutamatediglase (MurD) ligase, which catalyzes the immediate previous step of incorporation of \(D\)-glutamic acid in the biosynthesis of the peptidoglycan precursor. The refined model reveals the binding site for UDP-MurNAc-\(L\)-Ala-\(\gamma\)-D-Glu-meso-\(A_7\)pm, and comparison with the six known MurD structures allowed the identification of residues involved in the enzymatic mechanism. Interestingy, during refinement, an excess of electron density was observed, leading to the conclusion that, as in MurD, a carbamylated lysine residue is present in the active site. In addition, the structural determinant responsible for the selection of the amino acid to be added to the nucleotide precursor was identified.

Peptidoglycan, the polymeric mesh of the bacterial cell wall, plays a critical role in protecting bacteria against osmotic lysis. It consists of linear repeating disaccharide chains cross-linked by short peptide bridges. During the cytoplasmic steps involved in the biosynthesis of the peptidoglycan precursor, four ADP-forming ligases (namely the Mur ligases) catalyze the assembly of the peptide moiety by the successive addition of \(L\)-alanine, \(D\)-glutamate, diaminopimelic acid, or \(L\)-lysine, and, finally, dipeptide \(D\)-alanoyl-\(D\)-alanine to UDP-N-acetylmuramic acid (1, 2). Because all these enzymes are essential for cell viability, they are attractive targets for antibacterial chemotherapy. In *Escherichia coli*, these ligases are the products of the murC, murD, murE, and murF genes, located in the mra region (3).

Sequence comparison of the four *E. coli* Mur ligases shows several homologous regions, suggesting that these enzymes may be evolutionarily related and may use similar enzymatic mechanisms (4–6). In earlier publications, we reported the structure of UDP-N-acetylmuramoyl-\(L\)-alanine: \(D\)-glutamate ligase (MurD),\(^1\) both in the native form and complexed with substrates (7–9). MurD consists of three domains with topologies reminiscent of a nucleotide-binding fold; the N- and C-terminal domains have a dinucleotide-binding fold (the Rossmann fold), and the central domain displays a mononucleotide-binding fold, also seen in ATP-binding proteins. A comparison of six MurD structures reveals that large C-terminal rotation, loop rearrangement, and subdomain movements occur upon substrate binding (9). In addition, several potentially important residues for substrate binding and/or catalysis have been identified (10). Recently, the x-ray structure of the folypoly-\(\gamma\)-\(L\)-glutamate synthetase (FGS) of *Lactobacillus casei* has been reported (11). Despite low sequence identity, FGS and MurD are clearly both members of the Mur ADP-forming ligase superfamilly (9, 12).

In *E. coli*, MurE catalyzes the addition of meso-diaminopimelic acid (meso-\(A_7\)pm) to the nucleotide precursor, UDP-Nac-\(L\)-Ala-\(\gamma\)-D-Glu (UMAG), according to the reaction: UmAG + meso-\(A_7\)pm + ATP ↔ UDP-MurNAc-\(L\)-Ala-\(\gamma\)-D-Glu-meso-\(A_7\)pm (UMT) + ADP + Pi. As established with other Mur ligases, the MurE reaction presumably proceeds by phosphorylation of the C-terminal carboxyate of UmAG by the \(\gamma\)-phosphate of ATP to form an acyl phosphate intermediate, followed by nucleophilic attack by the \(\alpha\)-amino group of \(A_7\)pm to produce UMT (Fig. 1), ADP, and inorganic phosphate (13–15). This mechanism is supported by the three-dimensional structures of MurD and MurE complexes. Some bacteria (such as *E. coli* and *Bacillus subtilis*) contain meso-\(A_7\)pm, and others (such as *Streptococcus pneumoniae* and *Staphylococcus aureus*) contain \(L\)-lysine at the third position of the peptide side chain of cell wall peptidoglycan. In each case, the MurE enzymes have been shown to efficiently discriminate between these two amino acids in vitro, because they are only able to catalyze the addition of either meso-\(A_7\)pm or \(L\)-lysine to UmAG (16, 17). Because these two amino acids effectively coexist in bacterial cells, the high specificity of the MurE enzymes acts as a gatekeeper to ensure that only the specific substrate is incorporated in the

\(^1\) The abbreviations used are: MurD, UDP-N-acetylmuramoyl-\(L\)-alanine: \(D\)-glutamate ligase; FGS, folypoly-\(\gamma\)-\(L\)-glutamate synthetase; \(A_7\)pm, diaminopimelic acid; SeMure, selenomethionyl-MurE; UMAG, UDP-N-acetylmuramoyl-\(L\)-Ala-\(\gamma\)-D-Glu; UMT, UDP-N-acetylmuramoyl-\(L\)-Ala-\(\gamma\)-D-Glu-meso-\(A_7\)pm; rms, root mean square.
MurE. discuss the two structurally characterized ligases, MurD and residues involved in UMT binding. In addition, we compare and the localization of the active site and the identification of the presence of the UMT reaction product. The structure re-

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ration of the overproduced His 6-tagged MurE enzyme. The p

production of C-terminal His6-tagged proteins, has been recently de -

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Bacterial Strains, Plasmid Vectors, and Growth Conditions—E. coli strains JM83 ( ara Δ [lac-proAB] rpsL thi φ80 lacZ Δ M15) (20) and B180 (ΔmetA::Cm) (21) were used as plasmid hosts and for the prepa-

ration of the overproduced His6-tagged MurE enzyme. The pTrcHis60 plasmid, a pTrc99A (Amersham Pharmacia Biotech) derivative for the production of C-terminal His6-tagged proteins, has been recently de-

cribed (22). 2YT medium (23) or M9 minimal medium (24) was used for growing cells, with growth being monitored at 600 nm with a Shimadzu UV-1601 spectrophotometer. For strains carrying drug resistance genes, the antibiotics used were ampicillin (100 µg·ml⁻¹) and chloramphenicol (25 µg·ml⁻¹).

General DNA Techniques and E. coli Cell Transformation—Small and large scale plasmid isolations were carried out by the alkaline lysis method, and standard procedures were used for endonuclease digestions, ligation, and agarose electrophoresis (24). E. coli cells were made competent and transformed with plasmid DNA using the method of Dagert and Ehrlich (25).

Construction of Plasmids—A plasmid suitable for high level overpro-

duction of MurE (C-terminal His6-tagged form) was constructed as follows. Polymerase chain reaction primers were designed to incorpo-

rate a Ncol site (shown in boldface type) 5’ to the initiation codon (underlined) of murE, 5’-GGGACCGACTGCGATCCTGTAATTTGCAGCCGAC-3’, and a BglII site (in boldface type) 3’ to the gene without its stop codon, 5’-TACCAGATCCTTGGCAATCCACCCCCAGCAG-3’. These primers were used to amplify the murE gene from the E. coli chromosome, and then the resulting material was treated with Ncol and BglII and ligated between the same sites of vector pTrcHis60, resulting in pMLD117, a plasmid allowing expression of the gene under the control of the strong isopropyl- β-D-thiogalactopyranoside-inducible trc promoter.

Preparation of Crude Protein Extracts and Enzyme Purification—JM83 (pMLD117) cells were grown exponentially at 37 °C in 2YT-ampicillin medium (18 liters of culture in a fermenter). At an optical density of 0.1, isopropyl- β-D-thiogalactopyranoside was added at a final concentration of 1 mM, and growth was continued for 3 h (final optical density of 0.60) before collection of the cells. The cells were harvested, resuspended in buffer A, and disrupted by sonication. After centrifugation, the supernatant was loaded on a DEAE-cellulose column, and proteins were eluted with a linear 0–0.3 M NaCl gradient in buffer A. Fractions with high enzyme activity were combined and loaded onto a HiLoad 16/60 Superdex 75 size-exclusion column. The enzyme was eluted with buffer A and collected in 1-ml fractions. Fractions containing enzyme activity were pooled and stored at −20 °C as stock solutions in buffer A. The enzyme activity was determined by measuring transfer of the reporter group from UDP-glucosamine to muramyl-L-Ala. The reaction was started by addition of enzyme and stopped by the addition of a cold methanol-water mixture (91:9), and then the samples were spotted onto Whatman chromatography paper and developed with a ninhydrin solution. The amount of the UMT product was estimated by scanning the chromatograms with a densitometer (9).

Materials—DNA restriction enzymes and synthetic oligonucleotides were obtained from Eurogentec or New England Biolabs. Polymerase chain reaction amplification of DNA was performed in a Thermocycler 60 apparatus (Bio-med) using Taq polymerase (Appligene), and DNA fragments were purified using the Wizard purification system. UMAG was synthesized from UDP-N-acetylmeso-A2pm using purified MurD (18), and UMT was prepared as described (22). 2YT medium (23) or M9 minimal medium (24) was used for growing cells, with growth being monitored at 600 nm with a Shimadzu UV-1601 spectrophotometer. For strains carrying drug resistance genes, the antibiotics used were ampicillin (100 µg·ml⁻¹) and chloramphenicol (25 µg·ml⁻¹).

Crystal Structure of MurE from E. coli

This report describes the crystal structure of MurE, the final product of the UMT reaction. The enzyme from E. coli was overproduced as a C-terminal His6-tagged protein (pMLD117) on a trc promoter. The protein was purified to greater than 90% homogeneity by DEAE cellulose and Superdex 75 chromatography. The crystals belong to the space group P2₁2₁2₁, with unit cell dimensions a = 73.5 Å, b = 73.5 Å, and c = 73.5 Å. The crystals diffract to 2.0 Å resolution, and the Matthews number is 2.0 Å⁻³. The structure was solved by molecular replacement, with the MurE structure as the search model. The final model includes 7341 protein atoms, 405 water molecules, and 359 UMT molecules. The structure was refined to an R-value of 20.2% and a free R-value of 23.0%. The crystal structure of MurE provides insight into the mechanism of UMT and its role in peptidoglycan biosynthesis.
density was 1.3). The cells were harvested at 4 °C (about 45 g of wet weight), and the cell pellets were washed with cold 20 mM potassium phosphate buffer, pH 7.4, containing 1 mM b-mercaptoethanol and 0.5 mM MgCl2 (buffer A) and then stored at 220 °C. Cells from one-fifth of the preparation were suspended in 9 ml of buffer A and disrupted by sonication at 4 °C using a Bioblock Vibracell 72412 sonicator. The resulting suspension was centrifuged at 4 °C for 20 min at 200,000 × g, and the pellet was discarded. SDS-polyacrylamide gel electrophoresis analysis showed that MurE accounted for about 20% of the crude protein soluble extract (data not shown).

For the preparation of the MurE protein in which all methionine residues were replaced by selenomethionine (SeMurE), E. coli methionine-auxotrophic strain b180 was transformed with plasmid pMLD117, and the resulting strain was cultured in M9 medium (2 liter culture) supplemented with 0.4% glucose, ampicillin, and all the usual amino acids (100 μg/ml), except that L-selenomethionine was substituted for L-methionine. Expression of murE was induced at mid-log phase (optical density was 0.6) with 1 mM isopropyl-b-D-thiogalactopyranoside, and cell growth was allowed to continue for a further 3 h (final optical density was 1.8). The cells were then harvested, washed in buffer A, and disrupted by sonication, and the crude protein extract was prepared as described above.

The His6-tagged proteins (MurE and SeMurE) were purified on Ni2+-nitrilotriacetate-agarose essentially following the manufacturer’s recommendations (Qiagen): binding to the resin, washing with buffer A containing 20 mM imidazole and 300 mM NaCl, and elution of the adsorbed proteins by a discontinuous gradient of 20–300 mM imidazole (MurE and SeMurE behaved similarly and eluted at a concentration of imidazole of about 150 mM). The pooled fractions were dialyzed against 20 mM HEPES buffer, pH 7.4, containing 5 mM dithiothreitol and 200 mM NaCl and then concentrated on PM10 membranes (Millipore) to 12 mg/ml for use in crystallization experiments. SDS-polyacrylamide gel electrophoresis analysis of proteins performed on 12% polyacrylamide gels (26) showed that the His6-tagged proteins were at least 90% pure. Protein concentrations were determined by the Bradford method (27), using bovine serum albumin as a standard. Typically,
FIG. 5. Schematic drawing of the UMT binding in molecule B. For clarity, only hydrogen bond interactions are shown.

FIG. 6. Sequence alignment and secondary structure assignment. Four representative sequences of MurE ligases with secondary structural elements shown using ESPript (49). The E. coli (ECOLI) and B. subtilis (BACSU) enzymes incorporate meso-A2pm into the peptidoglycan, whereas the S. aureus (STAAU) and S. pneumoniae (STRPN) enzymes incorporate an L-lysine. Residues that are conserved in the 20 available MurE ligases are shown as red boxes, and those conserved in all members of the Mur ligase family are marked as green triangles.
15–20 mg of pure protein (MurE or SeMurE) were obtained per liter of culture. Amino acid and mass spectrometry (matrix-assisted laser desorption/ionization) analyses confirmed the composition and molecular mass of the two proteins as well as the 100% selenomethionine substitution in SeMurE.

**Enzyme Activity**—The meso-A2pm adding activity was assayed by measuring the formation of radioactive UMT. The reaction mixture (0.1 M Tris-HCl buffer, pH 8.6, 0.1 M MgCl2, 5 mM ATP, 0.2 mM UMAG, and 0.1 mM meso-[^14C]A2pm (1.5 KBq); total volume, 75 μl) was incubated for 30 min at 37 °C. The reaction was stopped by addition of 10 μl of glacial acetic acid and the radioactive substrate and product were separated by reverse-phase high pressure liquid chromatography on a Nucleosil SC6 column (4.6 × 150 mm; Altitech France, Templemars, France) using 50 mM ammonium formate buffer, pH 3.9, at a flow rate of 0.6 ml/min. Detection was performed with a radioactive flow detector (model LB506-C1; EG&G Wallace/Berthold, Evry, France) using the Quicksafe Flow 2 scintillator (Zinsser Analytic, Maidenhead, UK), and quantification was carried out using the Winflow software (EG&G Wallace/Berthold). The activity of the pure preparation of His6-tagged MurE enzyme was 625 nmol of meso-A2pm incorporated in UMT per min and mg of protein.

**Cryostalization and Data Collection**—MurE, expressed as a C-terminal His6-tagged protein, consists of 502 amino acids (molecular mass was 54,276.5 Da). Crystals of native and selenomethionyl MurE were grown in the presence of the reaction product, UMT. Drops of 2 μl of protein solution (10 mg/ml) were equilibrated against 1 ml of reservoir buffer. The crystals, space group C2221, had unit cell dimensions of a = 93.27 Å, b = 99.51 Å, and c = 234.34 Å, with two molecules in the asymmetric unit. Multiwavelength anomalous dispersion data collection was carried out on a single flash cooled crystal at three wavelengths on a BM14 beam line (European Synchrotron Radiation Facility, Grenoble, France). The long c axis was aligned so that it was almost coincident with the spindle axis, and data were collected at 2.8 Å resolution using a MAR ccd detector. The range of data collection was determined using STRATEGY (28). Data were processed using DENOZ (29). The relevant statistics are given in Table I. Data at 2 Å resolution were collected using a MAR ccd on the ID14-EH2 beam line (European Synchrotron Radiation Facility, Grenoble, France), a 10° sweep being made in increments of 0.25°. Data were processed using XDS (30).

**Multiwavelength Anomalous Dispersion Phasing, Model Building, and Refinement**—Multiwavelength anomalous dispersion data were input into the program SOLVE (31). All 24 selenium positions in the asymmetric unit were found, and experimental phases were calculated from these using a multiple isomorphous approach. The noncrystallographic symmetry was defined using FINDNCS (32). Density modification (33) was then used to extend the experimental phases to 2 Å using noncrystallographic symmetry averaging, solvent flattening, and histogram matching. The resulting experimental map was of excellent quality. The majority of the model was traced automatically using wARP (34). Refinement to 2 Å was carried out by sequential use of the CRYSTIAL program (35). The final model consists of two molecules of MurE (992 visible residues, of which 10 have been modeled with double conformations), 141 ligand atoms, and 394 water molecules. The stereochemistry of the final model was evaluated using the PROCHECK program (37). The coordinates of the MurE structure have been deposited with the Brookhaven Protein Data Bank (accession code 1e8c) and will be released 1 year after publication.

**RESULTS**

**Quality of the Refined Model**—The final model, which contains 992 residues, two UMT molecules, and 394 water molecules, had a crystallographic R factor of 20.2% (Rfree = 23.0%; Ref. 38) for all 72,674 reflections in the resolution range 46.7–2.0 Å (Table II). The root mean square (rms) deviations were 0.006 Å from ideal bond lengths and 1.3° from ideal bond angles. After density modification, the experimental density map was of good quality. Fig. 2 shows the chemical modification of Lys284 by covalent binding to three atoms (see “Discussion”). The asymmetric unit contains two molecules (A and B) of MurE, essentially identical in conformation. After superimposition, the rms deviation between 495 pairs of equivalent Ca was 0.45 Å. No symmetry was seen between the two molecules. The Ramachandran plot (39) for the present model showed all the nonglycine residues to be in allowed regions. The average temperature factors were slightly different for molecules A and B (29.9 and 31.4 Å², respectively) but identical for the two UMT molecules (39.5 Å²). A few residues at either end of the polypeptide chain had no visible electron density and were therefore not included in the model. However, 25 and 20 residues (molecule A and B, respectively) clearly showed holes in the electron density map. The damage was produced by third generation synchrotron radiation (40), the most frequent being decarboxylation of acidic residues. The side chains were built into the electron density, and a partial occupancy was assigned for the side chain atoms based on difference Fourier maps.

**Overall Protein Structure**—MurE consists of three globular domains formed from contiguous segments in the amino acid sequence (Fig. 3).

Domain 1 comprises residues 1–88 and consists of a five-stranded β-sheet surrounded by two helices (Fig. 4a). Comparison of its structure with the data base of known protein structures carried out using the DALI server (41) revealed no homology with known protein structures; the structure showing the greatest similarity is a fragment of the transferrin receptor (42) with a rms deviation of 3.9 Å for the 70 structurally equivalent Ca atoms.

Domain 2 (Fig. 4b) comprises residues 90–338 and consists of a central six-stranded parallel β-sheet surrounded by seven α-helices. The fold of the central β-sheet is similar to the classic “mononucleotide-binding fold” found in many ATP-binding proteins, and domain 2 will therefore be referred to as the ATP-binding domain. As expected, using DALI, the structures with the highest Z scores were the central domain of MurD (7) (Protein Data Bank code 1uag; Z score = 20.0) and the N-terminal domain of FGS (11) (Protein Data Bank code 1fgs; Z score = 9.7). Superposition of domain 2 on the corresponding domain of the two ligases gave an rms deviation of 3.1 Å for the 193 structurally equivalent Ca atoms of MurD and 3.5 Å for the 168 Ca atoms for FGS. The loop between β6 and α4 of the ATTPase domain (also referred to as the P loop) is probably involved in ATP binding.
Domain 3 (340–497) contains a six-stranded β-sheet with parallel strands (β17, β18, β19, β20, and β21) and an antiparallel strand (β16), and five surrounding α-helices (Fig. 4c). Surprisingly, as already observed for the C-terminal domains of MurD and FGS, domain 3 contains a Rossmann fold (Z score = 10.9 and 10.8 for MurD and FGS, respectively). Moreover, superposition of domain 3 on the corresponding domain of the two ligases gave an rms deviation of 2.7 Å for the 119 structurally equivalents Cαs for MurD and 2.4 Å for the 107 Cαs for FGS. The large insertion (464–481) between strands β22 and β23 has no structural equivalent in MurD and FGS.

UMT Binding Site—The product, UMT, binds in the cleft between the three domains. The experimental electron density map showed almost all of both UMT molecules. In the early stage of refinement, only the A_2pm density was difficult to interpret. As shown in Fig. 5, the bound UMT makes many polar interactions with the protein. The protein residues involved in UMT binding are located in the two loops connecting β1 with β2 and β2 with α2. The geometry of the uridine-ribose moiety of the two UMT molecules is C2_α-endo for the ribose ring pucker (χ = −114.5°) and an anti-orientation about the glycosyl bond. The uracil ring forms two hydrogen bonds with Ser2_26; these being between O2_ and the main chain nitrogen and between N3_ and Oy. In addition, it is inserted between a salt bridge (between Asp2_27 and Arg2_29) and Tyr2_50. Interestingly, C6_ and C5_ of the pyrimidine ring are exposed to the solvent, explaining how dihydrouridine can replace uridine in the uracil ring.

The pyrophosphate of UMT interacts with the loop connecting β2 with α2 (residues 42–47). The protein-phosphate interactions are made mainly through hydrogen bonds with main chain nitrogens, the α-phosphate oxygens forming hydrogen bonds with Gln4_44 and Ala4_45, and the β-phosphate oxygens with His4_43. One of the β-phosphate oxygens forms hydrogen bonds with ND1 of His4_44; the latter is the only residue to balance the negative charges of the pyrophosphate.

The N-acetylmuramic acid ring bridges the gap between domain 1 and the ATP-binding domain. Two groups of hydrogen bonds are important; Oα_ of Asn156 interacts with O4_ and Gln190 and Arg192 are in contact with the acetyl group. Interestingly, Arg192 forms three other hydrogen bonds with the carbonyl oxygen of L-Ala and Oα_ of γ-Glu. In the ATP-binding domain, three loops are close to the product (β8-α5, β9-α6, and α6-β10). Finally, the A_2pm-containing region of UMT forms five hydrogen bonds with the C-terminal domain, the atoms involved being located in two loops connecting secondary structure elements (β19-α14) and (β21-β22). These very important interactions are discussed below.

Sequence Alignment of MurE Sequences—An alignment analysis using the 20 MurE sequences currently available shows that 24 amino acid residues are strictly conserved; none of these are found in the N-terminal domain, whereas 14 occur in the ATP-binding domain and 10 are in the C-terminal domain. Fig. 6 shows the sequence alignment; for clarity, only four representative MurE ligase sequences were used (E. coli, B. subtilis, S. aureus, and S. pneumoniae). Of the 24 strictly conserved residues, only three (Thr116, Ser185, and Arg2_29) interact with the product, UMT. Eight others (Gly115, Gly118, Lys119, Glu182, His210, Asn310, Arg341, and Asp356) are invariant residues conserved in all the members of the Mur ligase family (MurC, MurD, MurE, MurF, and Mpl; Mpl protein (UDP-N-acetylmuramate: L-alanyl-γ,D-glutamyl-meso-diaminopimelateligase) catalyzes the formation of UDP-MurNAc-tetripeptide by addition of L-Ala-γ,D-Glu-meso-A_2pm to UDP-MurNAc (44)). Putative roles for these residues in reaction mechanism or structure of Mur ligases have been proposed (5–10). Excluding the seven glycine and proline residues, which may play a structural role, the remaining six of the 24 conserved amino acids are Thr2_14, Thr2_29, Tyr2_50, His119, Arg4_43, and Lys4_45. The first two form part of the P loop, found in the Mur ligase family, that has the characteristic finger print Xaa114-Gly-Xaa-Xaa-Xaa-Arg (43). A large anion hole is formed by the loop, which accommodates the phosphates of ATP (8). In MurE, the loop consists of residues 115–119 and connects β6 to α4. Tyr2_50 makes two hydrogen bonds, one with O7_ of Thr2_14 and the other with the carbamylated Lys224 (see below). The hydrophobic part of A_2pm lies against the ring of His359.

Finally, two buried salt bridges, located in the C-terminal domain, play a very important role. The first, between Arg4_43

<table>
<thead>
<tr>
<th>Residue in MurE</th>
<th>Residue in MurD</th>
<th>Location*</th>
<th>Proposed function in MurD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys119</td>
<td>Lys115</td>
<td>Central</td>
<td>ATP binding</td>
</tr>
<tr>
<td>Thr116</td>
<td>Thr117</td>
<td>Central</td>
<td>ATP binding</td>
</tr>
<tr>
<td>Glu182</td>
<td>Glu185</td>
<td>Central</td>
<td>ATP binding</td>
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<tr>
<td>Asp182</td>
<td>Asp185</td>
<td>Central</td>
<td>ATP binding</td>
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<tr>
<td>His210</td>
<td>His213</td>
<td>Central</td>
<td>ATP binding</td>
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<tr>
<td>Lys224</td>
<td>Lys226</td>
<td>Central</td>
<td>ATP binding</td>
</tr>
<tr>
<td>Asn271</td>
<td>Asn274</td>
<td>Central</td>
<td>ATP binding</td>
</tr>
<tr>
<td>Arg302</td>
<td>Arg305</td>
<td>Hinge</td>
<td>ATP binding</td>
</tr>
<tr>
<td>Asp306</td>
<td>Asp307</td>
<td>C-terminal</td>
<td>ATP binding</td>
</tr>
<tr>
<td>His310</td>
<td>Thr312</td>
<td>C-terminal</td>
<td>ATP binding</td>
</tr>
<tr>
<td>Lys345</td>
<td>Lys348</td>
<td>C-terminal</td>
<td>ATP binding</td>
</tr>
</tbody>
</table>

*a Location of the amino acid residues in the three domains.  
*b Hinge region between the central and C-terminal domains.  
*c UMA, UDP-N-acetylmuramyl-l-Ala.
and Asp<sup>182</sup> (in some orthologs, Asp<sup>182</sup> is replaced by a glutamate), links the first two amino acids of α15 and α16, stabilizing the domain. The second is between Lys<sup>465</sup> and Glu<sup>468</sup>. In addition, Lys<sup>465</sup> makes two hydrogen bonds, one with the almost buried side chain of Asn<sup>414</sup> and one with the main chain oxygen of Tyr<sup>460</sup>. Asn<sup>414</sup> and Glu<sup>468</sup> are functionally conserved in MurE sequences and are involved in the interaction with Ap<sub>pm</sub> (see below).

**DISCUSSION**

**Comparison with MurD**—An interesting comparison can be made between the structures of the MurD-UMAG and MurE-UMT complexes. Superposition of the central domain β-sheet of MurD and MurE clearly shows a well-conserved ATP-binding domain (Fig. 7). In addition, the two C-terminal domains are almost in the same position. As expected from the domain topology, the two N-terminal domains share no secondary structure elements. Interestingly, the uridine has a completely different orientation, and O<sub>4</sub> has moved 9.5 Å. Another striking detail in the comparison is the positional overlap between enzymatically important residues in both ligases (Table III). Most of these have already been identified in the Mur ligase family (5, 6, 8, 9). However, superposition of the two active sites reveals three new positions, these being Asp<sup>182</sup>, Thr<sup>221</sup>, and Lys<sup>348</sup> in MurD, corresponding to Asp<sup>209</sup>, His<sup>359</sup>, and Arg<sup>389</sup> in MurE. His<sup>359</sup> is strictly conserved in all MurE sequences, whereas Asp<sup>209</sup> and Arg<sup>389</sup> are replaced in some orthologs by functionally equivalent amino acids (Glu and Lys, respectively). In the MurE-UMT complex, the Arg<sup>389</sup> side chain does not interact with Asp<sup>209</sup>, in contrast to the situation in MurD, in which a salt bridge is formed between Lys<sup>348</sup> and Asp<sup>182</sup>. However, in MurE, the side chain of Arg<sup>389</sup> can move to interact with Asp<sup>209</sup>. In conclusion, all important residues in MurD have a corresponding residue in MurE. This suggests that MurD and MurE have the same catalytic machinery.

At this point, it is important to underline the role of Lys<sup>324</sup>. This amino acid is not conserved in the Mur ligase family, because MurC and Mpl have a phenylalanine or tyrosine at the equivalent position. The experimental density clearly shows chemical modification of Lys<sup>324</sup> in MurE. The same modification was also observed in all the “closed” structures of MurD for Lys<sup>398</sup> (8). Chemical interaction of the electron density form (Fig. 2) and the noncovalent interactions with neighboring atoms of the modified lysine led to the conclusion that Lys<sup>324</sup> is carbamylated. As observed in the MurD-UDP-N-acetylmuramoyl-1-Ala-ADP-Mg<sup>2+</sup> complex (8), this modification is probably crucial for Mg<sup>2+</sup> binding and, consequently, for the γ-phosphate positioning of ATP.

**Structural Implications for Catalysis**—In general, ATP-dependent amide-forming enzymes are believed to share a common mechanism involving an initial phosphorylation of the acid carboxylate. Subsequently, the acyl phosphate is attacked by the amino group, producing a tetrahedral intermediate, which ultimately collapses to the final product and inorganic phosphate (14). For ligation to occur between UMAG and Ap<sub>pm</sub>, MurE must (i) bring together the UMAG and ATP, (ii) correctly orient UMAG and ATP for acyl-phosphate intermediate formation, (iii) orient Ap<sub>pm</sub> for nucleophilic attack, and (iv) stabilize the tetrahedral intermediate, thereby lowering the activation barrier and accelerating catalysis. The structural determination of MurE has confirmed the location of the active site and identified the protein residues involved in the catalytic mechanism (Table III). The similar location of the 11 structurally conserved amino acids in MurE and MurD strongly supports the previous reaction mechanism proposed for MurD (8).

**MurE Ligases and Ap<sub>pm</sub> Structural Determinant**—The primary structures of peptidoglycans display a number of variations, mainly in the peptide moiety. Depending on the bacterium, MurE ligases add different amino acids at the third position, the most common being meso-A<sub>pm</sub>, L-lysine, L-ornithine, and LL-A<sub>pm</sub> (45). Mur ligase specificity has been studied in ten microorganisms; five use A<sub>pm</sub> at the third position, whereas the others use L-Lys (16). MurE ligases will only add Ap<sub>pm</sub> or L-Lys to UMAG, whereas MurF, which catalyzes the addition of d-Ala-d-Ala to UMT, is less specific for the occurrence of Ap<sub>pm</sub> or L-Lys in the tripeptide moiety. The substrate specificity of E. coli MurE ligase has been analyzed in detail using Ap<sub>pm</sub> and various analogs (17) in vitro, the relative specific activity of incorporation being 100, <0.1, and 2.6 for meso-A<sub>pm</sub>, L-Lys, and LL-A<sub>pm</sub>, respectively. Expression in E. coli cells of the S. aureus MurE enzyme, which catalyzes the addition of L-Lys, results in 50% incorporation of L-Lys into the peptidoglycan (46); interestingly, transpeptidation cannot occur if the acceptor unit bears an L-lysine residue. Of the 20 known MurE sequences, four belong to Gram-positive microorganisms (Enterococcus faecalis, S. aureus, S. pneumoniae, and Streptococcus pyogenes) and incorporate L-Lys at the third position. Of the four MurE enzymes shown in Fig. 6, the first two catalyze the addition of meso-A<sub>pm</sub>, and the last two catalyze the addition of L-Lys. The amino acids in E. coli MurE interacting with the free end of A<sub>pm</sub> are shown in Fig. 8. Asp<sup>413</sup>, Asn<sup>414</sup>, and Arg<sup>416</sup> are located on a loop connecting β19 to α14, and Gly<sup>464</sup> and Glu<sup>468</sup> are on a loop linking β21 to β22. Because Arg<sup>416</sup> is conserved in all Ap<sub>pm</sub>-adding enzymes and is replaced by Ala or Asn in the four L-Lys-adding enzymes, it should be the main structural determinant for Ap<sub>pm</sub> selection.

**Conclusion**—By solving the crystal structure of E. coli MurE, we have obtained a high resolution model of a closed active form of the enzyme. MurE shares the same three domain topology and a similar active site architecture with MurD. Comparison of the structures of MurE and MurD complexed with the final reaction product has allowed (i) the identification of key residues potentially involved in the enzymatic mechanism, one of these being, as in MurD, a carbamylated lysine and (ii) the explanation of the presence of a new fold in the N-terminal domain that is able to accommodate the extension of the UDP substrate. Finally, an arginine residue seems to be the main structural determinant involved in selection of the amino acid inserted at the third position in the peptide moiety of peptidoglycan precursors.

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