RimL is responsible for converting the prokaryotic ribosomal protein from L12 to L7 by acetylation of its N-terminal amino group. We demonstrate that purified RimL is capable of posttranslationally acetylating L12, exhibiting a $V_{\text{max}}$ of 21 min$^{-1}$. We have also determined the apostructure of RimL from *Salmonella typhimurium* and its complex with coenzyme A, revealing a homodimeric oligomer with structural similarity to other Gen5-related N-acetyltransferase superfAMILY members. A large central trough located at the dimer interface provides sufficient room to bind both L12 N-terminal helices. Structural and biochemical analysis indicates that RimL proceeds by single-step transfer rather than a covalent-enzyme intermediate. This is the first structure of a Gen5-related N-acetyltransferase family member with demonstrated activity toward a protein N$^\alpha$-amino group and is a first step toward understanding the molecular basis for N$^\alpha$-acetylation and its function in cellular regulation.

The posttranslational acetylation of N$^\alpha$-protein termini is a common occurrence in eukaryotic proteins where between 50 and 90% of proteins are N$^\alpha$-acetylated (1). However, in prokaryotes, N$^\alpha$-protein acetylation is limited to four known examples. The early secreted antigenic target (ESAT-6) of *Mycobacterium tuberculosis* is N$^\alpha$-acetylated, and its acetylation has been shown to alter its interaction with the 10-kDa culture filtrate protein (CFP10) (2). The three other examples are the prokaryotic ribosomal proteins S18, S5, and L12, which are N$^\alpha$-acetylated by the Rim proteins, RimI, RimJ, and RimL, respectively (3, 4). L12 and its N$^\alpha$-acetylated derivative, L7, have garnered the most interest because of its interesting physical and biological properties. L12 lacks tryptophan, tyrosine, cysteine, and histidine residues and is the most acidic *Escherichia coli* ribosomal protein (5). The dimeric L12/L7 complex is the only protein found in more than one copy in the ribosome (6) and is the only ribosomal protein that has no direct contact with rRNA, instead interacting indirectly via formation of a complex with the L10 protein (7). In addition, the efficiency of protein translation is correlated with the number of L12/L7 dimers bound with maximum efficiency occurring at a ratio of two dimers per ribosome (8, 9). This is due to the interaction of the C-terminal domains of L12/L7 with both elongation factors EF-Tu and EF-G and the promotion of GTP hydrolysis by these two elongation factors (10, 11). Finally, whereas S18 and S5 are always found in their $N^\alpha$-acetylated form, the relative proportions of L12 and L7 are correlated with the rate of growth and the cell cycle (12–14).

Although cotranslational N$^\alpha$-acetylation appears to be critical to the function and stability of numerous eukaryotic proteins (15), no known function has been ascribed to the acetylation of prokaryotic ribosomal proteins. Knock-out mutants lacking a functional RimL, RimL, or RimJ exhibit no distinguishable phenotype compared with wild type (16–18), and the L12/L7 ratio has not been found to affect ribosomal function (19–21). Questions continue to be raised concerning the physiological target(s) of the Rim proteins, their cellular effect, and how they catalyze the acetyltransferase reaction. In particular, the posttranslational N$^\alpha$-acetylation of dimeric L12 requires a different arrangement of monomers in RimL compared with that observed for eukaryotic histone acetyltransferases (22).

Here we present a biochemical and biophysical characterization of RimL from *S. typhimurium* (RimL$^{ST}$) and its three-dimensional structure in both apoform and in complex with CoA.

**MATERIALS AND METHODS**

**Determination of Protein Concentration**—Protein concentration was measured by bichoninic acid assay (Pierce) using bovine serum albumin as standard. All of the experiments were performed with a mixture of L12/L7 containing at least 60% L12. The ratio was determined by MALDI-TOF, assuming equivalent ionization of the two forms, that reported L12 concentrations have been corrected for the presence of L7.

**Cloning and Expression of rimLWT, rimLC134A, and L12**—The *rimL* gene was amplified by PCR using genomic DNA from *S. typhimurium* strain MM1278 and cloned into pET-28a (+) (Novagen), which produced RimL fused to an N-terminal thrombin-cleavable hexahistidine tag. Thrombin cleavage of the gene product leaves three additional non-native amino acids, glycine, serine, and histidine, at the N terminus of RimL$^{ST}$. The L12 gene, also from *S. typhimurium* strain MM1278 (L12$^{ST}$), was amplified by PCR using genomic DNA and cloned into pET-23a (+), which produced a C-terminal hexahistidine-tagged version of L12. The QuickChange kit (Stratagene) was used for the mutagenesis of C134 with pET-23a (+):rimL as the template. All of the constructs were confirmed by DNA sequencing. BL21 (DE3) cells harboring pET-28a (+):rimL or pET-28a (+):rimLC134A were grown in LB media at 37 °C until an $A_{600}$ of 0.5 was reached and induced by the addition of
Structure of RimL

Table 1

Data collection and refinement statistics

| Structure | Hg acetate* |
|-----------|-------------|
| Space group | P4_22 |
| Unit cell | a = b = 68.6, c = 89.6 |
| Resolution range (Å) | 30.2–2.4 (20.7–2.00) |
| Unique reflections | 15,720 |
| Completeness (%) | 95.2 (94.9) |
| Redundancy | 2.8 (1.7) |
| R瑛 refined | 34.0 |
| R瑛 (5% data) | 2.8 (6.1) |

Protein: CoA

Table 1 continued

| Protein | CoA |
|---------|-----|
| H2O/sulfate/malonate/Cl | 103/7/1 |
| Average B-factor (Å²) | 31.9 |
| Protein | 18.3 |
| CoA | 23.4 |
| H2O/sulfate/malonate/Cl | 37.2/31.7/8.9 |
| R.m. a.d. | 0.022 |
| Bond lengths (Å) | 2.0 |
| Bond angles (°) | 1.9 |

RimLST was performed in a similar manner with reactions utilized for heavy atom phasing, grew from solutions containing 0.8–1.2 M Hg acetate. A typical reaction contained 50 mM Hepes, pH 7.4, 50 µM Ac-CoA, 45 µM 4,4′-dithiobispyridine (4,4′-DTP), and 20 nM RimLWT or RimLCL134A in a final volume of 1 ml. The reaction rate was measured by following the increase in the absorbance at 324 nm resulting from the reaction of CoA with 4,4′-DTP (ε324 = 19,800 M⁻¹ cm⁻¹) at 25°C. There is no detectable reaction of 4,4′-DTP with RimLST or L12ST at the concentrations used in this work. Discontinuous and continuous assays using 4,4′-DTP yielded similar initial reaction rates, so continuous assays were chosen to monitor L12ST acetylation. Reactions were started by the addition of RimL after a 1-min preincubation at 25°C. Initial velocity data were analyzed by Lineweaver-Burke plots.

Demonstration of L12 Acetylase Activity—A typical reaction consisted of 120 µM [¹⁴C]Ac-CoA, 40 µM RimLST, 160 µM L12ST, and 50 mM Hepes, pH 7.5, in a final volume of 20 µl. Reaction mixtures were incubated for 1 and 12 h at 25°C. Protein separation and analysis were performed by SDS-PAGE with PhastGel 10–15% gradient gels. After electrophoresis, gels were stained, air-dried, wrapped in plastic film, and exposed for 24 h in an ImagePlate cassette and quantitated using ImageQuant software provided by the manufacturer. The time dependence of the acetylation of L12ST by RimLST was performed in a similar manner with reactions containing 100 µM [¹¹C]Ac-CoA, 1 µM RimLST, 200 µM L12ST, and 50 mM Hepes, pH 7.5, incubated at 25°C.

MALDI-TOF of RimL—Determination of the acetylation state of RimLST and complex formation with CoA was performed by MALDI-TOF spectrometry using an Applied Biosystems Voyager-DE mass spectrometer (Laboratory for Macromolecular Analysis and Proteomics-AECOM). Reactions consisted of 5 µg of RimLST incubated at 25°C for 12 h with or without 0.1 mM Ac-CoA in 50 mM Hepes, pH 7.5, in a 20-microliter volume. One sample of each reaction was subjected to cleavage with 25 ng of thrombin and 50 mM CaCl₂. Reactions were analyzed by MALDI-TOF.

Kinetic Assays—Kinetic studies were conducted at 25°C in a UVikon XL equipped with a constant temperature-circulating water bath and thermostats. A typical reaction contained 50 mM Hepes, pH 7.4, 50 µM Ac-CoA, 45 µM 4,4′-dithiobispyridine (4,4′-DTP), and 40 nM RimLWT or RimLCL134A in a final volume of 1 ml. The reaction rate was measured by following the increase in the absorbance at 324 nm resulting from the reaction of CoA with 4,4′-DTP (ε324 = 19,800 M⁻¹ cm⁻¹) at 25°C. There is no detectable reaction of 4,4′-DTP with RimLST or L12ST at the concentrations used in this work. Discontinuous and continuous assays using 4,4′-DTP yielded similar initial reaction rates, so continuous assays were chosen to monitor L12ST acetylation. Reactions were started by the addition of RimL after a 1-min preincubation at 25°C. Initial velocity data were analyzed by Lineweaver-Burke plots.

Dynamic light scattering was measured with a DynaPro MSX dynamic light-scattering instrument (Protein Solutions) with samples of thrombin-cleaved RimLST at 15 mg/ml in 20 mM TEA, pH 8.0, containing 100 mM (NH₄)₂SO₄.

Cryostalf—All four RimLST crystal forms were obtained by vapor diffusion under oil (Silicon-FISHER) by combining 3 µl of various precipitants and incubating at 18°C. For crystal form 2 and the two CoA binary complexes, the protein was first treated with thrombin to remove the hexahistidine tag and further purified on a Q-Sepharose column. Crystal form 1, which retained the hexahistidine tag and was utilized for heavy atom phasing, grew from solutions containing 0.8–1.2 M sodium malonate, pH 7.8, with RimLWT at 8 mg/ml. A second apo-structure was obtained from crystalline that grew from solutions containing 15–25% polyethylene glycol 3350, 200 mM NaCl, and RimLST at 15 mg/ml. The tetragonal binary complex (form 3) crystallized in 40–50% PEE 797 (pentaerythritol ethoxylate), 0.2 M (NH₄)₂SO₄, 5 µM Ac-CoA, 5 µM dithiothreitol, and RimLST at 50 mg/ml, whereas the monoclinic binary complex (form 4) crystallized in 40–50% PEP 426 (pentaerythritol propoxylate), 0.2 M KCl, 5 mM TCEP (tri(2-carboxyethyl)phosphine hydrochloride), 5 µM Ac-CoA, and RimLST at 20 mg/ml.

Data Collection—Prior to data collection, all of the samples were vitrified by immersion into liquid nitrogen and then mounted in the cryostream. Crystal form 1 and 2 were cryoprotected by including 25% trehalose or 20% isopropyl alcohol, respectively, in the mother liquor, whereas in the binary CoA complexes, the pentaerythritols acted as cryoprotectants. All of the x-ray data sets were collected on a MSC w-Raxis IV™ image plate detector using CuKα, radiation from a Rigaku X-ray generator.
RU-H3R x-ray generator and processed using DENZO/SCALEPACK (24). Data collection statistics are summarized in Table I.

**Structure Determination and Refinement**—The structure of RimL\textsuperscript{ST} was determined by SIRAS single isomorphous replacement utilizing quick-soak derivatization (25). A single crystal of RimL\textsuperscript{ST} (form 1) was soaked in the cryoprotectant mother liquor supplemented with 40 mM mercuric acetate for 10 min and then back-soaked in the same solution without mercuric acetate for 10 s prior to vitrification. The program SOLVE/RESOLVE (26) was used to locate the four mercury binding sites and to calculate the SIRAS phases to 2.4 Å. The figure of merit after SOLVE was 0.479 (0.555 at 2.4–2.5 Å), rising to 0.658 (0.822 at 2.4–2.5 Å) after density modification. The SIRAS SOLVE/RESOLVE map was of sufficient quality for the program MAID (27) to autobuild ~60% structure, whereas manual rebuilding resulted in an initial model that was ~85% complete. The second apostructure and two binary complexes were determined by molecular replacement as implemented in AmoRe (28). All of the model building and refinement were carried out using the programs O (29) and CNS (30). Details of the refined structures are reported in Table I. All of the figures were prepared with PyMOL (31) or MOLSCRIPT (32).

**RESULTS AND DISCUSSION**

The thrombin-cleavable N-terminal His\textsubscript{6} tag construction used to facilitate the purification of RimL\textsuperscript{ST} adds 20 amino acids to the native N terminus (MGSSHHHHHHSSGLVPRGSH-RimL\textsuperscript{ST}). MALDI-TOF analysis of the recombinant purified RimL\textsuperscript{ST} exhibited a mass of 22,679.0 Da corresponding to the demethionylated N-terminally His-tagged RimL\textsuperscript{ST} (predicted mass: 22,637.6 Da) with one acetyl modification (+42 Da). Thrombin cleavage resulted in a RimL\textsuperscript{ST} derivative with a mass of 20,883.0 Da, which corresponds to the expected mass of RimL\textsuperscript{ST} minus the 17 N-terminal residues lost due to thrombin cleavage (20,886.8 Da). This finding suggests that the acetylated residue was located within the N-terminal 17 residues. The Rim proteins have substrates with either a serine or alanine as the penultimate residue, and it is likely that the recombinant RimL\textsuperscript{ST} has catalyzed autoacetylation at the N-terminal glycine after demethionylation. When thrombin-cleaved RimL\textsuperscript{ST} was incubated with Ac-CoA, the mass of the protein was unchanged (20,884.0 Da), indicating that GSH-RimL\textsuperscript{ST} does not autoacetylate the less exposed N-terminal glycine residue. Autoacetylation of this particular tag by other Gcn5-related N-acetyltransferase (GNAT) family members, including the (6)-amino glycoside acetyltransferase from Salmonella enterica (AAC(6)-I\texttext{-}\textsuperscript{a}) has been previously documented (33). Interestingly, incubation of either the tagged or thrombin-cleaved forms with Ac-CoA resulted in additional higher masses (23,444.0 and 21,654.4 Da, respectively), representing 10–15% protein sample. The size difference suggests a tight complex between RimL\textsuperscript{ST} and CoA (+767.6 Da) and is consistent with the K\textsubscript{D} determined for Ac-CoA and/or a possible covalent complex between RimL\textsuperscript{ST} and CoA as observed in one of the crystal structures (see below).

In E. coli and Salmonella, the ratio of L12/L7 was found to vary with growth where the proportion as L12 was highest during logarithmic growth (12–14). To generate L12 substrate, varying with growth where the proportion as L12 was highest consistent with the expected mass of RimLST minus the 17 N-terminal residues. The Rim proteins have substrates with either a serine or alanine as the penultimate residue, and it is likely that the recombinant RimL\textsuperscript{ST} has catalyzed autoacetylation at the N-terminal glycine after demethionylation. When thrombin-cleaved RimL\textsuperscript{ST} was incubated with Ac-CoA, the mass of the protein was unchanged (20,884.0 Da), indicating that GSH-RimL\textsuperscript{ST} does not autoacetylate the less exposed N-terminal glycine residue. Autoacetylation of this particular tag by other Gcn5-related N-acetyltransferase (GNAT) family members, including the (6)-amino glycoside acetyltransferase from Salmonella enterica (AAC(6)-I\texttext{-}\textsuperscript{a}) has been previously documented (33). Interestingly, incubation of either the tagged or thrombin-cleaved forms with Ac-CoA resulted in additional higher masses (23,444.0 and 21,654.4 Da, respectively), representing 10–15% protein sample. The size difference suggests a tight complex between RimL\textsuperscript{ST} and CoA (+767.6 Da) and is consistent with the K\textsubscript{D} determined for Ac-CoA and/or a possible covalent complex between RimL\textsuperscript{ST} and CoA as observed in one of the crystal structures (see below).

In E. coli and Salmonella, the ratio of L12/L7 was found to vary with growth where the proportion as L12 was highest during logarithmic growth (12–14). To generate L12 substrate, cells expressing L12 were harvested early during logarithmic growth where L12 was greater than 70% total L12/L7. RimL\textsuperscript{ST} readily acetylates purified L12\textsuperscript{ST} followed by incubation with [\textsuperscript{14}C]Ac-CoA (Supplemental Figs. 1–3) with the majority of L12 converted to L7 within the first 10 min. Using 4,4’,-DTP to react with the product CoASH (ε = 19,800 M\textsuperscript{-1} cm\textsuperscript{-1} at 324 nm), we were able to spectrophotometrically determine the K\textsubscript{D} values for Ac-CoA (<0.5 μM) and L12\textsuperscript{ST} (1.0 ± 0.2 μM). In addition to this assay, we were able to quantitatively measure the activity of the enzyme when the conditions of the original spectrophotometric assay (Supplemental Figs. 1–3).

**General Fold**—The three-dimensional structure of RimL\textsuperscript{ST} was solved by isomorphous replacement with anomalous scattering from a single mercury derivative collected on a home source. The structure of RimL\textsuperscript{ST} was determined in two apo-crystal forms and subsequently in two CoA binary-complex crystal forms. The relatively compact 179 amino acid RimL\textsuperscript{ST} monomer exhibits the GNAT fold with the topology β0, β1, α1, α2, β2, β3, β4, α3, β5, α4, β6, and β7 (Fig. 1A). The β-strands pack into a predominately antiparallel β-sheet with the order β0, β1, β2, β3, β4, β5, β6, and β7. The sheet exhibits a V-like appearance due to the spaying of the only parallel-interacting strands β4 and β5. The α1 and α2 helices lie on one side of this central β-sheet with α3 and α4 helices on the other side.

**Dimerization**—Evidence from dynamic light scattering, ultracentrifugation, and gel filtration experiments suggests that RimL\textsuperscript{ST} exists as a homodimer in solution (data not shown). An identical monomer-monomer contact was observed in all four crystal forms, despite their unique crystallographic packing arrangements, suggesting that this is the physiological dimer (Fig. 1B). At the dimer interface, the outermost strand (β6) from each monomer interacts in an antiparallel fashion to form a continuous β-sheet that contains at its center the β5-β7-β6-β’-β’-β’-strands. This dimer interface is predominately formed by polar interactions, and a combined surface area of 2238 Å\textsuperscript{2} is buried upon its formation. Dimerization through C-terminal strands to form a continuous β-sheet across the dimer is common in prokaryotic GNAT enzymes (34). The construction of the RimL\textsuperscript{ST} dimer generates a rectangular trough 30-Å long, 13-Å wide, and 15–20-Å deep at the dimer 2-fold. Strands β5-β7 and β’-β’-β’ generate the floor of the trough with β2-β4 and α1-α2 as the long edges and the loops between β6 and β7, β3 and β4, and α1 and α2, which contain two 3\text{10} helices (3\text{10a}, 3\text{10b}) as the short edges. This trough provides a surface for which the protein substrate might bind and react with Ac-CoA, located in opposite corners at the bottom of the trough. The surface of the trough is lined with a mixture of both polar and aliphatic residues consistent with the nature of the protein substrate with which it must interact. Overall, the surface of RimL\textsuperscript{ST} has a mixture of positive and negative electrostatic regions, although there are three fully or partially exposed acidic side chains within the trough (Asp-40, Glu-88, and Glu-160), which give the trough a slight electronegative propensity.

A structural similarity search using the secondary structure matching algorithm (35) yielded matches to several GNAT family members. The highest scoring was the Bacillus subtilis protein YdaF (PDB code 1NSL). Interestingly, the entire dimer of YdaF and RimL\textsuperscript{ST} could be superimposed with an r.m.s.d. of 1.9 Å over 324 common Ca atoms. As such, YdaF and RimL\textsuperscript{ST} have a similar dimer interface and active site trough. YdaF has been proposed to be the B. subtilis homologue of RimL based on comparative sequence analysis (36), although it only exhibits 23% sequence identity with RimL\textsuperscript{ST}. In addition, the acetyltransferase activity of YdaF has not been demonstrated. Our structural superposition suggests that YdaF may indeed be the B. subtilis RimL acetyltransferase.

**Interaction of RimL\textsuperscript{ST} with CoA**—Despite cocryスタzing the protein with Ac-CoA, the two binary complex crystal forms do not exhibit electron density for the acetyl group, suggesting that the high energy thioester bond is hydrolyzed over the lifetime of the experiments. The interactions between CoA and RimL\textsuperscript{ST} are similar to those that have been observed in a number of GNAT family members (22). The adenosine moiety, while clearly visible in the electron density, is located on the back surface of the molecule away from the trough and does not interact substantially with the protein. In contrast, the pyrophosphate and pantetheine moieties of CoA form 15 direct and water-mediated hydrogen bonds with RimL\textsuperscript{ST} in addition to several van der Waals contacts (Fig. 2A). The pyrophosphate...
oxygens interact with the β4-α3 loop ("P-loop") through a number of backbone amides and a conserved water molecule. The splaying of the β4 and β5 strands is used to coordinate the pantetheine arm of CoA, which forms an anti-parallel strand-strand-like interaction with β4.

A comparison of the apoforms and CoA-bound forms indicates that RimLST undergoes a conformational change upon binding cofactor. Apocrystal forms 1 and 2 exhibited no electron density for residues 43–45 and 32–50, respectively. Upon binding CoA, the loop connecting α1 and α2 becomes ordered (Fig. 1B). Interestingly, in crystal form 1 where a portion of this flexible loop is ordered and binds a chloride ion, residues 39–41 are located within the active site pocket with the side chain of Asp-40 hydrogen bonded to the backbone amide of Tyr-98. This interaction is similar to that made with the acetyl group of AcCoA (see below). Upon binding CoA (form 4), residues 39–41 flip outward and form a 3_{10} helix. This conformational change creates interactions of hydrophobic residues Trp-34, Leu-35, and Leu-39 with the pantetheine arm of CoA. A similar arrangement of hydrophobic residues is seen in the corresponding loop of the B. subtilis protein YdaF in which Trp-36 and Leu-38 are blocking access to Ac-CoA in the apo-structure (36).

In addition to these localized conformational changes, more global changes are observed upon the binding of CoA. There is an increase in the splaying of β4 from β5 and therefore a global movement between the N- and C-terminal halves of the molecule. A superposition of the monomers of the apostructure (form 1) and the binary complex (form 4) yields a r.m.s.d. of 1.0 Å over 164 similar atoms. These changes are especially apparent when the six central β-strands (β5-β5') are used as the superpositional unit. Although the C-terminal residues exhibit a r.m.s.d. of 0.57 Å (98 common Cαs), the N-terminal residues exhibit a r.m.s.d. of 2.4 Å (228 common Cαs). Some of the largest deviations occur in the position of α1 and α2, which border the CoA and L12 binding site. The global movement within the first 100 residues and the ordering of the mobile loop between α1 and α2 change the relative positions and composition of residues that line the trough and therefore will affect protein substrate binding. It can be envisioned that changes in the structure may be used to provide a measure of cooperativity in which the binding of Ac-CoA enhances the binding of the second substrate, the primary amine. Such cooperativity has been observed in other GNAT proteins, especially sheep serotinin acetyltransferase (37), where the binding of Ac-CoA results in the formation of the proper active site pocket for serotonin, consistent with its ordered binding mechanism.

Kinetic Mechanism—There are two possible mechanisms for acetyltransfer, direct transfer from Ac-CoA to the amine of substrate in a ternary complex mechanism, or a ping-pong mechanism that includes an enzyme-acetyl intermediate. An enzyme bound intermediate has only been observed in one GNAT protein, the yeast histone acetyltransferase, Esa1, in which an acetyl-cysteine intermediate has been observed (38). All of the other GNAT proteins have been shown to catalyze direct transfer of the acetyl group from Ac-CoA to their substrate. RimLST contains three cysteines (Cys-74, Cys-134, and Cys-155). Cys-155 and Cys-74 are 13.5 and 21.0 Å from the reaction center, respectively, and are located near the protein surface, whereas Cys-134 is located ~4.0 Å away from the reaction center. In the apostructure, the side chain of Cys-134 faces...
away from the active site and is shielded within a small pocket. In the structure of the mercury derivative, the side chain of Cys-134 is bound to a mercury ion and is rotated into the active site. In addition, in the initial CoA-RimL\textsuperscript{ST} binary complex (form 3), the side chain of Cys-134 is again rotated into the active site pocket and is observed to be in a covalent disulfide with CoA (Fig. 2B). This covalent link may be one of the reasons that MALDI measurements of RimL\textsuperscript{ST} incubated with Ac-CoA consistently contained 10–15% of the enzyme whose mass was increased by the molecular mass of CoA. These observations suggested that RimL\textsuperscript{ST} might catalyze the reaction via a covalent acetyl-enzyme intermediate where Cys-134 is acetylated by Ac-CoA. However, Cys-134 is not a conserved residue among RimL\textsuperscript{ST} homologues where, in 50% aligned sequences, the residue is an alanine.

Therefore, we prepared the C134A mutation and performed steady-state kinetics. The C134A mutant form of RimL\textsuperscript{ST} exhibited no significant differences in the kinetic parameters. \(V_{\text{max}}\) is 12 ± 1 \(\text{min}^{-1}\) and the \(K_m\) for L12 is 1.8 ± 0.2 \(\mu\text{M}\) (Supplemental Figs. 1–3). The overall 3-fold reduction in \(V/K\) for L12 argues for a modest role of Cys-134 in the active site character and dynamics. These data argue that the enzyme does not use an enzyme-acetyl intermediate but rather a direct acetyl transfer chemical mechanism. In contrast to the WT enzyme, the C134A mutant exhibited no time-dependent loss of activity in the spectrophotometric assay in which dithiothreitol is absent from the assay mixture (Supplemental Figs. 1–3). This finding suggests that, in vivo, the activity of RimL\textsuperscript{ST} may depend on the intracellular thiol redox balance and the concentration of Ac-CoA.

Although the transfer of an acetyl group from a thioester to a primary amine is an energetically favorable reaction, the GNAT proteins facilitate this reaction in three primary ways. The first of these is the binding of the substrate that will be acetylated with the amine in the proper orientation for attack on the plane of the acetyl group (see below). Second, the interaction of the carbonyl of the acetyl group of Ac-CoA with a backbone amide (Tyr-98) in β4 facilitates not only the proper positioning of the acetyl group but also the polarization of the carbonyl and stabilization of the negative charge on the oxygen atom in the tetrahedral intermediate. Finally, both general acid and general base catalysis has been documented for GNAT superfamily members. In the modeled enzyme-Ac-CoA structure (Fig. 3C), Ser-141, which is hydrogen-bonded to Tyr-98, is positioned ~3.5 Å from the thiol of bound CoA and could function as a general acid by donating a proton to the initially formed thiolate anion of CoA. Glu-160, located ~6 Å away from the sulphydryl of bound CoA, is positioned to function as a general base to either deprotonate the α-amino group of L12 or to assist in deprotonation of the zwitterionic tetrahedral intermediate.

In all of the previously determined structures of GNATs, there is a β-bulge in β4 directly adjacent to the binding site, which coordinates the acetyl-carbonyl of Ac-CoA. Initially, it had been proposed that the positioning of two amide bonds due to the β-bulge might produce an oxyanion hole, facilitating the nucleophilic attack on the thioester carbonyl carbon by the primary amine of the second substrate (39). Subsequently, the structure of a number of GNATs, some with bound substrate, has shown that the orientation of the β-bulge differs, including structures with two protein carbonyl groups, instead of two amide groups pointing toward the active site. It also has been proposed that the β-bulge may be required to promote the splaying of β4 and β5 to create the pantetheine binding site (40, 41). RimL\textsuperscript{ST} does not contain a β-bulge, suggesting that the β-bulge is not necessary for the splaying of β4 from β5 and that the splaying is most likely influenced by a number of residues within the active site, and is determined globally by the protein. The differences in conformation of the β-bulge in GNAT
Binding of L12ST.—The only functionally characterized protein N-acetyltransferases in the GNAT superfamily are the histone acetyltransferases. These enzymes catalyze the region-specific acetylation of the ε-amino groups of lysine residues on the unstructured N-terminal histone “tails.” Most histone acetyltransferases are monomeric and are part of multienzyme complexes that catalyze acetylation of multiple protein substrates. The dimeric nature of both RimL and its protein substrate L12 and the posttranslational nature of the acetylation reaction suggest that RimL may use a very different set of interactions to achieve specific L12 acetylation.

The dimerization of RimLST via antiparallel β6 strand interactions creates a large central trough at the dimer interface where the protein substrate could be accommodated. Both the solution structure of dimeric L12 from E. coli and the crystal structure of L12 from Thermotoga maritima have been determined (42, 43). L12 consists of an N-terminal and C-terminal domain joined by a flexible linker (Fig. 3). The N-terminal domain is the dimerization domain and is composed of a four-helix bundle, whereas the C-terminal domain folds independently into a compact α/β-fold. L12 proteins have highly conserved sequences, and it is believed that the helical nature of the N-terminal domain is a conserved feature of all of the L12 proteins.

The N-terminal domain of L12 from E. coli (L12Ec) was used to model the interaction of L12 with RimLST, because the L12 proteins from E. coli and S. typhimurium are 98% identical. The N termini of L12Ec are not sufficiently exposed to penetrate the trough of RimLST. Therefore, we propose a model in which the α1 helices rotate away from the α2 helices of L12Ec and are inserted into the trough of RimLST. Because the α1 helices of L12Ec make interactions with the α2 helices of L12Ec, an effort was made to position these helices to make compansatory interactions with RimL. For example, the α2 helices of L12Ec make significant hydrophobic to hydrophobic and negative to positive electrostatic interactions with the α2 helices of RimLST, which define the upper sides of the trough. In this model, both α1 helices of the L12Ec dimer could be positioned without steric contacts within the RimLST trough. The N* terminal would be in close proximity to the acetyl group of Ac-CoA and the proposed base, Glu-160, in each of the RimL active sites.

This model would explain the specificity seen in prokaryotic N*-acetyltransferases that are thought to acetylate only one protein substrate. This is in contrast to the relatively nonspecific N*-acetyltransferases observed in eukaryotic N*-acetyltransferases where the active site is probably closer to the surface and whose substrates are cotranslationally acetylated as they exit the ribosome.

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