Structure and Evolution of a Novel Dimeric Enzyme from a Clinically Important Bacterial Pathogen

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Dihydrodipicolinate synthase (DHDPS) catalyzes the first committed step of the lysine biosynthetic pathway. The tetrameric structure of DHDPS is thought to be essential for enzymatic activity, as isolated dimeric mutants of Escherichia coli DHDPS possess less than 2.5% of the activity of the wild-type tetramer. It has recently been proposed that the dimeric form lacks activity due to increased dynamics. Tetramerization, by buttressing two dimers together, reduces dynamics in the dimeric unit and explains why all active bacterial DHDPS enzymes to date have been shown to be homo-tetrameric. However, in this study we demonstrate for the first time that DHDPs from methicillin-resistant Staphylococcus aureus (MRSA) exists in a monomer-dimer equilibrium in solution. Fluorescence-detected analytical ultracentrifugation was employed to show that the dimerization dissociation constant of MRSA-DHDPS is 33 nM in the absence of substrates and 29 nM in the presence of (S)-aspartate semialdehyde (ASA), but is 20-fold tighter in the presence of the substrate pyruvate (1.6 nM). The MRSA-DHDPS dimer exhibits a ping-pong kinetic mechanism (Kcat = 70 ± 2 s−1, Km_pyruvate = 0.11 ± 0.01 mM, and Km_AsA = 0.22 ± 0.02 mM) and shows ASA substrate inhibition with a Ki of 2.7 ± 0.9 mM. We also demonstrate that unlike the E. coli tetramer, the MRSA-DHDPS dimer is insensitive to lysine inhibition. The near atomic resolution (1.45 Å) crystal structure confirms the dimeric quaternary structure and reveals that the dimerization interface of the MRSA enzyme is more extensive in buried surface area and noncovalent contacts than the equivalent interface in tetrameric DHDPs enzymes from other bacterial species. These data provide a detailed mechanistic insight into DHDP catalysis and the evolution of quaternary structure of this important bacterial enzyme.

Staphylococcus aureus is a Gram-positive bacterium responsible for a wide range of clinical diseases (1) and an increased incidence of antibiotic resistance (2). Antibiotic resistant strains, such as methicillin-resistant S. aureus (MRSA), have significantly increased the mortality rate and financial burden on healthcare amenities worldwide (3–5). Accordingly, there is an urgent need to discover new antibiotics targeting MRSA and an equally urgent need to characterize novel drug targets. One such target is dihydrodipicolinate synthase (DHDP) (6).

DHDP catalyzes the condensation of ASA and pyruvate (Fig. 1A) in the first committed step of the biosynthesis of lysine in plants and bacteria, a pathway absent in humans. Given that bacterial DHDP is the product of an essential gene (7) and catalyzes a key step in the pathway yielding lysine, which is a crucial component of the S. aureus cell wall (8–10), DHDP is a valid but as yet uncharted antibiotic target (6). We have, therefore, been interested in understanding how DHDP functions for the development of inhibitors and also from a mechanistic standpoint.

We and others have extensively studied the catalytic mechanism of DHDP enzymes from various organisms (11–13). In Escherichia coli the key catalytic triad residues of DHDP have been identified as Thr-44, Tyr-107, and Tyr-133, which serve as a proton relay between the substrates and bulk solvent (14). Thr-44 and Tyr-133 are located in the active sites of each monomer, whereas Tyr-107 interdigitates across the tight-dimer interface such that Tyr-107 forms part of the active site in each adjacent subunit. Lys-161, which forms a Schiff base with the substrate pyruvate, and Arg-138 and Gly-186, which appear to stabilize ASA binding and cyclization, also play a role in catalysis.

The monomeric structure of DHDP is an (α/β)8 barrel with a C-terminal α-helical domain and is generally considered to form a homo-tetramer in a dimer of tight-dimers configuration (Fig. 1B). The self-association of two monomers to form the tight-dimer unit results in the generation of an allosteric site that binds lysine in E. coli DHDP (Fig. 1B) and mediates feedback inhibition. However, recent work in our laboratories has shown that mutant dimeric forms of E. coli DHDPs are less...
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FIGURE 1. E. coli DHDPS structure and reaction mechanism. A, the reaction catalyzed by DHDPS. B, the quaternary and tertiary structure of E. coli DHDPS. The monomers are labeled (a–d), and the active site for one monomer is indicated. The weak and tight binding interfaces are shown.

active than the wild-type tetramer due to relaxed substrate specificity (11). This is hypothesized to be a result of increased dynamics at the tight-dimer interface, which in turn alters the positioning of the key catalytic triad residues discussed above. The wild-type tetramer, which is formed from two tight-dimers buttressed together, reduces the dynamics at the tight-dimer interface and, thus, within the active site.

In this study we focused our investigation on the structure and function of DHDPS from MRSA (MRSA-DHDPS). We report that MRSA-DHDPS resides in a monomer-dimer equilibrium in solution that is stabilized by the substrate pyruvate and demonstrate the enzyme to be the first example of a functional, native dimer of DHDPS. We also present a 1.45-Å resolution x-ray crystal structure that highlights important structural features of the dimer. These findings provide insight into the evolution of the quaternary structure of an essential bacterial enzyme.

MATERIALS AND METHODS

Common laboratory methods were performed according to Sambrook and Russell (15), and reagents were obtained from Sigma-Aldrich, Australia, or Merck Australia, unless otherwise stated.

Mass Spectrometry—Electrospray ionization time of flight mass spectrometry was performed on an Agilent 6510 quadrupole-time of flight spectrometer coupled to a dual electrospray ionization source as described previously (16).

Circular Dichroism Spectroscopy—CD spectroscopy was performed using a Jasco J-815 CD spectrometer with sample prepared in 10 mM Tris-HCl, pH 8.0, in a 1-mm path length quartz cuvette with a step size of 0.5 nm and 2 s averaging time. Spectra were fitted using CDPRO (17) employing the CDSSTR algorithm and the SP43 protein data base (18).

Absorbance-based Analytical Ultracentrifugation—Sedimentation velocity studies of MRSA-DHDPS (3.1 μM) and E. coli DHDPS (3.1 μM) were performed at a wavelength of 229 nm using an XL-1 analytical ultracentrifuge (Beckman-Coulter) with a 4-hole An60-Ti rotor at 20 °C. Double sector centrifuge cells with quartz windows were loaded with 380 μl of sample and 400 μl of reference (20 mM Tris, 150 mM NaCl, pH 8.0). Sedimentation velocity data were collected at 40,000 rpm at 6-min intervals and fitted to the enhanced van Holde-Weischet method (19) using ULTRASCAN (20) or a continuous size-distribution model using SEDFIT (21).

Fluorescence-detected Sedimentation Velocity and Sedimentation Equilibrium Analyses—Fluorescence-detected sedimentation studies were conducted in a Beckman XL-A analytical ultracentrifuge equipped with a fluorescence detection system (Aviv Biomedical) using MRSA-DHDPS (0.39, 1.6, 6.3, and 25 nm) labeled with the Alexa Fluor 488 succinimidyl ester. The labeling procedure involved preincubation of 19.4 nmol of MRSA-DHDPS with 5 mM sodium pyruvate in 100 μl of 100 mM sodium carbonate, pH 8.3, at room temperature for 5 min to protect the active site lysine (Lys-163) from modification. To this, 194 nmol of Alexa Fluor 488 (0.5 μl) was added and incubated for a further 80 min at room temperature. These represent optimized conditions, such that (on average) one fluorophore was covalently linked per MRSA-DHDPS monomer. Unincorporated dye was removed by Sephacryl S200 gel filtration liquid chromatography on a 1.0-cm (diameter) × 30-cm (length) column equilibrated with 20 mM Tris, 150 mM NaCl, pH 8.0 (reference solution). Fractions containing labeled MRSA-DHDPS were incubated with 1.4 mM ASA to remove pyruvate from the active site as product. Unreacted substrates and product were removed by gel filtration liquid chromatography as described previously. The labeling procedure did not dramatically alter the activity of the enzyme (supplemental Fig. 1F). Double sector centrifuge cells with sapphire windows were loaded with 380 μl of sample and 400 μl of reference (sedimentation velocity) or 100 μl of sample and 120 μl of reference (sedimentation equilibrium). Sedimentation velocity data were collected continuously at 40,000 rpm and fitted to a continuous size-distribution model using SEDFIT (21). Sedimentation equilibrium data collected at 10,000 and 16,000 rpm were analyzed using SEDPHAT (22) after correction for the photomultiplier voltage and pseudo-absorbance normalization.

Coupled Kinetics Assay—Kinetic analysis of MRSA-DHDPS was performed using the DHDPS/dihydricopilinate reductase (DHDPR) coupled assay (14, 23). ASA was prepared according to the method of Roberts et al. (24). Dihydricopilinate reductase was considered to be in excess when a 2-fold concentration increase did not affect the initial rate. Assays
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FIGURE 2. Quaternary structure and kinetic analysis of MRSA-DHDPS. A, enhanced van Holde-Weischet analysis of 3.1 μM MRSA-DHDPS (○) and 3.1 μM E. coli DHDPS (●) plotted as boundary fraction versus standardized sedimentation coefficient (Svedberg). B, sedimentation velocity data of Alexa Fluor 488-labeled MRSA-DHDPS in the presence and absence of substrates. The concentration of the enzyme was 25 nM in each case. For the apo form (solid line) f′0 = 1.3, the s20,w = 3.3 S, and the r.m.s.d. = 24. In the presence of 2.0 mM ASA (short dash) the f′0 = 1.3, the s20,w = 3.3 S, and the r.m.s.d. = 24. In the presence of the substrate pyruvate (long dash) the f′0 = 1.3, the s20,w = 4.0, and the r.m.s.d. = 21. C, sedimentation equilibrium data of 25 nM MRSA-DHDPS at 10,000 rpm in the absence of pyruvate (○) and the presence of 2.0 mM pyruvate (□) overlaid with global nonlinear least squares best fits to a monomer-dimer equilibrium model. The full data and fits are shown in supplemental Fig. 2. D, initial rate versus MRSA-DHDPS enzyme concentration. E, apparent specific activity (SAapp) is plotted as a function of total MRSA-DHDPS concentration (expressed as the monomer concentration). The symbols represent apparent specific activity calculated from the data described in panel D above enclosed by error bars resulting from triplicate measurements. The solid line represents the weighted nonlinear least squares best-fit to supplemental Equation 1. The fit describes the apparent specific activity of a MRSA-DHDPS monomer-dimer equilibrium, yielding a SAapp (monomer) = 90 μmol min⁻¹ mg⁻¹, SAapp (dimer) = 160 μmol min⁻¹ mg⁻¹, and K21 = 1.0 ± 1.1 nM with an R² value of 0.94. This agrees well with our fluorescence-detected analytical ultracentrifugation results (Table 1, Fig. 2C, and supplemental Fig. 2). F, initial rate data (symbols) fitted to the ping-pong mechanism with ASA substrate inhibition (lines) (R² = 0.98, p > F = 5.3 × 10⁻²⁸) employing 26 nM MRSA-DHDPS.

were performed in 10-mm path length, 1.5-mL reduced-volume acrylic cuvettes in triplicate with 150 mM HEPES, pH 8.0, 0.16 mM NADPH, 0.09–3 mM pyruvate, and ASA, 26 nM DHDPS, and at least a 10-fold molar excess of DHDPR in a final volume of 0.7 mL. Cuvettes were preincubated for 10 min at 30 °C before reaction initiation with DHDPS (varied substrate analysis) or ASA (varied enzyme concentration analysis) with data collected using a temperature-controlled Cary5 UV-visible spectrophotometer (Varian). Rates were calculated from the initial linear portion of data and analyzed using ENSFITTER (Biosoft, Cambridge, UK).

Structure Determination—X-ray diffraction data from crystals of MRSA-DHDPS were collected at the Australian Synchrotron (Clayton, Australia) and were published elsewhere (25). Diffraction data were integrated and scaled using the MOSFLM (26) and SCALA (27) software. Initial phase estimation by molecular replacement was performed using PHASER (28), with a single monomer of E. coli DHDPS (PDB ID 1YXC (29)) as a search model. Structural refinement was performed using REFMACS (30) with iterative manual model building using WINCOOT (31). Non-crystallographic symmetry restraints were applied in the early stages of refinement and were released for most residues as the phases improved. Waters were added at later stages using WINCOOT and ARPWATERS (32). The final refinement rounds included the addition of hydrogen atoms and anisotropic refinement with REFMACS (30), which resulted in a 1.8% drop in the Rfree. The final model was checked with PROCHECK (33) and deposited in the PDB data bank (PDB ID 3DAQ).

RESULTS AND DISCUSSION

ApoMRSA-DHDPS Exists in a Monomer-Dimer Equilibrium in Solution—MRSA-DHDPS was expressed and purified as described elsewhere (25). The enzyme was initially characterized by electrospray ionization time of flight mass spectrometry and CD spectroscopy to show that the mass of the recombinant product was consistent with the amino acid sequence of the native protein after methionine cleavage (supplemental Fig. 1A) and that the enzyme was folded in aqueous solution (supplemental Fig. 1B).

Next we assessed the quaternary structure of the enzyme by native polyacrylamide gel electrophoresis. Given the conserved tetrameric structure of bacterial DHDPS, we surprisingly show that native MRSA-DHDPS (Rf = 0.46) migrates significantly farther on native PAGE than the native E. coli DHDPS tetramer (Rf = 0.13) (supplemental Fig. 1C), suggesting the oligomeric form of the MRSA enzyme is smaller than the E. coli DHDPS tetramer. Therefore, a more quantitative approach was sought to characterize the quaternary structure of MRSA-DHDPS.

Sedimentation velocity experiments were subsequently performed over a concentration range of 1.5–4.6 μM MRSA-DHDPS using absorbance-detected analytical ultracentrifugation. Representative raw data generated at 40,000 rpm for MRSA-DHDPS and E. coli DHDPS at the same initial concentration (3.1 μM) are compared in supplemental Fig. 1D. These data were fitted to an enhanced van Holde-Weischet model (19), which indicated that MRSA-DHDPS sediments with a standardized sedimentation coefficient (s20,w) of 4.2 S, compared with 6.5 S for E. coli DHDPS (Fig. 2A). These values are consistent with the sedimentation coefficients previously reported for dimeric and tetrameric species of native E. coli DHDPS, respectively (34). Similar results were obtained using the c(s) distribu-
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TABLE 1
Solution properties of MRSA-DHDPS in the presence and absence of substrates

| MRSA-DHDPS sample | $k_a$ (mM) | $K_D$ (nM) | Global reduced $\chi^{2b}$ |
|-------------------|------------|------------|--------------------------|
| Apoenzyme          | 5          | 5          |                          |
| +2.0 mM pyruvate   | 3.3        | 33         | $3.8 \times 10^{-2}$     |
| +2.0 mM ASA        | 4.0        | 1.6        | $4.3 \times 10^{-2}$     |
| +2.0 mM pyruvate   | 3.3        | 29         | $6.8 \times 10^{-2}$     |

$^a$ Weight-average standardized sedimentation coefficient.
$^b$ Values obtained from the global nonlinear least squares best fit to a monomer-dimer equilibrium (Fig. 2C, supplemental Fig. 2).

The MRSA-DHDPS Dimer Is Catalytically Active—Given that dimeric mutants of the *E. coli* enzyme showed reduced activity, we were very interested in determining if the MRSA-DHDPS dimer was catalytically active. The coupled assay was used to collect initial rate data for MRSA-DHDPS with varied concentrations of the two substrates, pyruvate and ASA. First, we measured the initial rate over an enzyme concentration range of 0.09 to 50 nM, which is below and above the $K_D^{2\to1}$. The data show that the relationship between initial rate and enzyme concentration deviates from linearity at enzyme concentrations below 2 nM (Fig. 2D). The reduced activity at low concentrations (approaching and below the $K_D^{2\to1}$ of 1.6 nM in the presence of pyruvate) suggests that the MRSA-DHDPS monomer is less active than the dimer. This is supported by Fig. 2E, which shows the apparent specific activity ($SA_{app}$) calculated from the data in Fig. 2D plotted as a function of total MRSA-DHDPS monomer concentration. Nonlinear regression analysis of these data fitted to a monomer-dimer equilibrium (Equation 1, supplemental material) yields an $SA_{app}$ of 90 μmol min$^{-1}$ mg$^{-1}$ for the monomer, 160 μmol min$^{-1}$ mg$^{-1}$ for the dimer, and a $K_D^{2\to1}$ of 1.0 ± 1.1 nM ($R^2 = 0.94$). This analysis demonstrates the monomer of MRSA-DHDPS has significantly reduced activity compared with the dimer and also results in a $K_D^{2\to1}$ that is consistent with our solution studies in the presence of pyruvate (Table 1). Statistically the fit was identical ($R^2 = 0.94$) when the specific activities were optimized, but $K_D^{2\to1}$ was fixed to 1.6 nM, the value calculated from our sedimentation equilibrium analyses (Table 1).

To determine the kinetic parameters of MRSA-DHDPS, initial rate data were measured with varying concentrations of both substrates (pyruvate and ASA) at an enzyme concentration of 26 nM, a concentration at which the enzyme is predominantly dimeric. The data obtained were fitted by least squares nonlinear regression to yield a best fit to a ping-pong mechanism, with ASA substrate inhibition ($K_{si} = 2.7 ± 0.9$ nM) (Fig. 2F). Furthermore, we show by measuring the activity of MRSA-DHDPS over a broad lysine concentration range that MRSA-DHDPS is insensitive to lysine-feedback inhibition (supplemental Fig. 1G). The catalytic turnover ($k_{cat}$) of MRSA-DHDPS was determined to be 70 ± 2 s$^{-1}$, which is similar to that observed for *E. coli* DHDPS (14). In addition, the $K_m$ values for pyruvate and ASA of MRSA-DHDPS were determined to be 0.11 ± 0.01 and 0.22 ± 0.02 mM, respectively. These correspond well with those reported for *E. coli* DHDPS, which are 0.26 ± 0.03 and 0.11 ± 0.01 mM for pyruvate and ASA, respectively. Given that the MRSA-DHDPS dimer is enzymatically active, we were interested in determining the three-dimensional structure of the dimer.

The Crystal Structure of MRSA-DHDPS Provides Insight into Its Functional Evolution—To understand the underlying molecular basis for catalysis by the MRSA-DHDPS dimer, we turned to X-ray crystallography. The crystal structure was solved in near-atomic resolution (1.45 Å) with a final $R_{free}$ of 16.1% and an $R_{work}$ of 13.2% (PDB ID 3DAQ). The final model included four polypeptide chains and nine glycerol molecules, including one in each of the four active sites, perhaps reflecting the similar size of glycerol to the enzyme substrate, pyruvate. One chloride ion was also observed bonded to T13 in each monomer and presumably comes from the crystallization buffer.

Pyruvate Shifts the Equilibrium in Favor of the Dimer—To investigate the quaternary structure of MRSA-DHDPS in the presence of substrates and to probe the stability of the dimer in solution, fluorescence-detected sedimentation studies were employed at low (nM) enzyme concentrations. Sedimentation velocity analysis of 25 nM MRSA-DHDPS showed a relatively broad and asymmetric $c(s)$ distribution with a weight-average sedimentation coefficient of 3.3 S, which remains unchanged in the presence of 2.0 mM ASA (Table 1; Fig. 2B). However, the addition of 2 mM pyruvate resulted in an altered $c(s)$ distribution with a weight-average $s_{20,w}$ of 4.0 S, suggesting pyruvate stabilizes the dimeric form of the enzyme (Table 1, Fig. 2B).

To quantify this phenomenon, sedimentation equilibrium experiments were conducted at 0.39, 1.6, 6.3, and 25 nM MRSA-DHDPS in the presence and absence of a saturating concentration of pyruvate (2.0 mM) and at two rotor speeds (10,000 and 16,000 rpm). Global nonlinear least squares analyses of the data at both speeds were performed with the mass fixed to the theoretical monomer, dimer, trimer, or tetramer mass of MRSA-DHDPS. The nonlinear least square global best fit was obtained to a dimeric model for the apo form and also in the presence of pyruvate, with global reduced $\chi^2$ values of $2.5 \times 10^{-1}$ and $5.5 \times 10^{-2}$, respectively (fits not shown). Subsequently, these data were globally fitted to multiple self-association models, including a monomer-dimer, monomer-trimer, and monomer-tetramer equilibrium. Not surprisingly, the best fit was obtained to a monomer-dimer equilibrium yielding a dimerization dissociation constant ($K_D^{2\to1}$) of 33 nM without substrate and 1.6 nM in the presence of pyruvate with global reduced $\chi^2$ values of $3.8 \times 10^{-2}$ and $4.3 \times 10^{-2}$, respectively (Fig. 2C, supplemental Fig. 2, Table 1) By contrast, the global nonlinear best fit yielded a $K_D^{2\to1}$ of 29 nM in the presence of ASA (Table 1). Taken together, native gel electrophoresis and analytical ultracentrifugation results indicate that native MRSA-DHDPS forms a tight dimer in solution that is not significantly changed in the presence of the substrate ASA, but is stabilized by more than 20-fold in the presence of the substrate pyruvate. This is important as the enzyme kinetic studies described below were conducted at a MRSA-DHDPS concentration that is 16.5-fold higher than the $K_D^{2\to1}$ calculated in the presence of pyruvate (Table 1).

The MRSA-DHDPS Dimer Is Catalytically Active—Given that dimeric mutants of the *E. coli* enzyme showed reduced
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Refinement statistics and geometric parameters are shown in Table 2. The Ramachandran plot showed that 99.6% of the modeled residues were in allowed regions. Only Tyr-109, which is part of the catalytic triad, was found to be outside the allowed regions. This has been previously observed in DHDPS enzymes from other sources (29, 35–38). The functional reason for the active site strain found in Tyr-109 is not known.

Consistent with the solution work, MRSA-DHDPS was found to be a dimer in the crystalline state (Fig. 3A) and structurally reminiscent of the tight-dimer unit of DHDPS seen in other organisms. Fig. 3B shows an overlay of the allosteric site residues of E. coli DHDPS (29) and the equivalent residues in the MRSA enzyme. Little structural conservation was observed, indicating why MRSA-DHDPS is insensitive to lysine inhibition (supplemental Fig. 1G). A number of side chains including Glu-84, His-56, and His-53, which are known to interact with lysine in E. coli DHDPS (29), are not conserved in the MRSA enzyme and instead are replaced by Lys-86, Lys-57, and Thr-55 (Fig. 3B).

The dimer interface of MRSA-DHDPS shows a greater solvent-inaccessible surface area than all other DHDPS crystal structures with demonstrated activity (Fig. 3C; Table 3). A relatively high number of hydrogen bonds and hydrophobic interactions were also observed, suggesting the dynamic fluctuations of dimeric E. coli DHDPS mutants may be restricted in MRSA-DHDPS by tighter binding across the dimer interface. A temperature factor analysis of the MRSA-DHDPS dimer shows that residues important in stabilizing the dimer interface have an overall reduced temperature factor compared with the average value for all residues in the structure and also the comparative residues in the E. coli homologue (supplemental Figs. 3, A and B). Similarly, the temperature factors of the key catalytic residues are also reduced compared with the average value across all residues (supplemental Fig. 3A).

The active site of MRSA-DHDPS is structurally well conserved (Fig. 3D). However, the orientation of Tyr-109 was quite different while still maintaining the conserved hydrogen-bonding network of the catalytic triad (Tyr-109—Thr-45—Tyr-135). The aromatic ring of Tyr-109 is twisted ~60° relative to the equivalent residue in the E. coli structure, providing a closer aromatic stacking interaction in MRSA-DHDPS compared with the E. coli enzyme (Fig. 3E). Along with the increased buried surface area and higher number of interface interactions compared with other DHDPS tight-dimers, the more extensive buttressing of Tyr-109 in MRSA-DHDPS may explain the increased activity compared with the E. coli dimeric enzymes. Increased/Altered dynamic motion in the dimeric E. coli mutants (compared with the wild-type tetramer) is hypothesized to specifically interfere with Tyr-107 of the catalytic triad (E. coli DHDPS numbering). For MRSA-DHDPS, this motif appears to possess stronger contacts and, thus, allows the formation of a catalytically competent and substrate-specific active site. This offers an alternative strategy to tetramerization, as observed in all other active DHDPS enzymes to date.

**Evolutionary Implications**—The increased buried surface area at the dimer interface and greater contacts to the key catalytic residue (Tyr-109) is a hitherto unseen strategy to combat the dynamic fluctuations observed in dimeric E. coli DHDPS mutants by Griffin et al. (16). This suggests the commonly

### Table 2

**Refinement statistics**

| Parameter                      | Value                  |
|--------------------------------|------------------------|
| Space group                    | P2_1                   |
| Unit cell parameters           | a = 65.4, b = 67.6, c = 78.0 |
| Refinement resolution          | 30.25-1.45 (1.49-1.45)  |
| Non-H atoms                    |                        |
| Protein                        | 9138                   |
| Glycerol                       | 54                     |
| Chloride ions                  | 4                      |
| Solvent (H2O)                  | 1759                   |
| Chloride ions                  | 4                      |
| Glycerol                       | 54                     |
| Protein                        | 9138                   |
| Mean isotropic B (protein) (Å²)| 10.6                   |
| Mean isotropic B (solvent) (Å²)| 25.1                   |
| Mean isotropic B (ligands) (Å²)| 12.9                   |
| Residues in Ramachandran plot (%)|                      |
| Most favored regions           | 92.3                   |
| Additionally allowed regions   | 7.3                    |
| Disallowed regions             | 0.4                    |
| r.m.s.d values from ideal geometry |                     |
| Bond lengths (Å)               | 0.007                  |
| Bond angles (degree)           | 1.27                   |
| Dihedrals (degree)             | 35.1                   |

* $r = \frac{2|F_{obs} - F_{calc}|}{|F_{calc}| + |F_{obs}|}$, where $F_{obs}$ and $F_{calc}$ are the observed and calculated structure-factor amplitudes, respectively.
* $R_{free}$ was calculated with 5% of the diffraction data and was selected randomly and omitted from the refinement.
observed DHDPs tetramer evolved from an ancient primordial monomer via a dynamically unstable dimer, consistent with the expected route of quaternary structure evolution described by Levy et al. (39). Given the tetramer is observed across a wide range of bacterial species, it seems more likely that the active MRSA-DHDPS dimer has resulted from divergent (rather than parallel) evolution. A comprehensive phylogenetic analysis may provide further insight into these observations.

Conclusions—The results of this study demonstrate that DHDPS from methicillin-resistant S. aureus exists in a monomer-dimer equilibrium that is significantly shifted in favor of the dimer in the presence of the substrate pyruvate. The MRSA-DHDPS tight dimer is the first native and active dimeric form of a DHDPS enzyme described to date. X-ray structural analyses show that the MRSA-DHDPS dimer has significantly increased contacts and greater buried surface area at the dimer interface compared with the tight-dimer interface in the archetypal tetrameric forms. This provides insight into the evolution of quaternary structure of DHDPS and suggests that two alternative mechanisms have evolved to overcome increased dynamics in the dimeric unit.

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