Structural Insights into the Catalytic Mechanism of Synechocystis Magnesium Protoporphyrin IX O-Methyltransferase (ChlM)*

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Mg-protoporphyrin IX O-methyltransferase (ChlM) catalyzes transfer of the methyl group from S-adenosylmethionine to the carboxyl group of the C13 propionate side chain of magnesium protoporphyrin IX. This reaction is the second committed step in chlorophyll biosynthesis from protoporphyrin IX. Here we report the crystal structures of ChlM from the cyanobacterium Synechocystis sp. PCC 6803 in complex with S-adenosylmethionine and S-adenosylhomocysteine at resolutions of 1.6 and 1.7 Å, respectively. The structures illustrate the molecular basis for cofactor and substrate binding and suggest that conformational changes of the two “arm” regions may modulate binding and release of substrates/products to and from the active site. Tyr-28 and His-139 were identified to play essential roles for methyl transfer reaction but are not indispensable for cofactor/substrate binding. Based on these structural and functional findings, a catalytic model is proposed.

Background: Magnesium protoporphyrin IX methyltransferase (ChlM) catalyzes the second step in the magnesium branch of tetrapyrrole biosynthesis.

Results: The SAM- and SAH-bound ChlM structures were obtained, and the ChlM active site was characterized.

Conclusion: Flexibility of two regions could be a key modulator for methyltransferase activity.

Significance: This work provides first structural insights into the catalytic mechanism of ChlM.

Chlorophylls are the primary pigments that harvest solar energy for the light reaction of photosynthesis. The biosynthesis of chlorophylls is a vital process in photosynthetic organisms and consists of multiple enzymatic steps (1, 2). Protoporphyrin IX is the last common tetrapyrrole precursor located at the branch point of heme and chlorophyll biosynthesis. The initial steps of the chlorophyll branch are tightly regulated by interplays of proteins involved in sequential conversions of protoporphyrin IX to magnesium protoporphyrin IX (MgP)2 and Mg-protoporphyrin IX monomethyl ester (MgPME). These interacting proteins include magnesium chelatase, a complex enzyme comprising three subunits (ChlH, ChlI, and ChlD), magnesium protoporphyrin IX O-methyltransferase (ChlM), genomes uncoupled 4 protein (Gun4), and redox regulators such as thioredoxin and the NADPH-dependent thioredoxin reductase C (3–5). In addition, the intermediates during the process, MgP and MgPME, are potential retrograde signaling molecules in plastid-containing photosynthetic organisms (6–8). The structural characterization of some of these proteins, including magnesium chelatase (9–12) and Gun4 (13, 14), has provided a basis to elucidate their regulatory mechanisms and the signaling issues. However, details about the catalytic mechanism are not well understood due to the lack of high resolution structural information, particularly for ChlH and ChlM.

ChlM (E.C. 2.1.1.11) catalyzes methyl transfer from the common methyl donor SAM to the carboxyl group of the C13 propionate side chain of MgP, resulting in the formation of MgPME and SAH. Genetic studies have shown that ChlM is essential for chlorophyll biosynthesis and chloroplast development (15, 16). The enzymatic kinetics of the cyanobacterium Synechocystis ChlM (SyChlM) have been elaborately studied (17, 18). These kinetic studies have established a random sequential mechanism in which SAM and MgP bind to SyChlM in either order. Sequence analysis revealed that SyChlM is homologous to small molecule methyltransferases, but structural information about the active site was not available. Furthermore, it has been shown that Synechocystis ChlH can stimulate SyChlM activity (19). Such a regulatory coupling of ChlH and ChlM is conserved from the purple bacteria to higher plants (20–23). An efficient intermediate channeling between ChlH and ChlM has been proposed to facilitate the flow of protoporphyrin IX to the chlorophyll branch (2, 3). To understand the molecular basis underlying the catalytic and regula-

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The atomic coordinates and structure factors (codes 4QDJ and 4QDK) have been deposited in the Protein Data Bank (http://wwpdb.org/).

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2 The abbreviations used are: MgP, magnesium protoporphyrin IX; MgPME, magnesium protoporphyrin IX monomethyl ester; ChlM, magnesium protoporphyrin IX O-methyltransferase; SyChlM, Synechocystis ChlM; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; ITC, isothermal titration calorimetry; r.m.s.d., root mean square deviation.
Crystal Structure of Cyanobacterial ChlM

In the present work we report the SAM- and SAH-bound SyChlM structures at resolutions of 1.6 and 1.7 Å, respectively. The structures demonstrate that the N-terminal region and a 21-residue insertion protruding from the core of the protein are flexible. Based on the SyChlM structure and supported by biochemical experiments on site-directed mutants, we identified two residues that are required for the methyl transfer reaction. In addition, these findings suggest a possible molecular basis for ChlM catalytic and regulatory mechanism.

EXPERIMENTAL PROCEDURES

Expression, Purification, and Crystallization—The chlM gene from Synechocystis sp. PCC 6803 was inserted into pET-22b (+) (Novagen) to generate the pET-22b (+)-SyChlM-His6 plasmid. The recombinant His-tagged SyChlM was expressed in Escherichia coli BL21 (DE3) cells and purified by nickel-nitrilotriacetic acid column (Qiagen) followed by size-exclusion chromatography using a HiLoad 16/60 Superdex 75 column (GE Healthcare). The purified protein was concentrated by ultrafiltration in a buffer containing 20 mM citrate sodium, pH 6.7, 150 mM NaCl, and 10% isopropyl alcohol) taken from a 1-mL reservoir.

Data Collection and Structure Determination—The harvested crystals were cryo-protected in crystallization solution supplemented with 20% (v/v) glycol and then shock-frozen in liquid nitrogen. X-ray diffraction data were collected at beamline BL17U of the Shanghai Synchrotron Radiation Facility at a wavelength of 0.9793 Å at 100 K. The data were indexed, integrated, and scaled using DINOZO and SCALEPACK as implemented in HKL2000 (24). Selenium positions in the SyChlM-SAM crystal and preliminary model-building were resolved using the program AutoSol in the PHENIX suite (25). Additional missing residues in the auto-built model were manually added according to the 2Fo – Fc and Fo – Fc electron density maps in Coot (26). The native SyChlM-SAM and SyChlM-SAHL structures were determined by molecular replacement using the selenomethionine-substituted SyChlM-SAM structure as the searching model. Automatic model building was performed using ARP/wARP (27), and manual model correction was done in Coot. The models were refined further in PHENIX, and the overall quality of the final structural models was assessed by PROCHECK (28). Statistics for data collection and structure refinement are summarized in Table 1. The protein structure figures were prepared using the program PyMOL.

MgP Docking—In silico docking of the substrate MgP to SyChlM was performed with AutoDock Vina 1.1.2 (29). The MgP coordinates were extracted from a high resolution x-ray crystal structure (Protein Data Bank code 1QSI (30)). Protein coordinates were taken from chain A of the SyChlM-SAHL structure. Before simulation, the cofactor SAH in the SyChlM-SAHL structure was replaced by SAM. Hydrogen atoms, Gasteiger partial charges, and ligand torsions were added using the program AutoDockTool in the MGLTools software (31). During simulation, the ChlM and SAM structures were kept

### Table 1

| Data collection | SyChlM-SAM | SyChlM-SAHL | SyChlM-SeMet |
|----------------|------------|-------------|-------------|
| Space group    | P2_1,2_1   | P2_1        | P2_1,2_1    |
| Resolution range (Å) | 50-1.6 (1.66-1.60) | 50-1.7 (1.76-1.70) | 50-2.5 (2.59-2.50) |
| Unit cell parameters | a, b, c (Å) | a, b, c (Å) | a, b, c (Å) |
| a, b, γ (°) | 90, 90, 90 | 90, 95.8, 90 | 90, 90, 90 |
| Wavelength (Å) | 0.9793 | 0.9793 | 0.9793 |
| Total reflections | 334,952 (27,799) | 200,818 (19,645) | 212,291 (21,588) |
| Unique reflections | 2,743 (2,305) | 54,275 (5,457) | 7,776 (747) |
| No. of protein molecules in asymmetric unit | 1 | 2 | 1 |
| Completeness (%) | 97.9 (84.3) | 99.5 (98.9) | 100 (100) |
| Rmerge (%) | 0.096 (0.317) | 0.125 (0.547) | 0.146 (0.432) |

* Rmerge = Σ[I – I_max]/ΣI, where I_max is the intensity of the measured reflection, and Im is the mean intensity of all symmetry related reflections.

** Rmerge = Σ[I – I_max]/ΣI, where I_max and I are observed and calculated structure factors. Rmerge = Σ[I – I_max]/ΣI, where I_max and I are observed and calculated structure factors.
rigid, and grid maps were calculated using $60 \times 40 \times 45$ grid points with a spacing of 0.55 Å. Grids were centered such that they covered the whole substrate binding site, and the most plausible model was selected based on the steric accessibilities of the C13 propionate side chain of MgP and the calculated binding affinity energy.

**Site-directed Mutagenesis—**SyChlM mutants were generated with the Fast Mutagenesis System kit (TransGen Biotech, Beijing) using pET-22b(+) -SyChlM-His6 as template. All mutant plasmids were sequenced to confirm the desired mutations. The procedure for purification of the SyChlM mutant proteins was the same as that of the wild type.

**Methyltransferase Assay—**SyChlM activity was measured using an enzyme-coupled continuous spectrophotometric assay (32, 33). The reaction mixtures contained 100 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5% polypropylene glycol P 400, 500 μM MnCl₂, 50 μM SAM, 50 μM MgP (Frontier Scientific), 10 μM SAH nucleosidase, 0.2 μM adenine deaminase, and 2 μM
The recombinant SAH nucleosidase and adenine deaminase were expressed and purified following the established procedures (32, 34). The assay was performed in a thermostatted 1-cm quartz cuvette at 30 °C. The coupled reaction was measured by continuously recording the absorbance at 265 nm for 5 min, which indicates the conversion of the reaction. ||FIGURE 2. Multiple ChlM sequences alignment and the cofactor binding pocket of SyChlM. A, multiple sequence alignment of ChlMs. Identical amino acids are in white on a red background, with the DXGCGXG motifs on a cyan background. The similar residues are in red and boxed. Dots indicate gaps introduced during alignment. Pink ellipses and green stars denote the residues involved in binding to the cofactor and to MgP, respectively. The two catalytically essential residues are marked with blue triangles. B, electron density maps of SAM and SAH. The 2Fo − Fc maps at 0.5 σ for SAM (top) and SAH (bottom) are shown. C, clipped view of the SAM binding pocket. SyChlM is in gray. D, residues interacting with SAM in the pocket. Residues involved in hydrophobic interaction with SAM are in green; residues involved in hydrogen bond formation are in yellow. The backbone of the DXGCGXG motif is in blue. Two waters forming hydrogen bonds with SAM are shown as red spheres. Hydrogen bonds are depicted as dotted dashes.||

SyChlM. The recombinant SAH nucleosidase and adenine deaminase were expressed and purified following the established procedures (32, 34). The assay was performed in a thermostatted 1-cm quartz cuvette at 30 °C. The coupled reaction was measured by continuously recording the absorbance at 265 nm for 5 min, which indicates the conver-
Crystal Structure of Cyanobacterial ChlM

Consistent with the color of SyChlM, the structure of apo-ChlM was determined at 2.7 Å resolution, revealing a compact protein fold that is characteristic of the ChlM family (1). The structure of ChlM after SAM binding was determined at 1.6 Å resolution, and the structure of SAH-bound ChlM was determined at 1.7 Å resolution. The secondary structural elements of ChlM are defined by DSSP (37), and the topology is depicted in Fig. 1A. An α/β/α sandwich forms the core of fold with the central seven-stranded β-sheet having a thickness of 22.9 Å.

Isothermal Titration Calorimetry—ITC experiments were performed on a MicroCal iTC200 calorimeter (GE Healthcare) at 20 °C. The purified SyChlM protein was changed into the buffer of 100 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 300 mM glycerol. The ligand SAH was directly dissolved in the above buffer, and MgP was dissolved in the buffer supplemented with 0.1% Tween 80. The concentrations of SAH and MgP were determined spectrophotometrically using the molar absorption coefficients of ε_{260 nm} = 15,400 M⁻¹ cm⁻¹ and ε_{408 nm} = 278,000 M⁻¹ cm⁻¹, respectively (35, 36). Each titration series consisted of 20 injections (2 µl) of ligand in the syringe into the protein sample (200 µl). Control experiments were carried out by injecting each ligand into the buffer, and the resulting heat of dilution was subtracted from the binding isotherm data. The first injection was ignored in the final analysis. The raw ITC data were processed using the Origin software and fitted to a single-site binding model.

RESULTS

Overall Structure of SyChlM—We obtained crystals of SyChlM in the apo-, SAM-bound, and SAH-bound forms. However, attempts to solve the apo structure were unsuccessful due to poor diffraction quality of the crystals. The structures of SyChlM bound to SAM and SAH were determined at resolutions of 1.6 and 1.7 Å, respectively (Table 1). The secondary structural elements of SyChlM are defined by DSSP (37), and the topology is depicted in Fig. 1A.

### TABLE 2

| Protein-Ligand | Kd (µM) | ΔG (kcal mol⁻¹) | ΔH (kcal mol⁻¹) | ΔS (cal mol⁻¹ K⁻¹) |
|----------------|--------|----------------|----------------|--------------------|
| SyChlM<sup>Wt</sup>-MgP | 6.0 ± 0.4 | -7.0 ± 0.4 | -10.4 ± 0.1 | -11.5 |
| SyChlM<sup>Y28A</sup>-MgP | 4.9 ± 0.7 | -7.1 ± 1.1 | -14.0 ± 0.6 | -23.1 |
| SyChlM<sup>H139A</sup>-MgP | 8.1 ± 0.3 | -6.8 ± 0.3 | -11.8 ± 0.1 | -17.0 |
| SyChlM<sup>H139N</sup>-MgP | 3.6 ± 0.3 | -7.3 ± 0.6 | -14.7 ± 0.3 | -25.2 |
| SyChlM<sup>H139A</sup>-MgP | 4.1 ± 0.2 | -7.2 ± 0.4 | -12.1 ± 0.1 | -17.0 |
| SyChlM<sup>Wt</sup>-SAH | 1.4 ± 0.1 | -7.8 ± 0.3 | -16.1 ± 0.0 | -28.0 |
| SyChlM<sup>H139A</sup>-SAH | 2.5 ± 0.2 | -7.5 ± 0.5 | -16.1 ± 0.2 | -29.3 |
| SyChlM<sup>H139N</sup>-SAH | 15.5 ± 1.8 | -6.4 ± 0.8 | -8.5 ± 0.3 | -7.0 |
| SyChlM<sup>H139A</sup>-SAH | 8.1 ± 0.2 | -6.8 ± 0.2 | -13.5 ± 0.1 | -22.9 |
| SyChlM<sup>H139N</sup>-SAH | 31.0 ± 1.7 | -6.0 ± 0.3 | -12.0 ± 0.4 | -20.4 |
| SyChlM<sup>SAH</sup>-MgP<sup>SAH</sup> | 2.7 ± 0.4 | -7.5 ± 1.1 | -7.6 ± 0.2 | -0.3 |
| SyChlM<sup>SAH</sup>-MgP<sup>SAH</sup> | 1.9 ± 0.1 | -7.7 ± 0.3 | -16.0 ± 0.1 | -28.3 |

<sup>a</sup> MgP titration to the SAH-saturated SyChlM; 50 µM SyChlM was mixed with 150 µM SAH and then titrated with 0.40 mM MgP. See “Discussion.”

<sup>b</sup> SAH titration to the MgP-saturated SyChlM; 50 µM SyChlM was mixed with 100 µM MgP and then titrated with 0.60 mM SAH.

FIGURE 4. Spectrophotometric methyltransferase assay. The reaction mixture was measured by recording absorbance at 265 nm for 5 min. Data are presented as the mean ± S.E. of at least three independent experiments.

FIGURE 5. ITC analysis of ligands binding. A, titration of SyChlM<sup>Wt</sup> with MgP. The top panel shows the heat response to injections, and the bottom panel shows the integrated heats of each injection and the fit to a single-site binding model. B, titration of SyChlM<sup>Wt</sup> with SAH. Top and bottom panels are the same as in A.
a strand order of 3-2-1-4-5-7-6, which is a characteristic feature of SAM–dependent methyltransferase (38, 39). The eight flanking α-helices are named sequentially in alphabetical order (αA to αH). In the SyChlM–SAM structure (Fig. 1B), electron density is missing for residues 168–188. In the SyChlM–SAH structure (Fig. 1C), residues 169–178 are observed as an α-helix (αG), which protrudes from the core of the protein. It is of note that the packing arrangements of SyChlM–SAM and SyChlM–SAH are in different space groups. Whereas SyChlM–SAM is a monomer in one asymmetric unit (ASU), there are two SyChlM–SAH monomers per ASU related by noncrystallographic symmetry. A superimposition of SyChlM in these three monomeric forms reveals that their major differences lie in the orientation of αG and the N-terminal sequence (including αA and the loop between αB and αC (Fig. 1D). These differences correlate with the increased B factors for these regions, indicating their conformational flexibility.

**SAM/SAH Binding Pocket—**Sequence alignment of ChlMs from different species reveals a DXGCGXG motif after β1 and a conserved aspartic acid at the end of β2 (Fig. 2A). These are the two conserved structural elements that are required for cofactor SAM binding among the Class I methyltransferases (39). In both SyChlM–SAM and SyChlM–SAH structures, the electron density for the cofactor SAM/SAH is well defined, and no significant differences were observed between SAM and SAH except the S-methyl group (Fig. 2B). Due to its higher resolution, the SyChlM–SAM structure is used to describe the SAM/SAH binding pocket. This pocket is covered by an N-terminal arm consisting of αA and αB (Figs. 1B and 2C) and is shaped by residues from helices αA and αC and loops β1–αD, β2–αE, β3–β4, and β4–αF. The SAM binding in this pocket is stabilized by interactions as follows (Fig. 2D). The adenine ring is surrounded by hydrophobic residues Val-12, Phe-16, Ile-92, Leu-121, and Val-136. Three ring nitrogen atoms, N1, N6, and N7, form a hydrogen-bond network with the carboxylic group of Asp-120, the backbone amine of Leu-121, and the phenolic hydroxyl group of Tyr-140. The ribose 2'- and 3'-hydroxyl groups form hydrogen bonds with the side-chain carboxyl group of Asp-91. The amine group of the SAM methylionine moiety forms a hydrogen bond with the backbone carbonyl group of Leu-134, and the carboxylic group interacts directly with the Nε2 nitrogen of His-44. The water-mediated hydroxyl bonds are observed between the methionine moieties and residues Asp-68, Ala-69, Cys-71, Val-73, Ser-75, and Leu-134.

**Substrate Binding Site—**We further tried to obtain crystals of SyChlM complexed with MgP by co-crystallization and soaking methods, but the solved structure revealed no bound MgP irrespective of the presence or absence of SAM/SAH. Based on the structure of SyChlM bound to SAM/SAH, we analyzed the substrate binding site of SyChlM by in silico docking using the program AutoDock Vina (29). The protein coordinates of the SyChlM–SAH structure were used for the docking model because the SyChlM–SAM structure misses αG, which protrudes from the protein core in the SyChlM–SAH structure and leaves the substrate binding site open (Fig. 3A). This site is a distinct deep hydrophobic cleft with a dimension of ~20 × 11 × 15 Å³. The MgP-docked model showed that the substrate MgP is bound by helices αA, αB, αC, and αG and loops β4–αF, β5–αG, αG–αH, and β6–β7 (Fig. 3B). The predicted interactions between MgP and SyChlM are summarized as follows: hydrophobic interactions between the tetrapyrrrole rings of MgP and the side chains of residues Tyr-15, Phe-16, Trp-24, Ile-27, Val-36, Ile-40, Ile-138, Leu-174, Phe-219, Tyr-220, and Tyr-221; hydrogen bonds between the propionate groups of MgP and the substrate.

### Table 3: Structural homologs of SyChlM identified by DALI with Z score above 18

| Protein | PDB  | Z*  | r.m.s.d. | Lali | Identity | Methyl acceptor | Reference |
|---------|------|-----|----------|------|----------|-----------------|-----------|
| NodS N-methyltransferase (Bradyrhizobium japonicum) | 3OFK | 20.8 | 2.3 | 184 | 11 | —NH₂ | 42 |
| tRNA (cmoU34)-methyltransferase, CmoA (E. coli) | 4GK | 19 | 2.9 | 193 | 15 | NA* | 43 |
| YeSo (Haemophilus influenzae) | 1IK8 | 19 | 2.7 | 189 | 16 | NA | 44, 53 |
| Phosphonate O-methyltransferase, Dhpl (Streptomyces lividus) | 3OU2 | 18.9 | 2.6 | 179 | 16 | —OH | 45 |
| Putative RNA methyltransferase PH1948 (Pyrococcus horikoshii) | 1NY7 | 18.8 | 2.1 | 161 | 16 | —NH₂ | 46 |
| Aclacinomycin-10-hydroxylase, RdmB (Streptomyces purpurascens) | 1QZ | 18.3 | 2.6 | 182 | 15 | NA | 47 |
| Nicotinamide N-methyltransferase (Homo sapiens) | 3ROD | 18.3 | 2.8 | 196 | 17 | —NH₂ | 48 |
| cyclopropane synthetase 1 (Mycobacterium tuberculosis) | 1KPG | 18.2 | 2.7 | 195 | 14 | —CH₂ | 49 |
| Geranyl diphosphate 2-C-methyltransferase (Streptomyces lasaliensis) | 4FS6 | 18.2 | 2.8 | 191 | 13 | —CH₂ | 50 |
| Glycine N-methyltransferase (Rattus norvegicus) | 1NRH | 18.2 | 2.9 | 192 | 19 | —NH₂ | 51 |
| Phosphoethanolamine N-methyltransferase (Plasmodium falciparum) | 3UJ8 | 18.1 | 3 | 194 | 13 | —NH₂ | 52 |

* Strength of structural similarity in S.D. above expected.
* r.m.s.d. of superimposed Co atoms.
* Lali, number of equivalent residues.
* Percentage of sequence identity over equivalent position.
* NA, not applicable.
MgP and the hydroxyl groups of residues Tyr-28 and Tyr-221; electrostatic interaction between the C13 propionate group of MgP and the Ne2 nitrogen of His-139. The aforementioned residues are conserved in ChlMs from different species with one exception, the purple bacterium *Rhodobacter*, suggesting a conserved mode of MgP binding (Fig. 2A). The active site of *Rhodobacter* ChlM (hereafter referred to as *RhBchM*) is addressed under “Discussion.”

Tyr-28 and His-139 Are Essential for Catalysis—The proximity of the C13 propionate group of MgP to the hydroxyl group of Tyr-28, the Ne2 nitrogen of His-139, and the S-methyl group of SAM (Fig. 3B) suggests that Tyr-28 and His-139 participate in the methyl transfer reaction. To address their potential involvement in catalysis, we mutated these two residues individually. Mutation of Tyr-28 to phenylalanine led to inclusion body formation during bacterial expression, and therefore, the activity of the Y28F mutant could not be assayed. The Y28A, H139N, H139Q, and H139A mutants lost >80% methyltransferase activity as measured in the described in vitro assay (Fig. 4). Because these two residues are also involved in MgP binding as suggested by in silico docking, the lost methyltransferase activity of the mutants could be a result of an impaired substrate affinity. To test this possibility, we measured MgP binding by ITC (Fig. 5A). The apparent $K_d$ value between the wild type *SyChlM* and MgP is 6.0 $\mu$M, and all mutants have comparable $K_d$ values as the wild type protein. These results indicate that the Y28A, H139N, H139Q, and H139A mutants largely retain the MgP binding affinity. Another explanation for the lost methyltransferase activity is that these mutations might disrupt the integrity of the cofactor binding pocket. Due to the instability of SAM (40), SAH was used for ITC analysis to evaluate the interaction between *SyChlM* and its cofactor (Fig. 5B and Table 2). The apparent $K_d$ value between the wild type *SyChlM* and SAH is 1.4 $\mu$M, and all mutants have increased $K_d$ values of ~2–22-fold. Whereas these mutations resulted in reduced cofactor binding affinity to various extents, no significant difference was observed in the methyltransferase assay in which the activity of each mutant was reduced to $0 \sim 20\%$ that of the wild type (Fig. 4). These results indicate that Tyr-28 and particularly His-139 are implicated in cofactor binding, but neither residue is dispensable. Taken together, we conclude that their essential role is in methyl transfer.

DISCUSSION

Structural Comparison—Although the structure of *SyChlM* is highly similar to a few members of the Class I SAM-dependent methyltransferases (Table 3), none of them catalyzes carboxyl methylation. The structures identified by Dali search (41) with $Z > 18$ include: Nods N-methyltransferase (42), tRNA (cmoS5U34)-methyltransferase (43), YecO (44), phosphonate O-methyltransferase Dhpl (45), a putative RNA methyltransferase (46), aclacinomycin-10-hydroxylase (47), nicotinamide N-methyltransferase (48), cyclopropane synthetase 1 (49), geryl diphosphate 2-C-methyltransferase (50), glycine N-methyltransferase (51), and phosphoethanolamine N-methyltransferase (52). These 11 structures can be superimposed to *SyChlM* with r.m.s.d. values ≤3 Å (Fig. 6A). Among them five catalyze N-methylation (42, 46, 48, 51, 52), two catalyze C-methylation (49, 50), two catalyze the conversion of SAM to carboxy-SAM (43, 44, 53), one is a hydroxylase (47), and the only O-methyltransferase Dhpl methylates the phosphonic acid group (45). Interestingly, like *SyChlM*, Dhpl has an unusual insertion that encompasses a helix called the “capping helix” (topologically equivalent to αG in *SyChlM*) (Fig. 6B). This insertion between β5 and α6 (equivalent to αH in *SyChlM*) of Dhpl plays a critical role in modulating substrate binding (45). It is likely that a similar binding mechanism can be applied to account for the formation of *SyChlM*-MgP complex in which the motion of αG allows conformational change during MgP recognition. This also suggests that the flexibility of the αG region together with the N-terminal region and the DXGCGXG motif may reduce the homogeneity of apo*SyChlM* molecules in the crystals and cause poor diffraction. Thermodynamic measurements using ITC reveal that both MgP and SAH can result in decreased motion of *SyChlM* as reflected by the decreased entropy values (Table 2). Thus, flexibility (as inferred from Fig. 1D) may be an intrinsic property required for binding the cofactor SAM or the substrate MgP.

Catalytic and Regulatory Mechanism—The SAM-dependent methyltransferases utilize an $S_2$ replacement mechanism in which the nucleophile (methyl acceptor) directly attacks the S-methyl of SAM (methyl donor) (39). Based on structures of *SyChlM*, the nucleophile, the carboxyl oxygen of the C13 propionate group, is likely to be properly positioned by Tyr-28 and...
His-139. Such an arrangement of the active site might facilitate a direct methyl transfer from the methyl donor to the negatively charged acceptor. It is likely that His-139 serves as the base responsible for deprotonation of the carboxyl of MgP; thus, the negatively charged carboxyl attacks the high energy S-CH3 bond. During this process Tyr-28 helps to position the carboxyl in the proximity for efficient nucleophile attack. The catalytic role of the Tyr-His pair is reflected by the loss of methyltransferase activity if either residue is mutated. This pair is conserved across the species with the exception of RhBchM in which the corresponding residues are Arg-43 and Tyr-135, which compensates for these structural changes compared with other ChlMs (Fig. 7A). Upon the completion of the methyl transfer, the resulting monomethyl ester of the product MgPME might cause a steric interference with the surrounding residues, thus pushing MgPME away from the substrate binding site.

A thiol-based redox regulation of the chlorophyll branch has been identified (5). The redox status of the cysteine residues in Arabidopsis ChlM has been demonstrated to be controlled by the NADPH-dependent thioredoxin reductase C (54). Whereas the three cysteines are buried within the protein interior in SyChlM-SAM and SyChlM-SAH structures, we suggest that the conserved cysteine of the DXGCGXG motif (Cys-71 in SyChlM) on the loop β1-αD (Fig. 2D) may undergo a large conformational change when no cofactor is present and that oxidation of this cysteine may disturb the cofactor binding so as to negatively regulate the methyltransferase activity.

The cofactor binding and the substrate binding sites of SyChlM are possibly controlled by two flexible regions (the N-terminal arm and the αG arm), respectively (Fig. 7B). Each arm may independently undergo a conformational change upon binding one ligand without obstructing the binding of the other one. This model would explain the random binding of the cofactor and the substrate to SyChlM as shown previously (17) and also by our ITC experiments, in which saturation of SyChlM with one ligand did not prevent binding to the other one (Table 2). Notably, SyChlM saturated with SAH appears less disordered during MgP binding as indicated by the least decreased entropy. We suggest that this is due to stabilization of the N-terminal arm of SyChlM in the presence of SAH. Furthermore, the stimulatory effect of ChlH (the protoporphyrin binding subunit of magnesium chelatase) on ChlM could possibly be achieved through the interaction between ChlH and the N-terminal arm of MgPME (the protoporphyrin binding subunit of magnesium chelatase). The N-terminal arm of MgPME might cause a steric interference with the surrounding residues, thus pushing MgPME away from the substrate binding site.

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