PhoU proteins are known to play a role in the regulation of phosphate uptake. In *Thermotoga maritima*, two PhoU homologues have been identified bioinformatically. Here we report the crystal structure of one of the PhoU homologues at 2.0 Å resolution. The structure of the PhoU protein homologue contains a highly symmetric new structural fold composed of two repeats of a three-helix bundle. The structure unexpectedly revealed a trinucletidal and a tetranuclear iron cluster that were found to be bound on the surface. Each of the two multinuclear iron clusters is coordinated by a conserved E(D)XXXD motif pair. Our structure reveals a new class of metalloprotein containing multinuclear iron clusters. The possible functional implication based on the structure are discussed.

Inorganic phosphate (P_i) uptake is of fundamental importance in the cell physiology of bacteria because P_i is required as a nutrient. *Escherichia coli* has developed a P_i acquisition system that allows the assimilation of P_i via a variety of systems. Two distinct systems for the uptake of P_i have been described: the low affinity phosphate inorganic transporter and the high affinity phosphate-specific transporter (PstSCAB) (1, 2). When the preferred P_i source is in excess, it is taken up by the phosphate inorganic transporter. When the extracellular P_i concentration is less than ~4 μM, the synthesis of the high affinity transporter is induced, and P_i is taken up by PstSCAB. The PstSCAB transporter belongs to the superfamily of ATP-binding cassette transporters and is encoded by the *pst* operon (3, 4). This operon contains five genes that are transcribed counterclockwise in the following order: *pstS*, *pstC*, *pstA*, *pstB*, and *phoU*. PstS is a periplasmic P_i-binding protein, and PstC and PstA are integral membrane proteins that mediate the translocation of P_i through the inner membrane. PstB is an ATPase that energizes the transport. The *Pst* operon is part of the phosphate (PHO) regulon that consists of 31 genes arranged in eight different operons in *E. coli* (5, 6). The genes and operons of the PHO regulon are co-regulated by a two-compartment system composed of the regulatory proteins PhoB and PhoR. When the concentration of P_i in the medium falls below ~4 μM, the sensor protein PhoR phosphorylates PhoB, and the phosphorylated PhoB binds to the PHO boxes in the control region of *Pst*, recruiting the σ^70_ subunit of the RNA polymerase and initiating transcription. When the P_i concentration in the medium is in excess, the PHO regulon is repressed. Repression of the PHO regulon requires not only an excess concentration of extracellular P_i, but also the intact PstSCAB transporter and the PhoU protein.

The *phoU* gene encodes a polypeptide of molecular mass ~27,000 Da. Although it is located in the *pst* operon, the encoded PhoU protein does not seem to participate in P_i transport (4). Besides its role as a repressor of the PHO regulon, PhoU was also reported to be involved in intracellular P_i metabolism (presumably related to the synthesis of ATP) (7).

Currently, there is a total of 44 entries of annotated PhoU protein homology sequences in the Protein Information Resource data base (www.pir.georgetown.edu). Only the *E. coli* PhoU protein was reported to be purified as a protein aggregate, and no structures for this protein family are available (8). Two PhoU homologues have been identified in *Thermotoga maritima*, and they are *Tm1260* and *Tm1734* (www.tigr.org). The *Tm1260* gene is located in a gene cluster similar to the *PhoU* gene in *E. coli*, and *Tm1734* is located in a gene cluster containing several hypothetical genes. The detailed molecular function for both PhoU homologues in *T. maritima* is unknown. However, these two PhoU homologues are highly sequence-related (25% identity and 54% homology). As part of the Berkeley Structural Genomics Project (www.strgen.org), we purified and solved the crystal structure of one of the two PhoU homologues (*Tm1734*) from *T. maritima* (*Tm_PhoU2*) at 2.0 Å resolution. This structure reveals a new class of metalloproteins containing multinuclear iron clusters.

**EXPERIMENTAL PROCEDURES**

**Cloning, Protein Expression, and Purification—**The *Tm_PhoU2* (gi number 49832311) gene was amplified by PCR using *T. maritima* genomic DNA template and primers designed for ligation-independent cloning (9). The amplified PCR product was prepared for vector insertion by purification, quantitation, and treatment with T4 DNA polymerase (New England Biolabs, Beverly, MA) in the presence of 1 mM dTTP. The prepared insert was annealed into the ligation-independent cloning expression vector pET21a (Novagen, Madison, WI) that expresses the cloned gene with an N-terminal His-tagged tobacco etch virus cleavage sequence, and transformed into chemical competent DH5α to obtain fusion clones. The seleno-l-methionine (Se-Met) isoform of *Tm_PhoU2* protein was expressed in *E. coli* B834 (DE3). Cell paste was prepared with Studier's autoinduction method (2).

For protein purification, cell paste was resuspended in buffer A...
containing 50 mM HEPES, pH 7.0, 100 mM NaCl, supplemented with Roche protease inhibitors (Roche Applied Science). Cells were opened with a Microfluidizer (Microfluidics, Newton, MA). After centrifugation, the cleared cell lysate was loaded on to a 5-ml HiTrap MC column (Amersham Biosciences). The column was first washed with 10 column volumes of buffer A with 10 mM BME followed by 10 column volumes of buffer B containing 50 mM HEPES, pH 7.5, 1 mM NaCl, and 10 mM BME. The target protein was eluted with buffer C containing 50 mM HEPES, pH 7.5, 100 mM NaCl, 10 mM BME using an imidazole gradient from 40 to 300 mM in 20 column volumes. Target protein was eluted at ~200 mM imidazole concentration. The peak fractions were pooled together and diluted 10-fold with buffer A. The diluted protein solution was then loaded onto a 5-ml HiTrapQ anion exchange column (Amersham Biosciences), and eluted with a linear NaCl gradient from 0 to 0.5 M NaCl in 20 column volumes. Protein target was eluted at ~200 mM imidazole concentration. The peak fractions were then concentrated to 10 mg/ml in 20 mM HEPES, pH 7.0, 300 mM NaCl, 10 mM BME for crystallization.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)—

The initial selenium atoms were determined using the HySS program (12). A total of 14 sites in the asymmetric unit were identified using the multiple wavelength anomalous dispersion data at 3.0 Å resolution, even though the protein monomer has only 12 seleniums. A calculation of the electron density map confirmed P3_121 to be the correct space group. Phasing and phase extension to 2.0 Å resolution by density modification were done with the crystallography NMR software program (13). The resultant electron density map was readily interpretable. The initial model was built with program O (14). The initial model building confirmed that the 14 sites found by HySS include 11 ordered selenium sites and three strong metal sites. Further experiments revealed that the three metal sites belong to two multinuclear iron clusters. Model refinement was carried out with the REFMAC5 program (15). The TLS method was utilized for structural refinement. At the final stage of refinement, iron clusters were built into the structural model, and water molecules were added. The final model of the Tm_PhO2 protein was refined to Rfree = 25.5% and R-factor = 21.7% at 2.0 Å resolution and contained residues 2-226, seven iron ions, one nickel ion, one calcium ion, two acetate ions, and 78 water molecules. The final refinement statistics are given in Table I.

RESULTS

The crystal structure of Tm_PhO2 was determined by multiple-wavelength anomalous dispersion using Se-Met protein. The protein crystallized in space group P3_121 and contained one monomer in the asymmetric unit. The final model of the Tm_PhO2 protein was refined to Rfree = 25.5% and R-factor = 21.7% at 2.0 Å resolution and contained residues 2-226, seven iron ions, one nickel ion, one calcium ion, two acetate ions, and 78 water molecules. The N-terminal methionine and nine C-terminal residues were not included in this model because of poor electron density. PROCHECK was used to assess the stereochemistry of this model (16). It shows 95.7% of the residues in the most favored regions of the a-b plot, and no outliers were present. The r.m.s. deviations of bond lengths and bond angles from the standard geometry were 0.012 Å and 1.17°, respectively.

Crystallographic data for Tm_PhO2 Se-Met crystals were collected at Advanced Light Source Beamline 5.0.2 (Lawrence Berkeley National Laboratory, Berkeley, CA) at 100 K with a Quantum 315 charge-coupled device detector and processed with HKL 2000 (11). The crystals belong to the space group P3_121 or P3_21 with approximate unit cell dimensions of a = b = 90.677 Å, c = 45.265 Å. The crystals contain one monomer/ asymmetrical unit corresponding to a solvent content of 44.7%. Data statistics are summarized in Table I. The data for iron peak wavelength and nickel peak wavelength were also collected at Advanced Light Source Beamline 5.0.2.

TABLE I

| Data collection | Selenium peak | Selenium inflection | Selenium remote (H) | Iron peak | Nickel peak |
|-----------------|---------------|---------------------|--------------------|-----------|------------|
| a = b = 90.677 c = 45.255 | 20-2.0 | 20-2.0 | 20-2.5 | 20-2.5 |
| Space group | P3_121 | 0.9797 | 0.9796 | 0.9600 | 1.7433 | 1.4879 |
| Wavelength (Å) | 40-300 | 312.197 | 209.326 | 55.210 | 122.249 |
| No. of reflections | 208.625 | 14.613 | 14.583 | 14.616 | 7.661 |
| Completeness (%) (final) | 98.71 (91.8) | 99.1 (95.4) | 98.9 (93.3) | 99.3 (94.9) | 97.2 (79.0) |
| Rmerge-R-factor | 0.043 | 0.012 | 0.012 | 0.012 | 0.012 |
| No. of protein atoms (non-hydrogen) | 1804 | 7 | 1 | 1 | 2 |
| Iron ions | 78 | 95.7 | 4.3 | 0 | 0 |
| Nickel ion | 95.7 | 4.3 | 0 | 0 | 0 |
| Calcium ion | 1 | 1 | 1 | 1 | 1 |
| Acetate ion | 2 | 2 | 2 | 2 | 2 |
| Water molecules | 78 | 78 | 78 | 78 | 78 |
| r.m.s. deviations from ideality | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 |
| Bonds (Å) | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 |
| Angles (degrees) | 1.17 | 1.17 | 1.17 | 1.17 | 1.17 |
| Residues in most favored regions (%) | 95.7 | 95.7 | 95.7 | 95.7 | 95.7 |
| Residues in generously allowed regions (%) | 0 | 0 | 0 | 0 | 0 |
| Residues in additional allowed regions (%) | 4.3 | 4.3 | 4.3 | 4.3 | 4.3 |
| Residues in disallowed regions (%) | 0 | 0 | 0 | 0 | 0 |
Crystal Structure of PhoU Protein

conformations, 19.6% of the amino acids in turns, and 3.65% of the amino acids in β-strand conformations (Fig. 1A). The overall structure of Tm_PhoU2 is composed of a six-helix bundle and a short two-stranded anti-parallel β-sheet attached at the C terminus. The six-helix bundle is composed of two-three-helix bundles connected by a 10-residue loop. The N-terminal three-helix bundle is composed of H1A, H2A, and H3A, and the C-terminal three-helix bundle is composed of H1B, H2B, and H3B. The interface between these two three-helix bundles is mediated through H1A-H1B and H3A-H3B and contains mainly hydrophobic residues.

The two three-helix bundles are structural repeats. The superposition of the two structural repeats of the three-helix bundle results in an r.m.s. deviation of 1.45 Å over 79 equivalent Ca atoms (Fig. 1B). The major difference between these two repeats is that H1A is 12 amino acids longer than H1B. The other two α-helices in the structural repeats can be well superimposed. The superposition of these two repeats also revealed that the protein sequences in these two repeats are related. In fact, the two iron clusters identified in this structure are found at the equivalent positions of these two structural repeats and coordinated by the same E/D/XXXD motif repeats (discussed below).

Tm_PhoU2 Structure Represents a New Structural Fold—The DALI program was used for a structural homology search (17). No match was found for the complete model of the Tm_PhoU2 protein. A search with the whole model led to structural homologues to the first or second repeat. Not surprisingly, a search with the two structural repeats of the three-helix bundle led to many similar structural homologues. The structures with the highest Z-scores are the STAT (signal transducers and activators of transcription) protein fragment (Protein Database accession number 1UUR, Z-score 11.0, r.m.s. deviation 2.8 Å over 103 Ca atoms with 7% sequence identity), the α-spectrin fragment mutant (Protein Database accession code 1CUN, Z-score 10.8, r.m.s. deviation 2.2 Å over 105 Ca atoms with 10% sequence identity), and the heat shock cognate 71-kDa fragment (hsc70) bag-family (Protein Database accession code 1HX1, Z-score 10.7, r.m.s. deviation 2.2 Å over 97 Ca atoms with 11% sequence identity). These proteins have very different sequences and molecular functions. They do not appear to be remotely related to the PhoU protein family. To the best of our knowledge, the structural fold composed of two repeats of a three-helix bundle for Tm_PhoU2 is unique and represents a novel structural fold in the protein data bank.

Two Multinuclear Metal Clusters—Metal binding of the Tm_PhoU2 protein was first suggested by the Se-Met phased protein structure of Tm_PhoU2, which was later confirmed using 2.5 Å resolution data collected at 1.7433 Å (iron peak wavelength) and 1.4879 Å (nickel peak wavelength) (Fig. 1A). The overall difference Fourier maps calculated using 2.5 Å resolution data collected at 1.7433 Å (iron peak wavelength) and 1.4879 Å (nickel peak wavelength) (Fig. 1A, B and C). Iron is a strong anomalous scatterer at both wavelengths (fobs ≈ 3.99 at 1.7433 Å and −2.99 at 1.4879 Å). The contribution of nickel is weak at 1.7433 Å (fobs ≈ −0.64) but strong for the wavelength of 1.4879 Å (fobs ≈ −3.92). The identification of nickel ion at the N-terminal iron cluster is based on the stronger anomalous difference peak at 1.4879 Å rather than that at 1.7433 Å, when both maps are contoured at the same level (Fig. 2B).

Trinuclear Iron Cluster—The trinuclear iron cluster is bound on the protein surface of the C-terminal repeat, coordinated by residues from H2B and H3B (Figs. 1C and 2C). All three iron ions (Fe-6, −5, and −7) in this cluster are well ordered and form a nearly isosceles triangle with a distance between Fe-5 and -6, Fe-5 and -7, and Fe-6 and -7 of 3.82 Å, 3.69 Å, and 6.68 Å, respectively. In the trinuclear cluster, Fe-5 can be described as a core ion and is partially buried inside the protein surface between H3B and H2B, coordinated by the terminal carboxylates of four conserved acidic residues: Asp152 and Asp156 from H2B and Glu191 and Asp195 from H3B. Fe-6 and -7 can be described as adjacent ions with Fe-6 bound on top of H3B, coordinated to Glu191 and Asp195, and Fe-7 bound on top of H2B, coordinated to Asp152 and Asp156. Toward the solvent region, the trinuclear iron cluster is internally capped by a modeled acetate ion with two oxygens coordinated by all three iron ions. The acetate ion was tentatively modeled based on the [Fobs − Fcalc] difference density map and the geometry of the trinuclear iron cluster. Although this difference density was modeled as an acetate ion, no acetate ion was used during purification and crystallization steps, and we are not sure about the actual identity of this difference density. A similar example for this modeling is the crystal structure of a ribonucleotide reductase R2 protein complexed with an oxo-centered trinuclear iron cluster, in which an acetate ion was found to coordinate two of the ions in the oxo-centered trinuclear iron cluster (18). Besides the modeled acetate group and protein residues, seven water molecules were also found to be involved in the coordination of the trinuclear iron cluster, with one water binding to Fe-5 and three waters each binding to Fe-6 and -7. The overall chemical environment around the trinuclear iron cluster is nearly symmetric. Fe-5 has a pentagonal bipyramidal coordination to seven oxygens. There are five equatorial oxygens: one from a well ordered internal water, two from the acetate oxygens, and two from the carboxyl oxygens of Asp152 and Glu191. The two axial oxygens for Fe-5 are from the carboxylates of Asp156 and Asp195. Among the five equatorial oxygens, four (except the internal water) are bridging oxygens between core iron Fe-5 and the two adjacent ions Fe-6 and -7. Fe-6 also has a pentagonal bipyramidal coordination to seven oxygens, in which the five equatorial oxygens are two water molecules, two bridging oxygens to Fe-5 (one from the acetate oxygen, another from the carboxyl oxygen of Glu191), and another carboxyl oxygen from Glu191. The two axial oxygens are one water molecule and one carboxyl oxygen of Asp195. Fe-7 has a slightly distorted octahedral coordination to six oxygens, which are three water molecules, two bridging oxygens to Fe-5 (one from acetate oxygen, and another from the carboxyl oxygen of Asp152), and one carboxyl oxygen from Asp156. Unlike Glu191, where both carboxyl oxygens are coordinated to Fe-6, Asp152 has only one carboxyl oxygen coordinating to Fe-7. The other carboxyl oxygen of Asp152 forms a hydrogen bond with the internal water that coordinates Fe-5, possibly stabilizing the internal water.
Tretranuclear Metal Cluster—The tetranuclear iron cluster is bound on the protein surface of the N-terminal repeat coordinated by residues from H2A and H3A (Figs. 1C and 2D). The tetranuclear cluster includes four metal ions: nickel, Fe-2, -3, and -4, in which nickel and Fe-2 and -3 form a similar triangle as the Fe-6–5–7 trinuclear cluster. Here, Fe-2 represents the core ion, whereas nickel and Fe-3 represent the adjacent ions similar to Fe-7 and -6, respectively. An acetate ion was also modeled into a similar position as that of the C-terminal cluster. This acetate ion is less well defined, indicated by its high B factors (average ∼70 Å²). Again, we are not sure about the actual identity of the difference density, which we modeled as an acetate ion. The nickel ion has a higher B factor compared with other iron ions (68 versus 30–47 Å² for iron ions), indicating that this metal ion is more mobile than other iron ions. Furthermore, it is possible that the nickel ion in this structure could be a result of an ion exchange between nickel and iron during protein purification because a nickel affinity column was used for purification.

The coordination for this tetranuclear iron cluster involves seven water molecules and eight protein residues including Glu⁴⁹, Glu⁵⁰, Asp⁵³, and Glu⁵⁶ from H2A and Glu⁹¹, Glu⁹⁴, Asn⁹², and Asp⁹⁵ from H3A. Fe-2 is partially buried inside the protein surface and is coordinated by four conserved residues: Glu⁴⁹ and Asp⁵³ from H2A and Glu⁹¹ and Asp⁹⁵ from H3A (Figs. 1C and 2D). Overall, Fe-2 has an octahedral coordination to six oxygens: one from an internal water molecule, one from the acetate oxygen, and four from the carboxyl oxygens of Glu⁴⁹, Asp⁵³, Glu⁹¹, and Asp⁹⁵. Here, Glu⁴⁹ and Glu⁹¹ provide the bridging oxygens. Fe-3 has a distorted pentagonal bipyramidal coordination to seven oxygens: two from water molecules, one from the acetate oxygen, and four others from the terminal oxygens of Glu⁹¹, Asp⁹⁵, and Asn⁹². Nickel has coordination to six oxygens: two from water molecules, two from carboxylates of Glu⁴⁹, and two others from carboxylates of Asp⁵³ and Glu⁵⁶. Because the nickel has a higher B factor, this coordination of the nickel ion shown is tentative. The extended iron ion, Fe-4, is coordinated by both H2A and H3A. Fe-4 has a slightly distorted octahedral coordination to six oxygens, three of which are from water molecules and three others from the carboxyl oxygens of Glu⁴⁹, Glu⁵⁶, and Glu⁹¹. Here, one water molecule and another carboxyl oxygen of Glu⁹¹ act as bridging oxygens between Fe-3 and -4.

E/DXXXD Motif—A conserved motif was identified in the PhoU family by identifying the protein residues that coordinate the N- and C-terminal iron clusters in the Tm_PhoU2 structure. A total of four repeats of the E(D)XXXD motif can be identified in the PhoU family (Fig. 3). For the first N-terminal repeat, this motif can be extended to EXXXDXXE(D). For the second and the fourth repeat, the motif is restricted to EXXXD, and for the third repeat, the motif is restricted to DXXXD. The four motif repeats can be grouped into two motif pairs. The two N-terminal motif repeats form the N-terminal motif pair and constitute the N-terminal tetranuclear iron cluster binding site, whereas the two C-terminal motif repeats form the C-terminal motif pair and constitute the C-terminal trinuclear iron cluster binding site. Both sites are located at the equiva-

Fig. 1. Overall structure of Tm_PhoU2. A, ribbon diagram of the Tm_PhoU2 monomer. The N-terminal three α-helix bundle is labeled as H1A, H2A, and H3A in light blue. The C-terminal three α-helix bundle is labeled as H1B, H2B, and H3B in cyan. The two C-terminal β-strands are labeled as B1 and B2 in red. Iron ions are shown as spheres in green, and the nickel ion is in pink. The N-terminal and C-terminal iron clusters are indicated. B, stereo view of the superposition of the two structural repeats. The N-terminal repeat is shown in red, and the C-terminal repeat is in green. The conserved residues in the E(D)XXXD motif pair are shown as sticks. C, left panel, an electrostatic surface presentation of the Tm_PhoU2 bound by two iron clusters; right panel, a close-up view of the electrostatic surface region of the trinuclear (top) and tetranuclear (bottom) iron cluster binding sites.
lent position of the two structural repeats, and conserved carboxyl residues from each motif pair form a highly acidic surface patch. In the coordination between each of the two E(D)XXXD motif pairs and their bound multinuclear iron cluster, all four conserved carboxyl residues in each motif pair are involved in coordination to the core iron ion. The two N-terminal conserved residues (Glu or Asp) provide the bridging oxygens and symmetrically bind to the core iron, whereas the two conserved C-terminal residues (Asp) provide each of its carboxyl oxygens coordinating the core iron ion and an adjacent iron.

Dimeric Structure—It is not clear whether the Tm_PhO2 protein functions as a monomer or as an oligomer. Gel-filtration chromatography indicates a protein size between a dimer and a monomer (~40 kDa). In the crystal, two Tm_PhO2 monomers form a dimer through the crystallographic dyad, resulting in a 12-helix bundle (Fig. 4A). The four helices bound with clusters in one monomer (H2A, H3A, H2B, and H3B) are parallel with their counterparts in another monomer and form
the dimer interface. There are a total of 64 interactions between the two monomers, including 30 hydrophobic interactions. A total of 5995 Å² surface area is buried between these two monomers, corresponding to 46% of the overall surface of the monomer. A crystal structure of the apo-PhoU protein from *Aquifex aeolicus*, recently solved in our laboratory in two different crystal forms, also shows a very similar dimer interface.3

The striking feature about the Tm_PhoU2 dimer is that a central pore is observed at the dimer interface (Fig. 4B). At both ends of this pore, a tetraneuronic iron cluster from one monomer and a trinuclear iron cluster from another monomer are close to each other and bind to the open acidic protein surface formed by conserved residues that may be functionally important.

**DISCUSSION**

The crystal structure of the Tm_PhoU2 protein revealed unexpected results. The Se-Met phased difference density map indicates that Tm_PhoU2 binds two metal clusters on the protein surface. The metal content was subsequently

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3 V. Oganesyan, N. Oganesyan, P. D. Adams, J. Jancarik, H. A. Yokota, R. Kim, and S.-H. Kim, unpublished results.
confirmed by the ICP-MS results and the anomalous difference density. The octahedral or pentagonal bipyramidal coordination for each individual iron ion in the two clusters is consistent with previously defined coordination geometry for the iron ion (18–20). The fact that a multinuclear iron cluster is coordinated by an EXXXD motif pair has never been described previously. However, an oxo-centered multinuclear iron cluster coordinated by protein-derived carboxylates has been structurally characterized in the ribonucleotide reductase R2 protein, although these carboxyl residues are not conserved (18). In ferritins, the formation of an iron core is crucial for iron storage, detoxification, and mobilization throughout all three kingdoms. All ferritins contain several conserved carboxylate residues that are clustered in a patch on the inside surface of the ferritin sphere. Mutation studies have been performed on some of these residues and resulted in diminished core formation. The carboxylates are thus believed to make up nucleation sites for the mineral core (21–25). Although we cannot completely rule out the possibility that the iron ion could be another metal, the evidence presented here strongly supports that the two multinuclear metal clusters in Tm_PhoU2 protein are multinuclear iron clusters. The nickel ion is likely to be the result of an ion exchange between iron and nickel during protein purification.

So far, structures reported for a trinuclear iron cluster or multinuclear iron cluster-binding proteins are only for proteins involved in iron transport and iron metabolism. This includes ferric ion-binding protein, a protein involved in iron uptake from the transferrin superfamily, which binds trinuclear oxoiron clusters in an open cleft with a conserved dityrosyl iron-binding motif (19–20), and the DpsA protein (Dps, DNA-protecting protein during starvation), a Dps-like ferritin family protein, which binds to iron clusters in a manner similar to that found in diiron-carboxyl oxygen-activating proteins (26). The Tm_PhoU2 structure defines a new class of metalloproteins that bind multinuclear iron clusters by its conserved E(D)XXXD motif pair in a manner not observed previously.

An intriguing feature about the Tm_PhoU2 structure is the highly symmetric nature in the protein sequence motifs, the structural fold, and the coordination between the protein and the multinuclear iron clusters. Such a symmetric coordination environment is only observed in small organic structures and is rarely found in protein structures. The Tm_PhoU2 structure bound by two multinuclear iron clusters gives the first example of how a protein uses its structural fold and conserved motifs to create a nearly symmetric coordination environment for multinuclear iron clusters.

The biological function of these carboxylate-coordinated multinuclear iron clusters bound to the Tm_PhoU2 protein is unknown. However, the structure of the Tm_PhoU2 protein suggests that the iron cluster binding for PhoU protein is likely to be conserved and functionally related. We speculate that the PhoU protein might use its conserved E(D)XXXD motif pair to recruit iron clusters, which may in turn act as a cofactor involved in unknown PhoU protein-related functions, such as P metabolism (7).

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