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The ybeY protein from *Escherichia coli* is a metalloprotein

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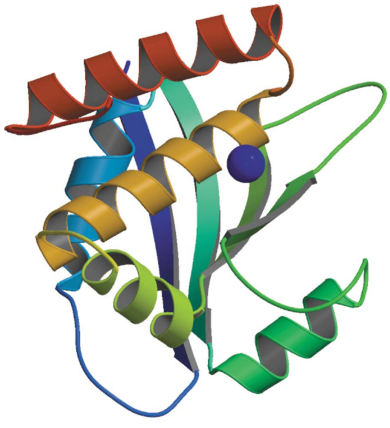

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The three-dimensional crystallographic structure of the ybeY protein from *Escherichia coli* (SwissProt entry P77385) is reported at 2.7 Å resolution. YbeY is a hypothetical protein that belongs to the UPF0054 family. The structure reveals that the protein binds a metal ion in a tetrahedral geometry. Three coordination sites are provided by histidine residues, while the fourth might be a water molecule that is not seen in the diffraction map because of its relatively low resolution. X-ray fluorescence analysis of the purified protein suggests that the metal is a nickel ion. The structure of ybeY and its sequence similarity to a number of predicted metal-dependent hydrolases provides a functional assignment for this protein family. The figures and tables of this paper were prepared using semi-automated tools, termed the *Autopublish* server, developed by the New York Structural GenomiX Research Consortium, with the goal of facilitating the rapid publication of crystallographic structures that emanate from worldwide Structural Genomics efforts, including the NIH-funded Protein Structure Initiative.

1. Introduction

The *Escherichia coli* hypothetical protein ybeY (SwissProt entry P77385; MW = 17 526 Da; 155 amino-acid residues) is the product of the gene *ybeY* and a member of the UPF0054 family (Bateman *et al.*, 2004). The sequence similarity of this protein to a number of predicted metal-dependent hydrolases would suggest a potential hydrolytic function (Tatusov *et al.*, 2001). Here, we report the structure of the ybeY protein determined by the multiple anomalous dispersion method (MAD) at a resolution of 2.7 Å (PDB code 1xm5). The structure provides new insights that help to functionally annotate this previously uncharacterized protein.

The figures and tables of this paper were prepared using semi-automated tools developed by the New York Structural GenomiX Research Consortium. This paper illustrates the ability of these tools to facilitate rapid publication of crystallographic structures that emanate from worldwide Structural Genomics efforts, including the NIH-funded Protein Structure Initiative.

2. Materials and methods

The coding sequence from the *E. coli ybeY* gene was cloned into the pet26b vector. A six-His tag was encoded at the C-terminus and was removed by proteolysis. The native protein and the selenomethionine-substituted protein were expressed in *E. coli* BL21 (DE3) cells and *E. coli* B834 cells, respectively. The proteins were purified to homogeneity by affinity chromatography on nickel-chelating Sepharose and subsequent gel filtrations were performed on a preparative Superdex-200 column. The *E. coli* protein (native and SeMet derivative) was concentrated to 15 mg ml^{-1} in the following buffer: 10 m*M* HEPES, 150 m*M* NaCl, 10 m*M* methionine, 10% glycerol, 1 m*M* DTT. Initial screening for crystallization conditions was performed by the hanging-drop vapour-diffusion method. Hampton matrix Index (Jancarik & Kim, 1991) was used and condition No. 82 yielded initial crystals. The reservoir (0.7 ml) for refined crystallization contained 20% PEG 3350, 0.2 *M* $MgCl_2$, 0.1 *M* Bis-Tris pH 6.5 and crystals were grown at 278 K. The protein drop

Table 1
Data-collection statistics.

Statistics in parentheses are for the highest resolution bin (2.7–2.8 Å).

		SeMet replacement		
	Native	Edge	Peak	Remote
Data collection	APS-19BM	NSLS-X29	NSLS-X29	NSLS-X29
Wavelength used (Å)	0.97919	0.97938	0.97919	0.96408
Data-collection temperature (K)	110	110	110	110
Space group	$P2_12_12_1$	$P2_12_12_1$		
Unit-cell parameters (Å, °)	a = 46.07, b = 119.61, c = 132.90, $\alpha = \beta = \gamma = 90$	a = 45.70, b = 119.05, c = 132.51, $\alpha = \beta = \gamma = 90$		
Resolution range (Å)	25.0–2.7	30.0–3.0	30.0–3.0	30.0–3.0
No. of unique reflections	19520	15084	15136	15109
Total No. of reflections	144379	89659	124244	108891
Redundancy	6.9	5.1	6.5	5.8
Completeness (%)	97.9 (99.5)	99.3 (94.3)	99.7 (97.8)	99.4 (94.3)
$R_{\rm merge}$†	0.059 (0.379)	0.117 (0.335)	0.098 (0.237)	0.120 (0.332)
$\langle I/\sigma(I)\rangle$	24.9 (4.2)	12.8 (3.8)	14.8 (5.2)	13.4 (4.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I(hkl)$.

(2 µl) was mixed with an identical volume from the reservoir. The crystals appeared after 2–3 d and reached a final size of 0.2 × 0.2 × 0.2 mm after one week. The crystallization conditions for the selenomethionine-substituted protein were the same as for the native protein.

Diffraction data for the native protein were collected to 2.7 Å resolution at the Advanced Photon Source beamline 19BM. Prior to data collection, crystals were transferred to a solution of mother liquor supplemented with 20% glycerol and flash-cooled to 100 K. The data were processed using the *HKL*2000 software suite (Otwinowski & Minor, 1997). The crystals exhibit diffraction consistent with space group $P2_12_12_1$. There are four molecules in the asymmetric unit, with unit-cell parameters a = 46.1, b = 119.6, c = 132.9 Å, a $V_{\rm M}$ value of 2.6 Å^3 Da^{-1} and a solvent content of 51.1%. In each molecule there are six selenomethionines with a mean phase error of about 20°.

A three-wavelength SeMet MAD experiment (0.97408, 0.97938 and 0.97919 Å) was performed at National Synchrotron Light Source beamline X29 and the structure was solved with the program *SOLVE* (Terwilliger & Berendzen, 1999). An initial model was automatically traced using the program *RESOLVE* (Terwilliger, 1999). The rest of the model was built manually with *O* (Jones *et al.*, 1991) and NCS restraints were used in the refinement by the program *CNS* (Brünger *et al.*, 1998). The final model contains 604 residues and four metal ions. The final data-collection statistics are shown in Table 1, phasing statistics in Table 2 and refinement statistics in Table 3. Atomic coordinates and structure factors have been deposited in the PDB and are available under accession code 1xm5.

X-ray fluorescence analysis was performed to detect the transition-metal content of the protein at National Synchrotron Light Source

Table 2
Phasing statistics from the program *SOLVE*.

	λ_1	λ_2	λ_3
R.m.s. anomalous $F_{\rm H}$ (%)	4.3	4.8	3.2
R.m.s. anomalous $F_{\rm H}/E$	1.5	1.7	1.5
	λ_1 *versus* λ_2	λ_1 *versus* λ_3	λ_2 *versus* λ_3
R.m.s. dispersive $F_{\rm H}$ (%)	3.5	6.7	3.2
R.m.s. dispersive $F_{\rm H}/E$	1.1	1.7	0.9
FOM	0.65 (*SOLVE*), 0.79 (*RESOLVE*)		

Table 3
Refinement statistics.

Refinement	
Resolution range (Å)	25.0–2.7
No. of reflections used for refinement	19520
No. of reflections used for $R_{\rm free}$	894
R factor†	0.234 (0.358)
$R_{\rm free}$‡	0.273 (0.363)
R.m.s. bonds (Å)	0.007
R.m.s. angles (°)	1.2
No. of non-H atoms	4777 plus 4 Ni^{2+} ions
Average B factor (Å^2)	73.2
R.m.s.d. B factor for bonded main-chain atoms (Å^2)	6.3
R.m.s.d. B factor for bonded side-chain atoms (Å^2)	9.3
R.m.s.d. B factor for angle main-chain atoms (Å^2)	10.2
R.m.s.d. B factor for angle side-chain atoms (Å^2)	13.9
Cruickshank DPI (Å)	0.40
Ramachandran plot (*PROCHECK*)	
Residues in most favored region	467 (87.8%)
Residues in additional allowed regions	59 (11.1%)
Disallowed regions	3 (0.6%)

† $R = \sum_h \big||F_{\rm o}(h)| - k|F_{\rm c}(h)|\big|/\sum_h |F_{\rm o}(h)|$. ‡ $R_{\rm free}$ was calculated using a 5% randomly selected subset of the total number of reflections.

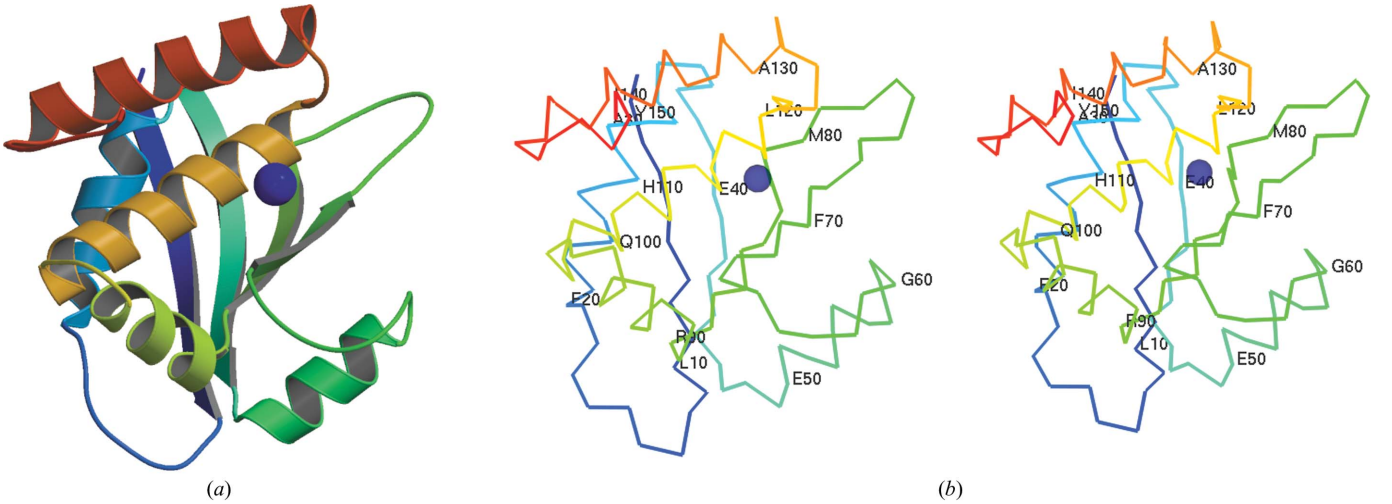

Figure 1
(*a*) Tertiary structure of the ybeY protein (chain *A* in asymmetric unit) prepared using *MOLSCRIPT* (Kraulis, 1991). (*b*) show a pair of stereo figures of the C$^\alpha$ trace with every tenth residue numbered. It is in the same orientation as (*a*).

beamline X9B according to established procedures (Chance *et al.*, 2004; Shi *et al.*, 2005). As outlined in the results, Ni was detected in the purified protein preparation and corresponding electron density was also observed in the crystal structure.

The figures and tables were prepared using the NYSGXRC *AutoPublish* web server.

3. Results and discussion

The structure of *E. coli* ybeY includes four identical proteins in an asymmetric unit. Quaternary structure prediction (Henrick & Thornton, 1998) suggests that this protein might exist as a homo-dimer. However, residue conservation was not observed on the hypothetical dimer interface to support this prediction. Thus, the oligomeric state of the ybeY protein cannot be confirmed without further experiments. The overall protein structure consists of six α-helices and four β-strands in a β-α-α-β-α-β-β-α-α-α fold (Fig. 1). The protein contains a conserved domain from the functionally uncharacterized protein family UPF0054 (pfam02130; Bateman *et al.*, 2004). There is a metal ion, most probably an Ni^{2+} ion, coordinated by NE2 atoms from residues His114, His118 and His124 within each protein subunit. The fourth ion-coordination site might be a water molecule, but this cannot be confirmed because of the relatively low resolution of the data. The nickel content of the purified protein used for crystallization was analyzed using quantitative X-ray fluorescence

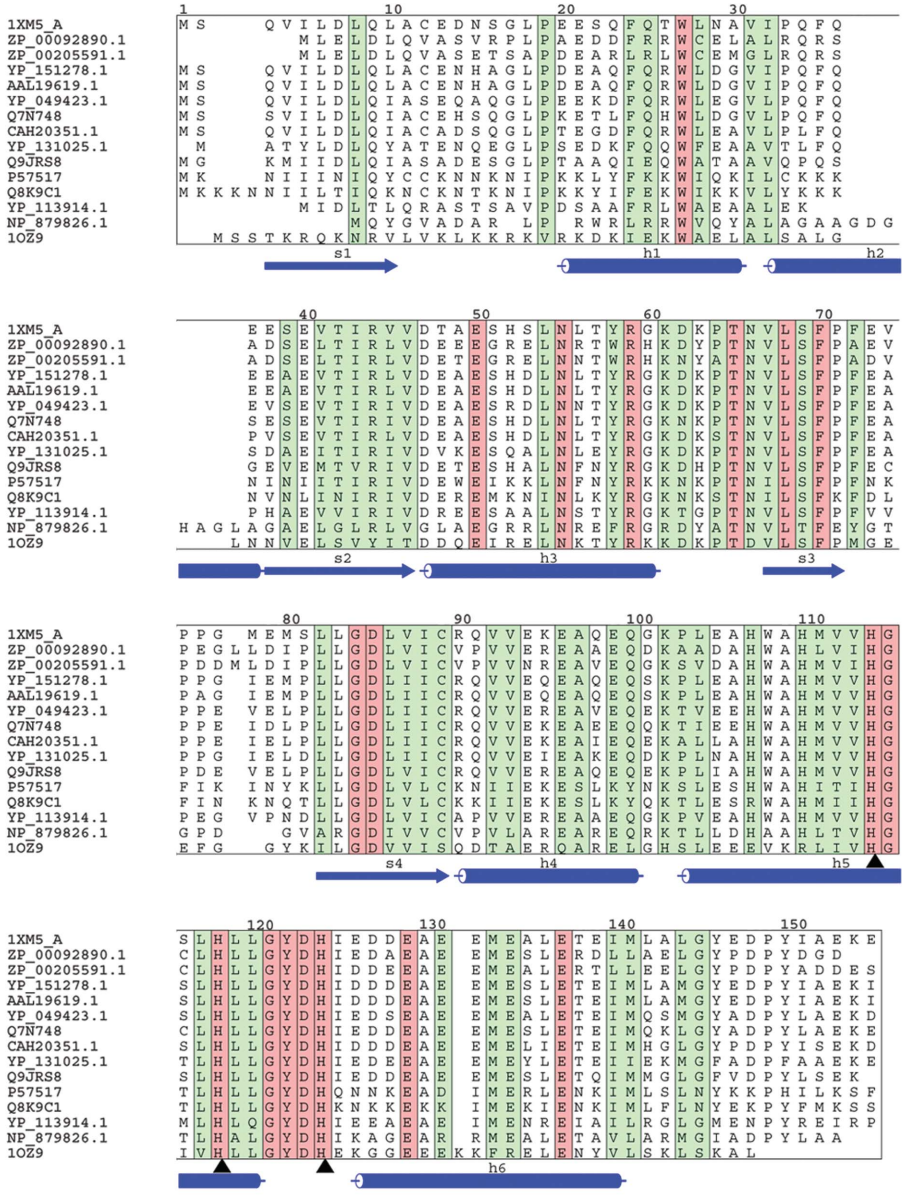

Figure 2
Alignment prepared by *CLUSTALW* (Thompson *et al.*, 1994), *AMAS* (Livingstone & Barton, 1993) and *ALSCRIPT* (Barton, 1993). Identical and conserved regions are marked in red and green, respectively (see definition in text). The secondary structure of 1xm5 was assigned using *DSSP* (Kabsch & Sander, 1983) and is indicated below each block. Cylinders stands for α-helix and arrows for β-strand. Metal-binding residues are indicated by black arrows. The species for each sequence is listed following their accession ID: 1XM5 (ybeY), *Escherichia coli*; ZP_00092890.1, *Azotobacter vinelandii*; ZP_00205591, *Pseudomonas syringae*; YP_151278.1, *Salmonella enterica*; AAL19619.1, *Salmonella typhimurium*; YP_049423.1, *Erwinia carotovora*; Q7N748, *Photorhabdus luminescens*; CAH20351.1, *Yersinia pseudotuberculosis*; YP_131025.1, *Photobacterium profundum*; Q9JRS8, *Actinobacillus actinomycetemcomitans*; P57517, *Buchnera aphidicola*; Q8K9C1, *Buchnera aphidicola*; YP_113914.1, *Methylococcus capsulatus*; NP_879826.1, *Bordetella pertussis*; 1OZ9 (AQ_1354), *Aquifex aeolicus*.

analysis (Chance *et al.*, 2004) and indicated a nickel:protein subunit stoichiometry of 0.5 ± 0.2 (data not shown). Previous experiments (Chance *et al.*, 2004; Shi *et al.*, 2005) have demonstrated that His-tagged proteins appear to show no more tendency to coordinate metal ions than do their non-His-tagged analogs. However, tetrahedral geometry with His-type ligands for Ni binding is uncommon, thus this metal-atom identification is tentative. Also, the occupancy is less than 100%.

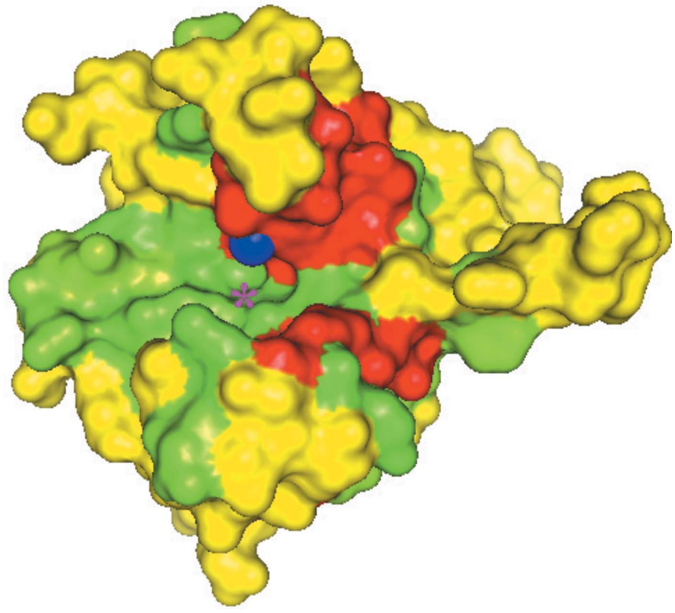

Figure 3
Surface-mapping representation prepared using *PyMol* (DeLano, 2002). Identical, conserved and less conserved residues are marked red, green and yellow, respectively. The Ni^{2+} ion is represented by a blue sphere. The approximate location of the putative fourth coordination site for Ni^{2+} ion is shown by a star.

A *BLAST* search (Altschul *et al.*, 1990) for proteins related to *E. coli* ybeY reveals homologous sequences from 132 unique species (with *BLAST* cutoff score higher than 100), all of which are of bacterial origin. Most of the related proteins are annotated as hypothetical UPF0054 protein (Bateman *et al.*, 2004) or predicted metal-dependent hydrolases (COG0319; Tatusov *et al.*, 2001). The structure around the metal-ion site supports a predicted function of metal-dependent hydrolase for this protein. 14 homologs of *E. coli* ybeY with identity ranging from 39 to 75% were selected and are presented in a multiple sequence alignment (Fig. 2). Conserved and identical positions are marked by green and red colors, respectively. A residue was considered conserved if at least six out of ten possible physicochemical properties (polar, small, proline, tiny, aromatic, aliphatic, positive, negative, charged, hydrophobic) are shared in a given sequential position (Livingstone & Barton, 1993). This is a rather strict definition of residue conservation. The metal-binding motif is entirely conserved in these sequences and is marked by black arrows in Fig. 2.

Using the data from Fig. 2, the solvent-accessible surface was mapped and colored by degree of conservation (Fig. 3). The protein depicted in Fig. 3 shows a cleft lined with conserved and identical residues in green and red colors, respectively. These include the three histidines that coordinate the metal ion (colored blue). Positions with lower physicochemical conservation scores are colored yellow. The putative fourth coordination site for the metal is located within a cleft, as indicated by a star in Fig. 3. The red-colored patch seen below the metal ion includes many of the entirely conserved residues seen in the stretch of residues from Asn55 to Asp85 in Fig. 2.

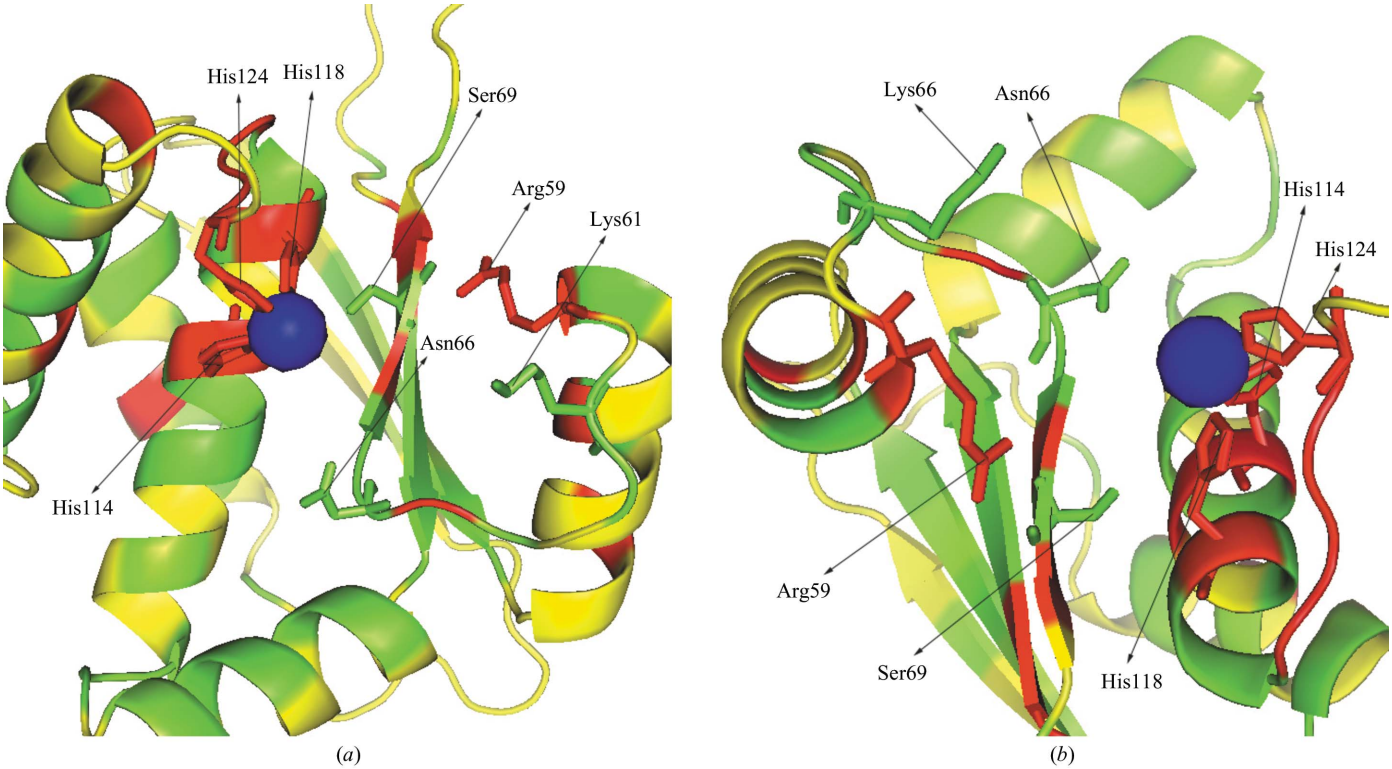

Figure 4
Cleft structure prepared by *PyMol* (DeLano, 2002). The cleft structure is shown in secondary structure colored by conservation score as in Fig. 3. Conserved residues in the cleft that are possibly involved in function are represented by sticks. Blue sphere is the Ni^{2+} ion. A side view and a top view are shown in (*a*) and (*b*), respectively.

The metal-binding site and the adjacent cleft are shown in Fig. 4. The sides of the helices that face the cleft are more conserved than the sides opposite the cleft. In addition to the three histidines coordinating the metal, Arg59, Lys61, Asn66 and Ser69 are either identical or have only very few substitutions in the multiple sequence alignment (Fig. 2). These residues might be important for the functional activity of the protein.

A search for structurally similar proteins was carried out using the *DALI* program (Holm & Sander, 1998). The closest protein structure is a hypothetical protein AQ_1354 from *Aquifex aeolicus* (PDB code 1oz9), which also belongs to the UPF0054 family (*DALI Z* score 19.6, r.m.s.d. 1.8 Å, 137 matched residues, 29.6% sequence identity for global alignment). All the other protein structures identified in the *DALI* search have *Z* scores less than 3.4. AQ_1354 is included in the sequence alignment of Fig. 2. It is predicted that AQ_1354 might be a monomer (Henrick & Thornton, 1998). The high structural similarity between AQ_1354 and ybeY implies that the ybeY protein might also be momomeric. The metal-binding site as well as some other relevant residues in the cleft are identical in ybeY and AQ_1354; however, AQ_1354 does not contain any metal atom in the structure. It was suggested that AQ_1354 might contain a metal in the binding motif and that the loss of metal might be the result of using 0.1 m*M* EDTA and 0.1 m*M* DTT in the protein purification and crystallization conditions (Oganesyan *et al.*, 2003).

The data in this paper, which include X-ray fluorescence analysis of purified ybeY protein in solution, the crystal structure of the protein and bioinformatics analysis, provides support for the hypothesis that the ybeY protein and its closely related structure and sequence homologs contain a functional metal atom. In addition, metal binding and potential hydrolase functions may be a common feature of members of the UPF0054 protein family.

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