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Molecular characterization of a homolog of the ferric-uptake regulator, Fur, from the marine bacterium *Marinobacter algicola* DG893

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Abstract

Full length recombinant iron regulatory protein, Fur, has been isolated and characterized from the algal-associated marine bacterium *Marinobacter algicola* DG893. Under nondenaturing conditions the Fur protein behaves on size exclusion chromatography as a dimer while it is monomeric under SDS PAGE conditions. ICP-MS and fluorescence quenching experiments show that Mb-Fur binds

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a single metal ion (Zn, Mn, or Co) per monomer. Electrophoretic mobility shift assays were used to probe the interaction of Mb-Fur with the purported Fur box in the promoter region upstream of the vibrioferrin biosynthetic operon. Interaction of Mb-Fur with a 100 bp DNA fragment containing the Fur box in the presence of 10 μ M Mn, Co or Zn(II) resulted in decreased migration of DNA on a 7.5 % polyacrylamide gel. In the absence of the Fur protein or the metal, no interaction is seen. The presence of EDTA in the binding, loading or running buffers also abolished all activity demonstrating the importance of the metal in formation of the promoter-repressor complex. Based on a high degree of similarity between Mb-Fur and its homolog from *Pseudomonas aeruginosa* (PA) whose X-ray structure is known we developed a structural model for the former which suggested that only one of the several metal binding sites found in other Fur's would be functional. This is consistent with the single metal binding stoichiometry we observed. Since the purported metal binding site was one that has been described as "structural" rather than "functional" in PA and yet the monometallic Mb-Fur retains DNA Fur box binding ability it reopens the question of which site is which, or if different species have adapted the sites for different purposes.

Keywords

Iron; Ferric uptake regulator; Metal binding; Homology model; 'Recombinant protein

Introduction

Iron, although one of the most important micronutrients in the marine environment is largely bio-unavailable due to its poor solubility and tendency to form colloidal and oxopolymeric species (Bruland et al. 1991; Tortell et al. 1999). The presence of organic ligands of as yet unknown structures, that tightly complex iron and increase its solubility, yet reduce the concentration of biologically available inorganic ferric species, further complicates iron speciation (Rue and Bruland 1995; Wu and Luther 1995; Gledhill and van den Berg 1994). Multiple iron fertilization experiments in high-nutrient-low-chlorophyll (HNLC) regions of the oceans have corroborated the importance of iron and its limitation to marine microorganisms (Boyd et al. 2000; Coale et al. 1996). In response to this limitation, diverse marine bacterial species excrete small organic compounds, called siderophores, which bind iron with exceptional affinity and facilitate its transport into cells (Vraspir and Butler 2009). One of the emerging structural features that seems to differentiate terrestrial from marine siderophores is the near universal presence in the latter of α - or β -hydroxy acid moieties (Barbeau et al. 2002; Sandy and Butler 2009). These chelating groups make the resulting iron complexes photolabile so that in the presence of light the bound Fe(III) is reduced to Fe(II) with the concomitant oxidation and loss of CO₂ from the ligand via an irreversible internal redox reaction (Barbeau et al. 2001).

Marinobacter belong to the class of γ -proteobacteria and these motile, halophilic or halotolerant bacteria all share the ability to use petroleum hydrocarbons as sole energy and carbon sources (Duran 2010). They are a ubiquitous species in the world's oceans, having been isolated from a wide variety of marine environments ranging from hydrothermal vents to Antarctic sea ice (Kaye et al. 2011; Glatz et al. 2006). They have also been identified as

members of the bacterial flora associated with other marine organisms. Indeed we and others have observed that among the most notable members of the bacterial communities associated with marine phytoplankton, including diatoms, coccolithophores and dinoflagellates (Kaeppl et al 2011; Alavi et al. 2001; Seibold et al. 2001; Green et al. 2004) were bacteria from several *Marinobacter* clades. While these algal-associated species were closely related to other *Marinobacter* species (e.g. *M. hydrocarbonoclasticus* or *Marinobacter* sp. DS40M8), most of the tested strains did not produce the types of siderophores commonly produced by free-living members of the genus (Barbeau et al. 2002; Martinez et al. 2003). Rather we have shown that only the extremely photolabile siderophore, vibrioferrin (VF), is produced by the two clades of *Marinobacter* that appear to be algal-associated. We have further shown that the photo-generated iron, Fe(III)' was highly bioavailable both to the producing bacterium and its algal partner (Amin et al. 2009). This led to the hypothesis that algal cells produced dissolved organic matter (DOM) that helped support bacterial growth and ultimately fuel the biosynthesis of VF through a light-dependent mutualism.

It has been known for many years that siderophores and other iron uptake systems are repressed at high levels of iron. This control is typically mediated via the global iron-response transcriptional regulator known as Fur (Escobar et al. 1999). Fur is a 17 kDa iron binding regulatory protein found in most Gram-negative bacteria which plays an important role in controlling the intracellular level of iron. The importance of acquiring enough iron to grow while avoiding the toxic effects of excess iron due to Haber–Weiss Fenton chemistry is crucial to survival, hence iron uptake and storage is tightly homeostatically controlled. The Fur protein is one component of this regulatory system. In general it is thought that in the presence of high iron an Fe²⁺-Fur complex forms which can bind to a regulatory Fur box sequence, (a conserved 19-base pair inverted repeat) in the promoter region of iron-regulated genes, repressing the transcription of those genes (Desai et al. 1996; Mey et al. 2005). These genes will only be translated when the internal iron concentrations are low, which causes the dissociation of Fe-Fur complex. The resulting apo-Fur can no longer bind to the Fur box sequence, which then allows the expression of many iron transport related proteins.

Here we assess in more detail the iron regulatory system in *Marinobacter* clades that produce VF via isolation and characterization of a Fur homolog. Understanding the role of Fur on the regulation of iron uptake and storage in the *Marinobacter* clades is essential to understanding their ecological relevance to the growth of algae in natural habitats and can serve as a promising step towards validating a “carbon-for-iron” based mutualism in bacterial-algal interactions in the marine environment.

Materials and methods

Bacterial growth

M. algicola DG893 cells were grown in artificial seawater (ASW) containing per liter: 15 g NaCl, 0.75 g KCl, 12.4 g MgSO₄·7H₂O, 3 g CaCl₂·2H₂O, 1 g NH₄Cl, 0.1 g β-glycerophosphoric acid and 2 g Casamino acids with the final pH adjusted to 8.0. The ASW was passed through Chelex-100 resin (BioRad) to remove trace metals and supplemented with the appropriate concentrations of FeCl₃ immediately prior to the start of growth.

Cloning and expression of Mb-Fur

Genomic DNA was isolated from *Marinobacter algicola* via extraction with xanthate buffer (Tillett and Neilan 2000). The complete *fur* gene (ZP_01893541.1 411 base pairs) was then amplified by standard PCR methods using Pfu polymerase and the primers 5'-TACTTATG CCGCGGCCGCTCAGGATTTGAGGGGTTTGA-3' and 5'-CACCTTCTAAGGATCCATGTCATCCGA AAACACCGA-3' (restriction sites underlined). After cleanup using QIAquick Gel Extraction Kit (Qiagen), PCR amplicons were digested with BamHI-HF and NotI-HF (New England BioLabs) and ligated into a similarly double digested pHis8 vector. The resultant Mb-Fur pHis8 plasmid encodes for amino acids 1 to 411 of *M. algicola* Fur with an amino-terminal histidine tag and a thrombin cleavage site. The plasmid was isolated using a QIAGEN Plasmid Midi Kit and the correct gene sequence verified by DNA sequencing (Retogene). Expression of recombinant Mb-Fur was carried out in BL21 *E. coli* cells (Stratagene). Transformed bacteria were cultured overnight in 2 L of LB broth supplemented with kanamycin sulfate (25 mg/mL) at room temperature on stir plates with 0.1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG). Cells were collected by centrifugation at 3000 $\times g$ for 20 min at 4 °C, washed one time in 80 mL of 1 $\times$ PBS, and pelleted by centrifugation at 3,000 rpm in a Sorvall 75006445 swinging bucket rotor for 20 min in sterile 50 mL conical centrifuge tubes prior to decanting and storage of cell pellets at -80 °C.

Purification of Mb-Fur

A tube containing the frozen cell pellet from 1 L culture was resuspended in 80 mL of lysis buffer (50 mM Tris-HCl pH 8.0, 500 mM NaCl). Slurried cells were disrupted on ice by four cycles of sonication for 45 s each with 1 s pulses at output setting 8 (Branson 450 Sonifier). The resulting lysate was clarified by centrifugation for 50 min at 12,000 rpm in an SS-34 rotor at 4 °C. The crude supernatant was filtered through 0.8 micron syringe-tip filters and loaded on to a pre-equilibrated 6 mL Ni Sepharose 6 Fast Flow column (GE Healthcare). The column was washed with 50 mL of lysis buffer to remove nonspecifically bound protein and then washed with 75 mL of running buffer (50 mM HEPES pH 8.0, 1 M NaCl, 25 mM imidazole, 1 mM DTT). Protein was eluted in 1 mL fractions with 20 mL elution buffer (50 mM HEPES pH 8.0, 1 M NaCl, 250 mM imidazole, 1 mM DTT). Peak fractions were filtered through 0.2 μ syringe-tip filters and further purified by gel filtration chromatography on a Superdex 200 16/60 size exclusion chromatography column in 50 mM HEPES pH 8.0, 100 mM NaCl, and 1 mM DTT. Peak fractions were then concentrated in an Amicon centrifugal concentrator (MWCO 10 kDa) to a final concentration of 10–12 mg/mL, divided into 50 μ L aliquots, flash cooled in liquid nitrogen, and stored at -80 °C. The histidine tag was removed using the Thrombin Clean-Cleave kit (Sigma) according to the manufacturer's recommendations. Complete histidine tag removal was verified by both SDS PAGE and Western blots.

Protein characterization and metal ion binding

Protein concentrations were determined by the Bradford assay (Bio-Rad) or via the calculated extinction coefficient at 280 nm (10,470 L/mole-cm). Metal ions were removed by dialysis against a buffer containing 50 mM EDTA, 50 mM HEPES pH 8.0, 100 mM

NaCl, 1 mM DTT and reconstituted using the same buffer minus the EDTA and including 10 mM Zn. The presence of transition metal ions was determined by ICP-MS using an Agilent 7500cs ICP-MS with argon as the carrier gas and commercially available multi-element standards (Fluka or High Purity Standards). Metal binding was also evaluated via fluorescence quenching experiments using tyrosine fluorescence excitation at 275 nm and emission at 305 nm on a SPEX fluorimeter. Metal binding stoichiometry was determined by plots of relative fluorescence intensity versus metal/Fur ratio. Binding constants were determined by fits of relative fluorescence intensity versus logarithm of metal concentration data to a three parameter sigmoidal dose–response curve as implemented in Sigma Plot 12.5.

Gel electrophoretic mobility shift assay

Binding of Fur to a purported Fur-box sequence in *Marinobacter algicola* DG893 was monitored using fluorescence based electrophoretic mobility shift assays (Molecular Probes). Primers (Forward 5'-TCTGGTTA ACTGTCAGCACAT-3' and Reverse 5'-GTAGCGCT TGATTTTAGAATCG-3') for a 100 bp fragment containing a purported 19 bp Fur-box sequence (TATAAT TTAAATCTTAATC) just upstream of the vibrioferrin biosynthesis operon (*pvsX*) were amplified by PCR and purified using NucleoSpin Gel & PCR Cleanup kit (Macherey–Nagel). The binding buffer consisted of 10 mM Tris pH 8.1, 150 mM NaCl, 100 mM DTT and samples were separated on a 7.5 % non-denaturing polyacrylamide gel in a 0.5× TA running buffer (no EDTA) at 200 V.

Protein model creation

The *Marinobacter algicola* Fur protein sequence (Accession #: WP_007153120) was submitted to the Local Meta-Threading-Server (LOMETS: <http://zhanglab.ccmb.med.umich.edu/LOMETS/>) (Wu and Zhang 2007). All available algorithms produced high confidence models, and the top ten were evaluated with ERRATv2 (Colovos and Yeates 1993). Of these, the top three scoring models were energy minimized using the MMTK steepest descent algorithm implemented in Chimera (Pettersen et al. 2004) and reevaluated with ERRAT and Procheck (Laskowski et al. 1993), using the SAVE server at UCLA (<http://nihserver.mbi.ucla.edu/SAVES/>). The best scoring model (ERRAT score 96.825) was one based on the *Pseudomonas aeruginosa* Fur protein structure (PDB: 1MZB:A) and was created using the MUSTER threading algorithm (Wu and Zhang 2008). The RMSD between the model and template (1.719 Å) and other crystal structures was calculated using the Protein structure comparison service Fold (PDBeFOLD) at the European Bioinformatics Institute (<http://www.ebi.ac.uk/msd-srv/ssm>) (Krissinel and Henrick 2004). Of the available crystal structures, 1MZB also showed the greatest sequence identity with *Marinobacter* (71 %) compared to the next closest match, the *Escherichia coli* Fur protein (2FU4: 60 %).

Results

Protein isolation and characterization

Approximately 10 mg/L of crude recombinant protein was routinely isolated from induced BL21 cells. Protein was isolated in soluble form without the formation of inclusion bodies or the need for denaturing conditions for isolation. Protein of greater than 90–95 % purity was

isolated via a combination of Ni affinity and size exclusion chromatography and its sequence confirmed by MALDI-TOF mass spectrometry (UCSD Mass Spectrometry Center). Under reducing and denaturing conditions (SDS-PAGE) Mb-Fur migrated as a single band with a molecular weight of approximately 17 kDa indicative of a monomer. In the absence of the reducing agent DTT, Mb-Fur, which contains a single cysteine residue, gave two bands on SDS PAGE indicative of a mixture of monomer and dimer suggesting formation of disulfide bridged dimers on air oxidation. Under non-denaturing but reducing conditions (50 mM HEPES pH 8.0, 100 mM NaCl, 1 mM DTT) Mb-Fur eluted from a calibrated SEC column as a homogeneous band with an apparent molecular weight of 34 kDa, indicating that the apoprotein is a dimer in solution.

Metal binding

Metal binding to Mb-Fur is summarized in Table 1. The protein as isolated contained 0.6 mol Ni and 0.06 mol Zn/mole protein. Other metals were present at less than 0.01 mol metal/mole protein. The presence of Ni is undoubtedly due to purification of the protein via Ni affinity chromatography as loss of Ni from the column was evident after processing large amounts of the crude protein. Removal of the his tag and overnight dialysis against 50 mM EDTA gave a protein that contained ca. 0.1 mol metal/mole protein of Ni or Fe and less than 0.01 of other metal ions. Dialysis of this “metal free” protein against 10 mM Zn(II) gave a protein that contained 1.1(1) mol Zn/mole protein monomer.

Since Mn(II) or Co(II) are often used as surrogates for Fe(II) binding in EMSA assays we also evaluated the binding of these metal to Mb-Fur via fluorescence quenching experiments. As Mb-Fur contains no tryptophan residues, the fluorescence emission at 305 with 275 nm excitation seen with the recombinant protein must be due to one or more tyrosine residues. The closest tyrosine residue is approximately 14 Å away from the expected metal binding site and the fact that both the paramagnetic ions Mn(II) and Co(II), but not diamagnetic Zn(II) decrease the fluorescence yield upon metal binding suggests that the quenching is the result of non-radiative resonance energy transfer from the tyrosine excited state to the nearby metal. Evaluation of the metal binding properties as determined by this fluorescence quenching (Fig. 1) gave a Mn/protein monomer ratio of 1.08(4) with a dissociation constant of 1.0(1) μM. Cobalt appears to bind an order of magnitude less well than manganese with a Co/protein ratio of 1.1(1) and a K_d of approximately 60 μM.

Protein model analyses

The overall structure of the *Marinobacter* Fur protein model is similar to that of its template, the Fur protein (PDB:1MZB) from *Pseudomonas aeruginosa* (Fig. 2). The Fur N-terminal binding domain of *Marinobacter* is highly similar to that of *Pseudomonas*, with a sequence identity of 75 % and an RMSD of 0.467. In comparison, the C-terminal domains show greater diversity with a sequence identity of only 64 % and an RMSD of 0.630. Many proteins of this family include a histidine-rich motif in the C-terminal domain with a consensus sequence of HHHXHX₂CX₂C (Fillat 2014). However, the first histidine and last cysteine of this motif are not conserved in *Marinobacter* (Fig. 3).

The *Marinobacter* Fur model includes two potential metal binding sites. The first site has four coordinating ligands, two histidines and two glutamic acids, and is similar to the Zn site in *Pseudomonas* (Fig. 4). However, in the proposed second site, the model predicts that a potential metal ligand, His 125, is not in a position to contribute to metal binding (Fig. 5). This distortion may be due to the replacement of a conserved asparagine at position 125 in *Pseudomonas* with a serine in *Marinobacter* (Fig. 6). (See also Supplemental Table 1 for PDBeFOLD comparisons).

Electrophoretic mobility shift assays

EMSAs were used to probe the interaction of Mb-Fur with the purported Fur box in the promoter region upstream of the vibrioferrin biosynthetic operon. Interaction of Mb-Fur with a 100 bp DNA fragment containing the Fur box in the presence of 10 μ M Mn(II) resulted in decrease migration of DNA on a 7.5 % polyacrylamide gel. In the absence of the Fur protein or the Mn, no interaction is seen (Fig. 7). The presence of EDTA in the binding, loading or running buffers also abolished all activity demonstrating the importance of the metal in formation of the promoter-repressor complex. We also tested Co and Zn(II) for their ability to promote Fur-DNA binding and found that they both promoted binding of Mb-Fur to DNA at the 10 μ M level. Higher concentrations of metal actually gave a progressively weaker response which was traced to incipient precipitation of the protein at high metal concentrations.

Discussion

Members of the Fur superfamily have been found in all known groups of prokaryotes. Common features of these proteins include an N-terminal DNA binding domain linked by a hinge region to a C-terminal dimerization domain, and at least one metal binding site. To date, half a dozen crystal structures of proteins involved in ferric uptake regulation (Fur) have been published, and these and other biophysical studies reveal a surprising diversity in terms of metal binding stoichiometry, ligation and geometry (Table 2).

Katigbak and Zhang (2012) identify three types of Fur metal binding sites, based on the make-up of the amino acids in each site. One type is thought to be purely structural in nature and features a zinc atom tetrahedrally bound to four cysteines found in two CXXC motifs. These are found in *Helicobacter* and *Escherichia* Fur crystal structures, as well as some Zur and PerR molecules (Fillat 2014), but not in *Pseudomonas* or *Marinobacter*. The second and third type of binding site is made up of some combination of 2–3 histidines and 1–2 glutamic or aspartic acids. These amino acids are well conserved, but their metal coordination differs in the different crystal structures with four, five and six coordinate modes being found. Although iron has been shown to be able to bind to these sites (Adrait et al. 1999), more recent work suggests that some primarily play a structural role and physiologically bind zinc (Pohl et al. 2003). There remains debate, however, about which is the iron-sensing site (Ahmad et al. 2009; Katigbak and Zhang 2012). Two of these sites appear to be present in *Marinobacter*, which has some elements of sites seen in *Pseudomonas* and *Vibrio* (Table 3).

Although we have as yet been unsuccessful in growing crystals of Mb-Fur for X-ray analysis, our homology model suggests that the overall structure is similar to *Pseudomonas* but important differences are seen in the metal binding sites. The so-called structural site is likely intact in Mb-Fur with Zn(II) proposed to bind in a tetrahedral arrangement to Glu81, His33, Glu101 and His90. The second site, which was proposed to be the regulatory site in *Pseudomonas* Fur and which was made up of two histidine, one glutamic and one bidentate aspartic acid residue, appears to be unable to bind metals in the corresponding Mb-Fur structure as the second His residue appears to have rotated out of the metal binding cavity, leaving only His87, Asp89 and Glu108 as potential ligands. Our metal binding studies are consistent with this view and clearly show only a single divalent metal (Zn, Co or Mn) binds to Mb-Fur. Nevertheless we have also shown that metal bound (but not apo) Mb-Fur interacts with the Fur box sequence in the promoter region upstream of the vibrioferrin biosynthetic operon. It is worth noting as well that biophysical studies on the *Pseudomonas* protein suggest the binding of only one metal (zinc) rather than the two reported in the crystal structure (Lewin et al. 2002). Together these results raise questions about the functional role of the different metal binding sites implicated in the various Fur structures and whether or not some of the reported binding sites for zinc may be artifacts of the crystallization conditions which typically include high concentrations of zinc in the crystallization buffers. Alternatively, these could represent real adaptations of different bacteria to different environmental pressures and metal usage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Plot of fluorescence intensity (cps) versus log of the metal concentration for Mn(II) (closed circles) and Co(II) (closed triangles). The solid lines are the fits to the data with the model described in the materials and methods

Fig. 2. Fur protein monomer structures of the *Marinobacter algicola* model (a) and (b) *Pseudomonas aeruginosa* (PDB:1MZB). The model was created using MUSTER implemented in LOMETS using 1MZB. ERRAT score = 96.825

Fig. 3.
Histidine-rich motif conservation in Marinobacter and in FUR-family crystallized sequences. Numbering based on Marinobacter

Fig. 4. Comparison of proposed metal binding site 1 of *Marinobacter algicola* model (a) and (b) *Pseudomonas aeruginosa* (PDB:1MZB)

Fig. 5. Comparison of proposed metal binding site 2 of *Marinobacter algicola* model (a) and (b) *Pseudomonas aeruginosa* (PDB:1MZB). In *Pseudomonas*, His124 is a key ligand, while in *Marinobacter*, the analogous amino acid, His125, is predicted to be unavailable as a binding partner

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M-algicola      BRQQTAKERGYEIVDHSLLLYVKP-IKS-----
maq_Maqu_336   BRQQTAAEBHGYEIVDHSLLLYVKP-IKSDKK--
mhc_MARHY320   BRQQTAAEBHGYEIVDHSLLLYVKP-IKSDKK--
mad_HP15_308   BRQQTAAEBHGYEIVDHSLLLYVKP-IKS-----
mbs_MRBBS_33   DRQQTAAEBHGYEIVDHSLLLYVKP-IKS-----
hch_HCH_0122   KRQKEIABEBHGYEIVDHSLLLYVKP-KKK-----
mpc_Mar181_0   KRQKEIABEBHGYDIEEBHSLIMYVRP-KKK-----
rmw_Mmwy11_3   KRQKEIABEBHGYDIEEDHSLIIVYVRP-KKK-----
rme_Marme_05   QRQKEIABEBHGYDIEEDHNLVIYVRP-KSK-----
pnc_NCGM2_07   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
prp_M062_251   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
paf_PAM18_48   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pag_PLES_514   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pau_PA14_630   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pdk_PADK2_25   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
paer_PA1R_gp   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
paes_SCV2026   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
paem_U769_26   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
paep_PA1S_gp   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pae_PA4764     KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
lmzb          KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pael_T223_26   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
psg_G655_251   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pdr_H681_211   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pap_PSPA7_54   KRQKEIVREERGFEIVDHNLVLYVRK-KK-----
pfv_Psefu_36   KRQKEIVREERGFEIVDHNLVLYVRK-TAK-----

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Fig. 6.

Partial alignment of Fur proteins showing asparagine in *Pseudomonas* replaced by serine next to conserved histidine in *Marinobacter*

Fig. 7.

Binding of Mb-Fur to a 100 bp segment of DNA containing the Fur *box sequence* from the promoter region of the vibrioferrin biosynthesis operon. The mobilities of the free and bound forms are indicated by the *arrows*. Binding reaction resolved on a 7.5 % native polyacrylamide gel and visualized with SYBR Green fluorescence. **a** 10 μ M Mn, **b** 10 μ M Mn+100 μ M EDTA, **c** 0 μ M Mn, **d** no DNA **e** no Fur, 10 μ M Mn, 10 μ M Co, 10 μ M Zn

Table 1

Metal binding properties of Mb-Fur

Metal	Metal bound/monomer		Reconstituted	K _d (M)
	As isolated	After dialysis versus EDTA		
Zn(II)	0.06	< 0.01	1.1(1)	nd
Fe(II)	< 0.01	0.1	nd	nd
Ni(II)	0.60	0.1	nd	nd
Mn(II)	< 0.01	< 0.01	1.08(4)	$1.0(1) \times 10^{-6}$
Co(II)	< 0.01	< 0.01	1.1(1)	$6(3) \times 10^{-5}$

Table 2

Characteristics of published Fur structures

PDB	Species	# of metal binding sites	Stoichiometry	Ref.
1mzb	<i>Pseudomonas aeruginosa</i>	3	Homo 2-mer	Pohl et al. (2003)
2fu4	<i>Escherichia coli</i>	2	Homo 2-mer	Pecqueur et al. (2006)
2w57	<i>Vibrio cholerae</i>	2	Homo 2-mer	Sheikh and Taylor (2009)
2xig	<i>Helicobacter pylori</i>	3	Homo 4-mer	Dian et al. (2011)
4ets	<i>Campylobacter jejuni</i>	2	Homo 2-mer	Butcher et al. (2012)

Table 3

Fur metal binding site 2 coordination

	His 32	Glu80-1	Glu80-2	His89	Glu100
<i>Pseudomonas</i> (1mzb)					
<i>Helicobacter</i> (2xig)	His42	Glu90-1	Glu90-2	His97	Glu110
<i>Vibrio</i> (2w57)	His33	Glu81-1	Glu81-2	His88	His90
<i>Martinsbacter</i>	His33	Glu81-1	Glu81-2	His88	His90
					Glu101