doi:10.1016/j.jmb.2007.06.072 J. Mol. Biol. (2007) 372, 583–593

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Crystal Structure of the Transcriptional Regulator CmeR from *Campylobacter jejuni*

Ruoyu Gu[1]†, Chih-Chia Su[2]†, Feng Shi[3], Ming Li[1], Gerry McDermott[4] Qijing Zhang[3] and Edward W. Yu[1,2]*

[1]*Department of Physics and Astronomy, Iowa State University, Ames, IA 50011 USA*

[2]*Department of Biochemistry Biophysics and Molecular Biology, Iowa State University Ames, IA 50011, USA*

[3]*Department of Veterinary Microbiology, College of Veterinary Medicine Iowa State, University Ames, IA 50011, USA*

[4]*Department of Anatomy School of Medicine University of California San Francisco, CA 94143 USA*

**Corresponding author*

The CmeABC multidrug efflux pump, which belongs to the resistance-nodulation-division (RND) family, recognizes and extrudes a broad range of antimicrobial agents and is essential for *Campylobacter jejuni* colonization of the animal intestinal tract by mediating the efflux of bile acids. The expression of CmeABC is controlled by the transcriptional regulator CmeR, whose open reading frame is located immediately upstream of the *cmeABC* operon. To understand the structural basis of CmeR regulation, we have determined the crystal structure of CmeR to 2.2 Å resolution, revealing a dimeric two-domain molecule with an entirely helical architecture similar to members of the TetR family of transcriptional regulators. Unlike the rest of the TetR regulators, CmeR has a large center-to-center distance (54 Å) between two N termini of the dimer, and a large flexible ligand-binding pocket in the C-terminal domain. Each monomer forms a 20 Å long tunnel-like cavity in the ligand-binding domain of CmeR and is occupied by a fortuitous ligand that is identified as glycerol. The binding of glycerol to CmeR induces a conformational state that is incompatible with target DNA. As glycerol has a chemical structure similar to that of potential ligands of CmeR, the structure obtained mimics the induced form of CmeR. These findings reveal novel structural features of a TetR family regulator, and provide new insight into the mechanisms of ligand binding and CmeR regulation.

Keywords: TetR family; *Campylobacter jejuni*; transcriptional regulator; X-ray crystallography; multidrug resistance

Introduction

Campylobacter jejuni is the leading bacterial cause of food-borne diarrhea in the USA and other developed countries.[1] It is also a significant enteric pathogen for young children in developing countries. This Gram-negative enteric organism colonizes the intestinal tracts of animals and has become increasingly resistant to antimicrobials due to the possession of multidrug efflux transporters and acquisition of various resistance mechanisms, compromising the effectiveness of antibiotic treatment. According to the genomic sequence of NCTC 11168, *C. jejuni* harbors 13 putative antibiotic efflux transporters of the ATP-binding cassette (ABC), resistance-nodulation-division (RND), multidrug and toxic compound extrusion (MATE), major facilitator (MF), and small multidrug resistance (SMR) families.[2,3] At present, CmeABC and CmeDEF, which belong to the RND family, are the only two antibiotic efflux transporters that have been functionally characterized in *Campylobacter*.[4–6]

The CmeABC efflux system is the main efflux pump in *C. jejuni* and consists of three members, including an outer membrane channel (CmeC), an inner membrane drug transporter (CmeB), and a periplasmic membrane fusion protein (CmeA). These three proteins are encoded by a three-gene operon (*cmeABC*) and form an efflux system that extrudes a variety of toxic compounds directly out of *C. jejuni*.[5] The substrates extruded by CmeABC include commonly used antibiotics (e.g. fluoroquinolones, macrolides, ampicilin, tetracycline, chloramphenicol, cefotaxime, rifampin), metal ions (e.g. Co^{2+} and Cu^{2+}), and lipophilic compounds (e.g.

† R.G. and C.S. contributed equally to this work.

E-mail address of the corresponding author: ewyu@iastate.edu

SDS and various bile salts). Thus, CmeABC contributes significantly to the intrinsic and acquired resistance of *Campylobacter* to structurally diverse antimicrobials.[5–8] In addition, this efflux system is essential for *Campylobacter* colonization in the animal intestinal tract by conferring resistance to the bile acids that are normally present in the animal intestinal tract and have bactericidal effect.[9]

The expression of *cmeABC* is controlled by the transcriptional regulator CmeR.[10] The *cmeR* gene is located immediately upstream of the *cmeABC* operon and encodes a 210 amino acid residue protein that shares N-terminal sequence and structural similarities with members of the TetR family of transcriptional repressors.[11,12] Like other members of the TetR family, the N-terminal domain of CmeR contains a predicted DNA-binding helix-turn-helix (HTH) motif, while its C-terminal region has unique sequences and is expected to be involved in the binding of inducing ligands.[10,11] *cmeR* is transcribed in the same direction as *cmeABC*, and the intergenic region between *cmeR* and *cmeA* contains the 16 bp inverted repeat (IR) operator site for *cmeABC*. As a transcriptional regulator, CmeR binds directly to the IR operator and represses the transcription of *cmeABC*.[10] This regulating process is similar to those of the other TetR family members, such as AcrR of *Escherichia coli*,[13] MexR of *Pseudomonas aeruginosa*,[14] MtrR of *Neisseria gonorrhoeae*,[15] and QacR of *Staphylococcus aureus*,[16] in which they bind specifically to the promoter sequences of *acrAB*, *mexAB*, *mtrCDE*, and *qacA*, respectively, thus inhibiting the expression of the corresponding efflux pump(s). Deletion of *cmeR* or mutations in the IR operator releases the repression, resulting in the over-expression of CmeABC, which, in turn, leads to the enhanced resistance to multiple antibiotics.[10]

In addition, bile compounds, including both conjugated (e.g. taurodeoxycholate) and non-conjugated (e.g. cholate), induce the expression of *cmeABC* by inhibiting the binding of CmeR to the promoter of *cmeABC*, suggesting that bile compounds are inducing ligands of CmeR.[17] It is possible that CmeR can be induced by other unidentified ligands. How inducing ligands bind to CmeR and modulate the expression of CmeR-controlled genes is not known. On the basis of the predicted structural features, we hypothesize that binding of inducing ligands to the C-terminal domain of CmeR triggers conformational change in the N-terminal DNA-binding region. This change in conformation results in the release of CmeR from its operator DNA, and thus allows transcription from its cognate promoter. As an initial step to examine this hypothesis and elucidate the mechanisms that CmeR uses to regulate gene expression, we present here the crystal structure at 2.2 Å resolution of the CmeR regulator.

Results

Overall structure of CmeR

We used the multiple-wavelength anomalous dispersion method to solve the selenomethionyl-substituted (SeMet) CmeR crystal structure from

Table 1. Data collection, phasing and structural refinement statistics

	Native	SeMet inflect. point	SeMet peak	SeMet remote
A. *Data collection*				
Wavelength (Å)	0.9795	0.9798	0.9795	0.9662
Space group	*P*21212	*P*21212	*P*21212	*P*21212
Cell constants				
a (Å)	93.3	93.0	93.0	93.0
b (Å)	37.4	37.3	37.3	37.3
c (Å)	57.6	57.5	57.5	57.5
Resolution (Å)	2.24 (2.33–2.24)	2.10 (2.18–2.10)	2.07 (2.28–2.07)	2.07 (2.14–2.07)
Completeness (%)	99.6 (97.7)	97.6 (85.1)	93.4 (84.1)	97.2 (80.3)
No. reflections	297,224	168,174	179,712	179,712
No. unique reflections	10,172	12,493	14,087	12,897
R_{sym} (%)	5.5(26.2)	5.8(25.0)	5.2(26.4)	5.1(27.3)
Average I/σ	16.6(4.7)	23.3(3.8)	22.3(5.3)	18.2(3.2)
B. *Phasing*				
Selenium atom sites			3	
Resolution range of data used (Å)			50–2.80	
Overall figure of merit			0.59	
C. *Refinement*				
R_{work} (%)	21.9			
R_{free} (%)	24.3			
rms deviation from ideal				
Bond angles (deg.)	0.8			
Bond lengths (Å)	0.006			
Ramachandran analysis				
Most favored regions (%)	91.4			
Allowed regions (%)	7.5			
Generously allowed regions (%)	1.1			
Disallowed regions (%)	0.0			