Journal of Structural Biology 183 (2013) 512–518

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Alternative binding modes of L-histidine guided by metal ions for the activation of the antiterminator protein HutP of *Bacillus subtilis*

Balasundaresan Dhakshnamoorthy [1], Hiroshi Mizuno, Penmetcha K.R. Kumar *

RNA Processing Group, Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology, Central-6, 1-1-1 Higashi, Tsukuba 305-8566, Japan

ARTICLE INFO

Article history:
Received 6 December 2012
Received in revised form 11 May 2013
Accepted 29 May 2013
Available online 5 June 2013

Keywords:
Zn^{2+}
Mg^{2+}
Coordination
HutP
L-Histidine
Antitermination

ABSTRACT

Anti-terminator proteins control gene expression by recognizing control signals within cognate transcripts and then preventing transcription termination. HutP is such a regulatory protein that regulates the expression of the histidine utilization (*hut*) operon in *Bacillus subtilis* by binding to *cis*-acting regulatory sequences in *hut* mRNAs. During the anti-termination process, L-histidine and a divalent ion are required for hutP to bind to the specific sequence within the *hut* mRNA. Our previous crystal structure of the HutP-L-histidine-Mg^{2+}-RNA ternary complex demonstrated that the L-histidine ligand and Mg^{2+} bind together such that the backbone nitrogen and carboxyl oxygen of L-histidine coordinate with Mg^{2+}. In addition to the Mg^{2+}, other divalent ions are also known to efficiently support the L-histidine-dependent anti-termination of the *hut* operon, and the best divalent ion is Zn^{2+}. In this study, we determined the crystal structure of the HutP-L-histidine-Zn^{2+} complex and found that the orientation of L-histidine coordinated to Zn^{2+} is reversed relative to that of L-histidine coordinated to Mg^{2+}, i.e., the imidazole side chain nitrogen of L-histidine coordinates to Zn^{2+}. This alternative binding mode of the L-histidine ligand to a divalent ion provides further insight into the mechanisms responsible for the activation of RNA binding during the *hut* anti-termination process.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Bacteria exploit a variety of mechanisms to regulate transcription elongation, to control gene expression in response to change in their environment. Among these, a common path is the modulation of mRNA secondary structures by RNA-binding proteins, either to pause transcription near the terminator region or to allow synthesis of the full-length transcript. The latter mechanism of transcription regulation is referred as anti-termination. Several of these anti-termination proteins have been described in *Escherichia coli* and *Bacillus subtilis*, including LacT, BgIG, SacT, SacY, TRAP and PyrR (Arnaud et al., 1996; Aymerich and Steinmetz, 1992; Babitzke and Yanofsky, 1993; Houman et al., 1990; Lu et al., 1996). Another protein that belongs to this class is HutP, which regulates the histidine utilization (*hut*) operon in *B. subtilis* through the anti-termination of transcription (Oda et al., 1988; Wray and Fisher, 1994). As shown in Fig. 1, The *hut* operon consists of six open reading frames. The HutP gene is located near the promoter, and five additional genes, *hutH*, *hutU*, *hutI*, *hutG* and *hutM*, are located downstream from *hutP* and the promoter (Chasin and Magasanik, 1968; Kimmhi and Magasanik, 1970; Oda et al., 1992; Yoshida et al., 1995). Expression of the operon is regulated by catabolite repression and L-histidine-induced transcription anti-termination. The mRNA transcript sequence between the *hutP* coding region and the coding region for the five histidine utilization genes is predicted to form a stem-loop structure that functions as a terminator. In addition, a *cis*-acting regulatory sequence required for histidine-mediated induction of *hut* structural gene expression has been identified. This *cis*-acting regulatory sequence forms an anti-terminator structure and is located just upstream of the *hut* histidine utilization genes, partially overlapping the downstream terminator (Oda et al., 2000).

HutP is a 16.2 kDa protein consisting of 148 amino acid residues. HutP also exists in three other *Bacillus* species *B. anthracis*, *B. cereus* and *B. halodurans* and these proteins exhibit 60% sequence identity. Sequence comparison of the *Bacillus* HutP proteins revealed that the C-terminal amino acid residues are more conserved than the N-terminal residues. Interestingly, the HutP protein binds to the terminator region within the *hut* mRNA and enhances *hut* structural gene expression only when activated by L-histidine. Several lines of evidence have indicated that HutP regulates the expression of the downstream genes of the *hut* operon by an anti-termination mechanism (Oda et al., 1988; Wray and Fisher, 1994; Oda et al., 2000).

* Corresponding author. Fax: +81 29 861 6773.
E-mail address: pkr-kumar@aist.go.jp (P.K.R. Kumar).
[1] Present address: Department of Biochemistry and Molecular Biology, Gordon Center for Integrative Science, University of Chicago, Chicago, IL 60637, USA.

1047-8477/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jsb.2013.05.019

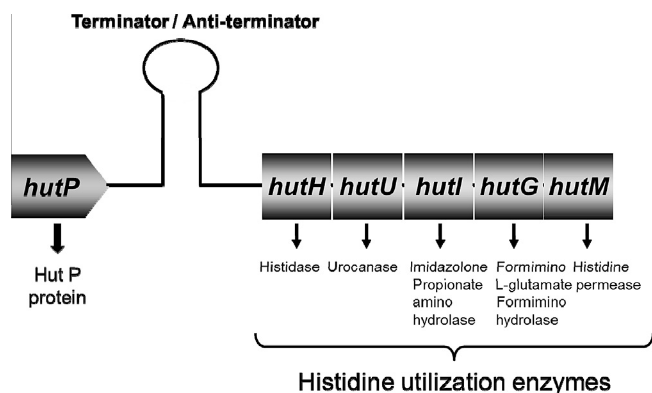

Fig.1. Schematic representation of the *hut* operon of *Bacillus subtilis*. The HutP, terminator/anti-terminator and structural genes are shown.

Metal ions in active metal-binding sites in proteins and RNAs are essential in many cellular processes. Divalent metal ions, such as Mg^{2+}, Zn^{2+}, Cu^{2+} and Ca^{2+}, are often associated not only with maintaining structure but also with the catalytic or regulatory activities of proteins that constitute some of the fundamental chemical life processes (Dudem and Lim, 2003). We previously reported that divalent metal ions in combination with the L-histidine ligand play an important role in the HutP-mediated regulation of the expression of the *hut* structural genes of *B. subtilis* by an anti-termination mechanism (Kumarevel et al., 2005a,b; Gopinath et al., 2008). The relationship between divalent metal ions and the L-histidine ligand is unique, and of particular importance to understanding the anti-termination mechanism in *B. subtilis*. The crystal structure of the HutP-L -histidine-Mg^{2+}-RNA ternary complex demonstrated that Mg^{2+} has six coordination bonds, three with histidine residues, one each with the backbone nitrogen and carboxyl oxygen of the L-histidine ligand, and one with a water molecule (Kumarevel et al., 2005b). L-histidine-Mg^{2+} binds indirectly to the UAG motif of the target RNA via Arg98 and Thr99 residues, indicating an important role of Mg^{2+} and L-histidine in mediating HutP–RNA interactions.

Further analyses of the effectiveness of various types of metal ions have been performed (Kumarevel et al., 2005a). Among 15 divalent metals, only Cu^{2+}, Yb^{2+} and Hg^{2+} failed to support HutP–RNA interactions, whereas Mn^{2+}, Zn^{2+} and Cd^{2+} were the best metal ions. Monovalent ions, such as Na^{+} and K^{+}, could not substitute for the divalent ions in the HutP–RNA interactions. Previously, we also solved the crystal structures of the HutP-L-histidine-Mn^{2+} and HutP-L-histidine-Ba^{2+} complexes (Kumarevel et al., 2005a) and showed that those divalent metal ions have six-coordinate geometries, as observed for Mg^{2+} (Kumarevel et al., 2005b). In contrast to these metal ions, Zn^{2+} is expected to be four coordinate because the coordination number of Zn^{2+} in proteins is preferably four (Dokmanic et al., 2008). To investigate the Zn^{2+} coordination geometry in the ternary HutP complex and to determine why Zn^{2+} is the best metal ion to mediate the activation of RNA binding, the crystallization of the HutP-L-histidine-Zn^{2+} complex was attempted. After many trials, crystals were obtained under conditions different from the crystallization conditions of the complexes using Mg^{2+}, Mn^{2+} and Ba^{2+}. The crystal structure was solved at a resolution of 2.5 Å, and the structure of HutP is very similar to that of the HutP-L-histidine-Mg^{2+}-RNA complex[15]. Exceptions were observed in the active site: (1) Zn^{2+} forms a distorted four-coordinate complex, and (2) the orientation of the L-histidine ligand coordinated to Zn^{2+} is reversed relative to the orientations with Mg^{2+}, Mn^{2+} and Ba^{2+}, resulting in the coordination of the Nε atom of the imidazole ring of the L-histidine ligand to Zn^{2+}. In this paper, we describe these structural differences and comparisons in relation with the RNA-binding activity.

2. Materials and methods

2.1. Expression and purification of the HutP protein

The single point mutant Val 51Ile originated spontaneously during our studies on *hut* operon in *B. subtili*. This mutant HutP has higher affinity to the Hut mRNA compared to the wild-type in the presence of L-histidine and metal ions. The nucleotide sequence encoding HutP containing the Val 51 Ile mutation was amplified and cloned into the pET5a vector (Promega, USA). The resultant plasmid was transformed into *E. coli* strain BL21 (DE3). The HutP protein was overexpressed at mid-log phase by the addition of isopropyl-β-D-thiogalactopyranoside (1 mM) and was purified as described previously (Kumarevel et al., 2004a). To remove the endogenous bound metal ions, the purified HutP protein was initially denatured in 7 M urea followed by dialyzed for 4–5 days against HutP equilibration buffer (15 mM Hepes, pH 7.4, 50 mM NaCl) containing 10 mM EDTA and was then dialyzed in the absence of EDTA in HutP equilibration buffer. These samples were used for crystallization.

2.2. Crystallization of the HutP-L-his-Zn^{2+} complex

Crystals of the HutP-L-histidine-Zn^{2+} ternary complex were grown at 20 °C by the hanging drop vapor diffusion method. The drops consisted of 2 μl of protein solution (10 mg/ml), 1 μl of zinc acetate (10 mM), 2 μl of 0.2 M L-histidine and 1 μl of well solution (30% PEG2K MME, 0.1 M Hepes, pH 7.6, and 0.5 M KBr). Diffraction data were collected with a charge-coupled device (ADSC) detector in the BL-NW12 at the Photon Factory, Tsukuba, Japan. Data sets for the ternary complex structure were processed at 2.3 Å with the HKL2000 suite of programs. The ternary complex crystals belonged to the P3 space group.

2.3. Structure determination

The crystal structure of the HutP-L-histidine-Zn^{2+} complex was determined by molecular replacement using the native HutP protein as a search model (PDB ID 1VEA). Both molecular replacement and structure refinement were carried out using CCP4i (Collabora-

Table 1
Data collection and refinement statistics of Hutp-L-histidine-Zn^{2+}complex.

Data collection	
Space group	P3
Unit cell (Å,°)	$a = b = 129.33$, $c = 76.67$ and $\gamma = 120.0$
Solvent Content (%)	47.01
Number of molecules in ASU	12
Resolution range (outer shell)	50–2.3(2.38–2.30)
No. of independent reflections	57983
Redundancy	5.0(2.7)
Completeness(%)	90(52)
R_{merge}	11.9(51.5)
Refinement statistics	
Resolution (Å)	37.11–2.5
No. of reflections used for refinement	46338
R_{factor}	0.219
No. of reflections used for R_{free}	2508
R_{free}	0.297
Average B-factor (Å^2)	57.16
RMS deviation from ideal geometry	
Bond lengths (Å)	0.011
Bond angle (°)	1.626

Values in parentheses refer to the high-resolution shell.

Download English Version:

https://daneshyari.com/en/article/5914383

Download Persian Version:

https://daneshyari.com/article/5914383

Daneshyari.com