doi:10.1016/j.jmb.2011.01.049 J. Mol. Biol. (2011) 407, 556–570

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Insights into Metalloregulation by M-box Riboswitch RNAs via Structural Analysis of Manganese-Bound Complexes

Arati Ramesh*, Catherine A. Wakeman and Wade C. Winkler*

Department of Biochemistry, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Received 24 October 2010;
received in revised form
18 January 2011;
accepted 25 January 2011
Available online
15 February 2011

Edited by J. Doudna

Keywords:
riboswitch;
magnesium–RNA interaction;
RNA folding;
RNA structure;
X-ray crystallography

The M-box riboswitch couples intracellular magnesium levels to expression of bacterial metal transport genes. Structural analyses on other riboswitch RNA classes, which typically respond to a small organic metabolite, have revealed that ligand recognition occurs through a combination of base-stacking, electrostatic, and hydrogen-bonding interactions. In contrast, the M-box RNA triggers a change in gene expression upon association with an undefined population of metals, rather than responding to only a single ligand. Prior biophysical experimentation suggested that divalent ions associate with the M-box RNA to promote a compacted tertiary conformation, resulting in sequestration of a short sequence tract otherwise required for downstream gene expression. Electrostatic shielding from loosely associated metals is undoubtedly an important influence during this metal-mediated compaction pathway. However, it is also likely that a subset of divalent ions specifically occupies cation binding sites and promotes proper positioning of functional groups for tertiary structure stabilization. To better elucidate the role of these metal binding sites, we resolved a manganese-chelated M-box RNA complex to 1.86 Å by X-ray crystallography. These data support the presence of at least eight well-ordered cation binding pockets, including several sites that had been predicted by biochemical studies but were not observed in prior structural analysis. Overall, these data support the presence of three metal-binding cores within the M-box RNA that facilitate a network of long-range interactions within the metal-bound, compacted conformation.

Introduction

All organisms require metal ions for many essential molecular functions, including catalytic mechanisms and cellular structural properties. It is injurious to bacterial growth when metal concentrations are inappropriately increased or decreased; therefore, specific mechanisms are required for maintaining intracellular pools. Metal-sensing regulatory (metalloregulatory) proteins coordinate metals with gene expression of transporters, metal-sequestering proteins, detoxification genes, and other factors.[1–3] Many examples of such metalloregulatory proteins have been discovered and characterized, mostly for control of transition metals. Less studied are regulatory mechanisms for the most prevalent ions in the intracellular environment of a bacterium, namely, potassium and magnesium, which are present at concentrations of approximately 110 mM and 0.5–1.0 mM, respectively.[4–7] More recently, two different

*Corresponding authors. E-mail addresses:
arati.ramesh@utsouthwestern.edu;
wade.winkler@utsouthwestern.edu.

Abbreviations used: NMIA, *N*-methylisatoic anhydride; AUC, analytical ultracentrifugation; SHAPE, selective 2′-hydroxyl acylation analyzed by primer extension; PDB, Protein Data Bank.

metal-sensing regulatory RNAs have been discovered to function as metalloregulatory elements for the control of magnesium homeostasis.[8,9] In both of these instances, increased magnesium levels resulted in an altered RNA structural conformation for an RNA element located within the mRNA 5′ leader region. This conformational change is coupled with the regulation of downstream genes. Understanding the molecular basis for these RNA-based mechanisms will provide important insights into metal-induced RNA folding pathways and will reveal the general importance of magnesium homeostasis control strategies.

Metal ions share a complicated relationship with nucleic acids,[10–13] playing a critical role in the formation of both secondary and tertiary structures. In general, positively charged metal ions aid in neutralization of the highly negative RNA backbone, enabling the formation of complex bends, folds, and long-range contacts characteristic of complex RNA structures. However, RNA-chelated ions have only been observed in small numbers; there are not enough ions to adequately shield the electronegative backbone by themselves. Instead, a concentration gradient of fully hydrated metals becomes aligned with the electrostatic potential surrounding the RNA, thus creating a diffuse "atmosphere" of ions. Various degrees of interaction between metal ions and RNA have been reported, ranging from loose or indirect association to direct coordination of the metal ion. The latter often involves the displacement of one or more water molecules from the inner-hydration shell of the metal ion and the substitution, instead, with functional groups from the RNA. Magnesium ions typically contact RNA oxygen atoms (mostly the nonbridging phosphoryl oxygens of the backbone) and, to a lesser extent, the 2′-OH groups of the ribose sugar and nucleobase oxygens (e.g., guanine O6 carbonyls and pyrimidine O4 groups). Less frequently, magnesium ions contact RNA nitrogens, such as the guanine N7 nitrogen.[14] It is through the cumulative effect of these interactions that many RNAs compact into their native conformational state. These interactions have been well illustrated by classic structural studies of ribozymes such as the P4–P6 domain of the group I intron and, more recently, riboswitch RNAs.

Riboswitches are cis-acting regulatory RNAs in bacteria that associate with metabolites and regulate expression of downstream genes.[15–18] They are generally composed of two portions: a signal-responsive aptamer domain and a downstream region that directly exerts regulatory control over proximal genes. Most often, the binding of the appropriate metabolite ligand is coupled with regulatory control of translation initiation or premature transcription termination,[15–19] although at least one riboswitch class controls the intracellular lifetime of its associated mRNA.[20] There are over 20 reported riboswitch classes that associate to a variety of small-molecule ligands, including amino acids, amino sugars, nucleobase-containing metabolites, and enzyme cofactors. Additionally, two different riboswitch classes have been discovered, which respond not to metabolites but to magnesium ions instead.[8,9] Specifically, the *Salmonella enterica mgtA* magnesium transport gene is subjected to regulatory control by a magnesium-responsive cis-acting RNA element located within the *mgtA* 5′ leader region. Also, the M-box RNA, which was originally described as an "orphan" riboswitch,[21] controls gene expression specifically in response to fluctuations of intracellular magnesium.[9] This function is consistent with the observation that the M-box riboswitch is typically located upstream of the three primary classes of magnesium transport genes: CorA, MgtA, and MgtE.[22] *In vitro* studies of the M-box riboswitch demonstrated that magnesium is capable of eliciting a dramatic conformational change for the aptamer domain.[9] However, this observation by itself is not surprising. Indeed, recent biophysical data have suggested that divalent ions alone can promote a near-native, compacted conformation for several riboswitch RNA classes, a structural configuration that resembles in part the final metabolite-bound state.[23–25] Although most riboswitches are likely to exhibit a structure-dependent relationship with monovalent and divalent ions, metal-dependent folding of the M-box RNA, by definition, must exhibit unique performance characteristics that enable it to function as a magnesium-responsive genetic element within the intracellular environment. Understanding the basis for regulation by the M-box riboswitch will help reveal the underlying principles used by metal-sensing regulatory RNAs in bacteria and may lead to the molecular engineering of synthetic metalloregulatory RNAs as genetically encoded metal sensors. Given the pivotal relationship between magnesium homeostasis mechanisms and bacterial pathogenesis,[7,26,27] the analysis of the M-box riboswitch is also likely to provide clues into the mechanisms used by intracellular bacterial pathogens for modulation of the divalent ion conditions within the phagosome where they reside.

Equilibrium measurements of folding of the M-box aptamer tertiary structure by hydroxyl radical footprinting and analytical ultracentrifugation (AUC) revealed that divalent ions triggered a compacted tertiary conformation.[9] Mutation of highly conserved residues prevented formation of this conformation. Also, the presence of cobalt(III) hexammine, which is isosteric with hexahydrated magnesium but is unable to make inner-sphere coordinations due to tight coordination of the amine ligands, did not elicit formation of the tertiary conformation. Together, these results suggested that individual cation sites were specifically required for tertiary structure formation, including a requirement