doi:10.1016/j.jmb.2006.03.059 J. Mol. Biol. (2006) 359, 679–689

JMB

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

New Roles for Key Residues in Helices H1 and H2 of the *Escherichia coli* H-NS N-terminal Domain: H-NS Dimer Stabilization and Hha Binding

Jesús García[1], Cristina Madrid[2], Antonio Juárez[2] and Miquel Pons[1,3]*

[1]*Laboratory of Biomolecular NMR, Institut de Recerca Biomèdica-Parc Científic de Barcelona, Josep Samitier 1-5 08028 Barcelona, Spain*

[2]*Departament de Microbiologia Universitat de Barcelona Diagonal, 645 08028 Barcelona Spain*

[3]*Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès 1-11 08028 Barcelona, Spain*

**Corresponding author*

Bacterial nucleoid-associated proteins H-NS and Hha modulate gene expression in response to environmental factors. The N-terminal domain of H-NS is involved in homomeric and heteromeric protein–protein interactions. Homomeric interaction leads to the formation of dimers and higher oligomers. Heteromeric interactions with Hha-like proteins modify the modulatory properties of H-NS. In this study, we have used NMR and mutagenesis of the N-terminal domain of H-NS to identify the Hha-binding region around helices H1 and H2 of H-NS. Two conserved arginine residues, R12 and R15, located in the same side and in adjacent turns of helix H2 are shown to be involved in two different protein-protein interactions: R12 is essential for Hha binding and does not affect H-NS dimer formation, and R15 does not affect Hha binding but is essential for the proper folding of H-NS dimers.

Our results demonstrate a close structural connection between Hha–H-NS interactions and H-NS dimerization that may be involved in a possible mechanism for the modulation of the H-NS regulatory activity by Hha.

Keywords: nucleoid-associated proteins; protein–protein interactions; protein NMR; H-NS mutagenesis; Hha

Introduction

Members of the H-NS family of proteins play roles both as architectural proteins and as modulators of gene expression in Gram-negative bacteria.[1] The H-NS protein, best characterized in different enteric bacteria such as *Escherichia coli* and *Salmonella enterica* serovar Typhi, participates in modulatory processes that respond to environmental changes such as osmolarity, pH or variations of temperature.[2] H-NS-modulated operons usually contain two target sequences, which have been often characterized as AT-rich curved DNA stretches. A current hypothesis that is supported by experimental evidence considers that, upon binding of H-NS to their target sequences, an oligomerization process occurs. A DNA loop is then generated, thereby bringing both target sites into closer contact. The resulting nucleoprotein complex influences expression of the genes affected.[3–5] Temperature and/or other environmental factors may influence the ability of H-NS itself to dimerize/oligomerize and to facilitate the interaction with DNA. In fact, it has been shown recently that temperature or osmolarity variations modify the oligomerization state of H-NS.[6,7] In addition to specific effects of environmental factors on the ability of H-NS to oligomerize, post-translational modifications[8] or interaction with other proteins[9] are processes that most likely influence the modulatory properties of H-NS.

The H-NS protein consists of two domains connected by a flexible linker.[1] The ability of the H-NS protein to generate higher-order oligomers and to interact with other proteins depends on the N-terminal domain. The C-terminal domain contains the DNA-binding motif, and appears to play a role in protein–protein interactions.[10]

Several truncated forms of H-NS: $H\text{-}NS_{64}$,[11] $H\text{-}NS_{57}$[12] and $H\text{-}NS_{46}$[13] (containing the first 64, 57 and 46 residues, respectively, after the N-terminal methionine) have been used to obtain structural data on the N-terminal domain of H-NS. $H\text{-}NS_{46}$ has been reported to be the smallest dimerization

Abbreviations used: HSQC, heteronuclear single-quantum correlation; TCEP, Tris(2-carboxyethyl)-phosphine; WT, wild-type.

E-mail address of the corresponding author: mpons@ub.edu

unit, with a minimum tendency to form larger oligomers.[13] Two different dimer structures have been observed in similar, but not identical, constructs. In both cases, the secondary structure comprises three helical segments (H1 to H3) separated by loops. However, H-NS_{46} from either *E. coli*[13] or *Vibrio cholerae*[14] has antiparallel topology, while H-NS_{57} from *Salmonella typhimurium* forms a parallel dimer.[12] In both models, the core of the homodimer is a coiled-coil structure formed by intermonomer hydrophobic interactions between residues of helices 3, although helix 1 and 2 are involved in dimer-stabilizing interactions.

The different experimental conditions under which data were collected and the presence of additional residues in the C terminus may explain the different folds. A recent report suggesting that the active form of H-NS is a tetramer with simultaneous parallel and antiparallel topologies between different pairs of protomers provides a model to reconcile both structural models for N-terminal H-NS dimers.[10]

Extensive mutational studies have been carried out to identify specific residues or regions involved in H-NS function. Several point mutations in the N-terminal domain of H-NS produce functionally defective H-NS proteins. Mutations located in helix H3, such as mutations in L26[15] or L30,[16] resulted in dominant negative activity, although it has been postulated that these mutations cause important structural effects, disturbing the coiled-coil structure of the H-NS homodimer.[13] This assumption has been supported recently by two-hybrid experiments showing that L26P, L30P and L33P mutations affect the *in vivo* self-association of H-NS to form tetramers, although *in vivo* dimerization was hardly affected.[10]

H-NS high-order oligomerization is also affected by modifications in helix H2. It has been reported that both a truncated version of H-NS containing residues 13–90, or a mutant containing a helix-disrupting proline residue at position 17 in helix H2 show impaired oligomerization. This has been suggested as evidence for the interaction in a head-to-tail manner of H2 with the H-NS linker domain, assumed to be responsible for oligomerization processes.[12] Point mutations in H2 including arginine residues in positions 12 or 15 lead to H-NS loss of repression function *in vivo*. Replacement of those residues by either histidine or cysteine resulted in derepression of the *proV* operon *in vivo* under low osmolarity conditions. The purified R12C mutant protein showed oligomerization and *in vitro* DNA-binding properties similar to those of wild-type (WT) H-NS. In particular, both proteins bind to the *proV* promoter with nearly identical affinity.[17] Other authors have shown that the R12E/R15A H-NS double mutant is able to bind an intrinsically curved DNA *in vitro*, although with a significant reduction in the binding affinity compared to that of WT H-NS. These authors reported that, in the context of the H-NS_{64} fragment or of the full-length protein, those mutations do not alter the folding of the protein. On the basis of these observations, a role in nucleic acid binding has been proposed for the N-terminal domain of H-NS, involving specifically the positively charged residues R12, R15 and K19 that are aligned along one side of the amphipatic helix H2.[13]

The dimerization domain of H-NS is involved in heteromeric interactions with H-NS-related proteins, such as StpA, and with members of the Hha family of proteins that lack direct DNA-binding activity.[15,18] Hha has been described as a modulator of bacterial virulence in response to environmental factors such as osmolarity or temperature.[19] Protein–protein interaction studies have shown that Hha-like proteins interact with members of the H-NS family of proteins,[20] and Hha–H-NS complexes have been shown to modulate gene expression. Interaction with Hha strengthens the ability of H-NS to repress under non-permissive conditions, among others, the *hly* operon,[21] the *htrA* gene,[22] or the *tra* gene from the conjugative plasmid R27.[23]

In a previous study from our group, the interaction between *E. coli* Hha and H-NS_{64} was confirmed by fluorescence anisotropy and by NMR, using ^{15}N-labelled Hha and unlabelled H-NS_{64}. The interaction was shown to occur with a stoichiometry of one Hha molecule per two H-NS_{64} monomers or one Hha molecule per H-NS_{64} dimer, and involved a substantial conformational change in Hha that exposed residues deeply buried in its hydrophobic core.[18]

Here, we present an NMR study of the interaction between ^{15}N-labelled H-NS_{46} and Hha. H-NS_{46} was chosen for this study because it is the smallest construct reported to contain a functional dimerization domain of H-NS and its three-dimensional structure is known.[13] In addition, we have studied the effect of two single-point mutations involving residues R12 and R15 of H-NS that are known to cause a strong loss of function phenotype *in vivo*. Interestingly, in spite of their proximity in helix H2 of H-NS, we have found that both mutations have completely different effects *in vitro*, involving two different protein–protein interactions: R12 is required for Hha binding, while R15 is involved in H-NS dimerization but is not essential in the heteromeric interaction with Hha.

Results

Hha binding to H-NS_{46}

Figure 1(a) shows a heteronuclear single-quantum correlation (HSQC) NMR spectrum of [^{15}N]H-NS_{46} displaying a single set of well-dispersed amide signals. H-NS_{46} forms a dimer in solution with a dissociation constant lower than 5 nM.[13] The NMR data confirm that the dimer is well folded and symmetrical in solution. HSQC spectra display separate signals from each NH pair and provide a powerful tool to identify residues involved in