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On the Origin of the Selectivity of Plasmidic H-NS towards Horizontally Acquired DNA: Linking H-NS Oligomerization and Cooperative DNA Binding

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Abstract

The nucleoid-associated protein H-NS is a global modulator of the expression of genes associated with adaptation to environmental changes. A variant of H-NS expressed in the R27 plasmid was previously shown to selectively modulate the expression of horizontally acquired genes, with minimal effects on core genes that are repressed by the chromosomal form of H-NS. Both H-NS proteins are formed by an oligomerization domain and a DNA-binding domain, which are connected by a linker that is highly flexible in the absence of DNA.

We studied DNA binding by means of oligomer-forming chimeric proteins in which domains of the chromosomal and plasmidic variants are exchanged, as well as in monomeric truncated forms containing the DNA-binding domain and variable portions of the linker. Point mutations in the linker were also examined in full-length and truncated H-NS constructs. These experiments show that the linker region contributes to DNA binding affinity and that it is a main component of the distinct DNA binding properties of chromosomal and plasmidic H-NS.

We propose that interactions between the linker and DNA limit the flexibility of the connection between H-NS oligomerization and DNA binding and provide an allosteric indirect readout mechanism to detect long-range distortions of DNA, thus enabling discrimination between core and horizontally acquired DNA.

Introduction

Genetic diversity originating from horizontal gene transfer (HGT) is a major contributor to evolution and responsible for the capacity of bacteria to colonize new environments, including human hosts. The acquired genes are retained when they confer an adaptive advantage in a certain niche; however, gene acquisition may bring about a fitness cost.[1,2] Understanding the origin of the fitness cost of HGT may uncover strategies for fighting the acquisition of antibiotic resistance by gene transfer. One of the components of fitness cost is the integration of acquired genes in existing regulatory networks.[3,4] This cost grows roughly with the square of the total number of genes (old and new) when they are regulated as a single pool.[5] However, when HGT and core genes are regulated independently, the fitness cost is significantly reduced.

Enterobacterial species have developed precisely this strategy through at least two independent mechanisms related to the H-NS protein. H-NS is an abundant nucleoid-associated protein that contributes to DNA compaction and acts as a global regulator of the expression of a large number of genes in Gram-negative bacteria.[6] Around 5% of the genes of *Escherichia coli* are modulated by H-NS.[7] H-NS also plays a key role in silencing externally acquired (xenogeneic) DNA,[8–11] including the major virulence genes of pathogenic *E. coli*, *Salmonella*, *Shigella* and *Yersinia*,[11,12] among others.

H-NS-like proteins are not encoded only in the bacterial chromosome. Several conjugative plasmids also encode H-NS homologues. Baños *et al.* demonstrated that chromosomal and plasmidic H-NS proteins have distinct functional properties.[13] The H-NS variant encoded in the IncHI plasmid R27 ($H\text{-}NS_{R27}$) rescued the phenotype of a *Salmonella* mutant lacking chromosomal H-NS ($H\text{-}NS_C$) for HGT genes but had no effect on most of the core genes. Furthermore, they showed that the set of genes regulated by $H\text{-}NS_{R27}$ coincided mostly with those that required a co-regulator of the Hha/YmoA family for proper silencing by $H\text{-}NS_C$. The presence of two redundant mechanisms for the same function suggests that selective regulation of genes acquired through HGT confers a significant evolutionary advantage.

Here we describe a systematic study aiming to identify the key structural elements responsible for the distinct DNA binding and regulatory properties of *Salmonella* $H\text{-}NS_C$ and $H\text{-}NS_{R27}$.

Both H-NS proteins contain an oligomerization domain and a DNA-binding domain (DBD), which are separated by a linker that is highly flexible in the absence of DNA.

Two dimerization regions connected by a long α-helix form the oligomerization domain. The N-terminal region, including residues 1–47, forms stable dimers.[14] The second dimerization site includes residues 57–83 and was recently described by Arold *et al.* in the crystal structure of an oligomer formed by a construct including the first 83 residues of H-NS.[15] In the crystal structure, H-NS oligomers adopt a superhelical structure with individual H-NS molecules linked by alternating head-to-head and tail-to-tail dimers.

The structures of the H-NS DBDs of *Salmonella* and *E. coli* have been solved in the apo-form.[16,17] The structures of the folded domains are very similar and are formed by a two-stranded antiparallel β-sheet (residues 97–100 and 105–109; residue numbers refer to *Salmonella* H-NS), an α-helical segment (residues 117–126) and a short 3_{10} helix (residues 130–133).

A DNA complex of the DBD of Ler, a member of the H-NS family, has recently been described.[18] Analysis of the complex and an extensive series of Ler and H-NS mutants demonstrated that the isolated Ler and H-NS DBDs recognize specific distortions in the minor groove of DNA that deviate from the canonical B DNA form.[16,18–20] Thus, the recognition of target DNA by individual H-NS molecules follows an indirect readout model. The affinity of the isolated DBD of Ler and H-NS was shown to be rather low. However, DNA binding involves H-NS oligomers in which the cooperative binding of multiple DBDs increases affinity.[21] While the interaction of individual H-NS molecules involves short DNA stretches and is sensitive to local DNA distortions, the interaction between H-NS oligomers and DNA has the potential to sense DNA on a longer length scale. This sensing capacity thus enables the recognition of general features that could allow the distinction between broad DNA classes that may be associated with core or HGT genes. We hypothesized that specific DNA recognition depends on the spatial arrangement of DBDs along the oligomer, constrained by the oligomer's structure, which should be compatible with the shape of the DNA region to which they bind. Thus, allosteric interplay between oligomerization and DNA binding, potentially modulated by the linker region, may provide a mechanism for long-range indirect readout of broad DNA classes.

The distinct specificity of $H\text{-}NS_C$ and $H\text{-}NS_{R27}$ provides an opportunity to test this hypothesis. With this in mind, we prepared chimeras combining domains from the two H-NS variants, measured their DNA binding characteristics and compared them with the two natural H-NS forms. We further refined our analysis by zooming in on specific regions and exploring point mutations in the linker region shown to be a main locus for the differences observed between $H\text{-}NS_C$ and $H\text{-}NS_{R27}$.

Results

Protein chimeras combining $H\text{-}NS_C$ and $H\text{-}NS_{R27}$

The amino acid sequences of $H\text{-}NS_C$ and $H\text{-}NS_{R27}$ are shown in Fig. 1a. The secondary structure elements observed in the truncated constructs of chromosomal H-NS are indicated. Sequence differences between the two variants are concentrated mostly in the H3 helix of the oligomerization domain (O/o), the linker region (L/l) and the α-helix of the DBD (D/d). Capital letters refer to the domains of $H\text{-}NS_C$, and small letters refer to those of $H\text{-}NS_{R27}$. Four chimeras were prepared (Fig. 1b). Chimera α (oLD) contained the oligomerization domain of $H\text{-}NS_{R27}$ and the linker region and DBD of $H\text{-}NS_C$. Chimera β (Old) had the complementary arrangement (oligomerization domain of $H\text{-}NS_C$ and linker region and DBD of $H\text{-}NS_{R27}$). Chimera γ (OlD) contained the linker region of $H\text{-}NS_{R27}$ connecting the oligomerization domain and DBD of $H\text{-}NS_C$. Finally, chimera δ (oLd) had the linker region of $H\text{-}NS_C$ in the context of the oligomerization domain and DBD of $H\text{-}NS_{R27}$.

DNA binding was studied by electrophoretic mobility shift assays (EMSAs) using a 540-bp fragment ($proU_{540}$) containing the regulatory region of the *proU* promoter. The interaction of *proU*, a representative core gene, with H-NS has been extensively studied.[21,22] In a previous transcriptomic analysis, *proU* was insensitive to $H\text{-}NS_{R27}$ modulation.[13]