doi:10.1016/j.jmb.2006.09.043 J. Mol. Biol. (2006) 364, 777–798

JMB

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Mechanism of Chromosome Compaction and Looping by the *Escherichia coli* Nucleoid Protein Fis

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Fis, the most abundant DNA-binding protein in *Escherichia coli* during rapid growth, has been suspected to play an important role in defining nucleoid structure. Using bulk-phase and single-DNA molecule experiments, we analyze the structural consequences of non-specific binding by Fis to DNA. Fis binds DNA in a largely sequence-neutral fashion at nanomolar concentrations, resulting in mild compaction under applied force due to DNA bending. With increasing concentration, Fis first coats DNA to form an ordered array with one Fis dimer bound per 21 bp and then abruptly shifts to forming a higher-order Fis–DNA filament, referred to as a low-mobility complex (LMC). The LMC initially contains two Fis dimers per 21 bp of DNA, but additional Fis dimers assemble into the LMC as the concentration is increased further. These complexes, formed at or above 1 μM Fis, are able to collapse large DNA molecules *via* stabilization of DNA loops. The opening and closing of loops on single DNA molecules can be followed in real time as abrupt jumps in DNA extension. Formation of loop-stabilizing complexes is sensitive to high ionic strength, even under conditions where DNA bending-compaction is unaltered. Analyses of mutants indicate that Fis-mediated DNA looping does not involve tertiary or quaternary changes in the Fis dimer structure but that a number of surface-exposed residues located both within and outside the helix-turn-helix DNA-binding region are critical. These results suggest that Fis may play a role *in vivo* as a domain barrier element by organizing DNA loops within the *E. coli* chromosome.

Keywords: chromosome structure; non-specific DNA binding; DNA looping; nucleoprotein filament; single-DNA molecule micromanipulation

Introduction

The prokaryotic nucleoid is a condensed but highly dynamic nucleoprotein structure. The *Escherichia coli* nucleoid contains a single circular chromosome comprising about 1.5 mm of DNA packaged into a volume of about 0.2 μm^3. Even further condensation is required during rapid growth when several chromosome equivalents are present in the cell due to multiple ongoing replication cycles. Because bacteria do not contain histones or stably wrapped nucleoprotein complexes analogous to nucleosomes, packaging of the chromosome into the prokaryotic nucleoid must differ radically from DNA packaging in eukaryotic cells. The *E. coli* chromosome is thought to be organized as a dynamic ensemble of topologically isolated supercoiled loops or domains.[1,2] Several independent

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Abbreviations used: HTH, helix-turn-helix; LMC, low-mobility complex; BSA, bovine serum albumin; Fis-OP, Fis site-specifically coupled to the chemical nuclease 1,10 phenanthroline-copper; orthophenathroline-copper.

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lines of evidence suggest the connections isolating these domains are spaced every 10 kb on average during rapid growth, but determination of the domainin factors responsible for this organization has been elusive.[3,4]

Additional compaction is believed to be mediated by a set of abundant nucleoid-associated, DNA-bending proteins. The dominant nucleoid proteins in growing *E. coli* are HU, IHF, H-NS (and its paralog StpA) and Fis.[5–7] Single-DNA studies have shown that HU compacts DNA when bound at subsaturating concentrations.[8–10] Sequence-independent binding by IHF has also been shown to compact λ phage chromosomes *in vitro*.[11] AFM experiments have shown that H-NS can form filaments containing two DNA duplexes, suggesting that H-NS may have a domainin activity.[12] Cellular levels of Fis vary widely depending on growth conditions but, as noted below, Fis can be the dominant DNA-binding and bending protein in the cell.[13,14] A nucleoid architecture role for Fis was suggested by Schneider *et al*.[15] based on observations of enhanced branching of Fis-bound supercoiled DNA. Here, we show that Fis can function both to directly stabilize loops in linear DNA (i.e. has domainin activity *via* stabilizing DNA nodes) as well as to promote DNA compaction by means of its bending activity.

Fis has been studied most extensively in the context of its ability to regulate specific DNA transactions. The protein was identified initially as a factor required for the Hin and Gin-catalyzed site-specific DNA inversion reactions and, as such, was named *f*actor for *i*nversion *s*timulation.[16,17] Subsequently, Fis was found to regulate λ phage site-specific recombination reactions, *ori*C and plasmid replication, and specific transcription reactions.[5,18] Each of these diverse reactions is typically controlled by one or more Fis dimers binding specific sites on DNA. Selective Fis binding to specific DNA sites at the low nanomolar range can be demonstrated by nuclease and chemical footprinting assays in the presence of excess mixed-sequence or homopolymeric competitor DNA. These sites are related by a highly degenerate 15 bp sequence motif, which can

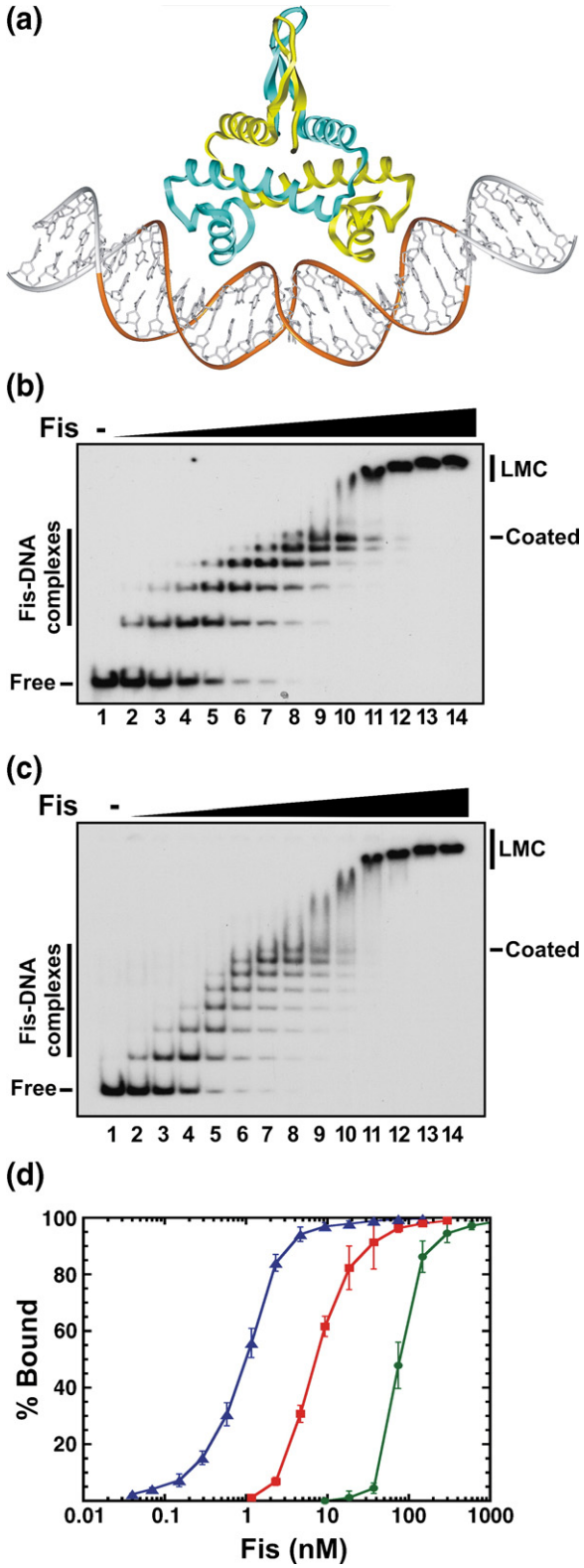

Figure 1. Sequence-independent Fis binding to DNA. (a) A model of a Fis–DNA complex. The two helix-turn-helix motifs, spaced by 25 Å, bind successive major grooves on one side of the DNA helix, forcing a bend into the double helix. The mobile N-terminal β-hairpin arms protrude from the side opposite the DNA. The gold ribbon over the DNA backbone denotes the 21 bp minimal binding site. The structure of Fis in this and subsequent Figures is from the K36E mutant, in which residues 10–98 that include the β-hairpin arms are resolved,[24] and it is modeled onto DNA with an overall curvature of about 50°.[20] (b) Gel mobility-shift assay of Fis binding to a 100 bp DNA fragment from phage λ. Fis was added in twofold increments, beginning at 0.15 nM (lane 2) and ending at 600 nM (lane 14). No Fis was added in lane 1. The locations of individual Fis–DNA complexes, the "coated" complex, the "low-mobility complex" (LMC), and the unbound (Free) DNA are denoted. (c) Same as (b), except that a 149 bp fragment from the yeast actin gene was the DNA substrate. (d) Fis binding isotherms of the complex bound by one or more Fis dimers (left curve, blue triangles), the coated complex containing seven or more Fis dimers (middle curve, red squares), and the LMC complex (right curve, green circles) assembled on the actin DNA fragment. Points represent the mean and standard deviations obtained from ≥4 independent experiments. Hill coefficients for the first complex, the coated complex, and the LMC are 1.5, 1.5, and 3.5, respectively.