Experimental report

16/03/2016

Proposal: 9-13-515 **Council:** 10/2012

Title: Distribution of bacterial architectural protein H-NS around DNA

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DNA/Protein/H2O/D2O/KCL/MgCl2

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D11	2	2	08/05/2013	10/05/2013
D22	2	0		

Abstract:

Nucleoid Associated Proteins (NAPs) play a key role in the compaction and expression of the prokaryotic genome. The mechanism involves stiffening of the duplex and bridging of distant, like-charged segments by protein-mediated attraction. In previous SANS work, we measured the distribution of polyamines around DNA. Here, we will focus on the distribution of a generic NAP, the bacterial protein H-NS. For this purpose, we propose SANS experiments with solvent contrast matching in order to derive the DNA-H-NS and H-NS partial structure functions. The structure functions will be interpreted in terms of a radial distribution of protein density and possible protein density correlation along the duplex in register with the phosphate moieties.

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Structure of the H-NS–DNA nucleoprotein complex†

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Nucleoid associated proteins (NAPs) play a key role in the compaction and expression of the prokaryotic genome. Here we report the organisation of a major NAP, the protein H-NS on a double stranded DNA fragment. For this purpose we have carried out a small angle neutron scattering study in conjunction with contrast variation to obtain the contributions to the scattering (structure factors) from DNA and H-NS. The H-NS structure factor agrees with a heterogeneous, two-state binding model with sections of the DNA duplex surrounded by protein and other sections having protein bound to the major groove. In the presence of magnesium chloride, we observed a structural rearrangement through a decrease in cross-sectional diameter of the nucleoprotein complex and an increase in fraction of major groove bound H-NS. The two observed binding modes and their modulation by magnesium ions provide a structural basis for H-NS-mediated genome organisation and expression regulation.

1 Introduction

The prokaryotic genome is highly compacted in the nucleoid, despite the lack of scaffolding in terms of lipid membranes and/or chromatin organization.[1] Although much work has been done to elucidate the mechanisms involved in stabilising the compacted state, the structural arrangement of the condensing agents near DNA is not clear. In previous small angle neutron scattering (SANS) works, we have measured the distribution of ions, including polyamines, around DNA.[2–6] More recently, we have investigated the structure of the bacterial protein Hfq bound to DNA.[7] Here, we describe the structure of the complex formed by the generic nucleoid associated protein H-NS (histone-like nucleoid structuring protein, M_w = 15.6 kDa) with double-stranded DNA. H-NS plays a global role in gene regulation and represses hundreds of genes, most of which are involved in the adaptation to stress, virulence and chemotaxis.[8] It exists as a dimer as well as higher oligomeric forms and binds DNA with a preference for curved or AT-rich sequences. These sequences serve as nucleation sites for oligomerization of the protein along the duplex; thus covering regions as long as 1.5 kbp *in vivo*.[9] H-NS binding results in an increase in bending rigidity and/or bridging of distant, like-charged segments of the DNA molecule by protein-mediated attraction.[10,11] The relative importance of bridging and stiffening depends on buffer composition and, in particular, the presence of divalent cations (Mg^{2+}).[12,13] The formation of the nucleoprotein complex has been proposed to be the structural basis for H-NS-mediated gene silencing.[14–17]

Detailed structural information on the binding of H-NS to DNA is scarce. Two distinguishable H-NS binding states have been identified, depending on the interaction of specific and nonspecific DNA target sites.[18] H-NS specifically binds to the minor groove of double-stranded DNA with a short C-terminal loop.[19] Nonspecific binding is thought to be predominantly controlled by electrostatics and is much more prone to variation in ionic strength. Here, we describe a SANS study of H-NS complexed to rod-like DNA fragments (contour length 54 nm) in solution with monovalent and divalent salts. The contributions to the scattering (structure factors) from DNA and H-NS are obtained using solvent contrast variation. The H-NS to DNA base-pair ratio was 1 : 6, so that the DNA fragments are almost fully covered with protein. Information on the arrangement of H-NS about B-form DNA is obtained by comparison of the H-NS structure factor with coarse-grained model calculations involving the radial distribution in amino acid density. Key structural features of the nucleoprotein complex are derived, including the cross-sectional radius of gyration and the extent to which

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