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Synergistic role of DNA-binding protein and macromolecular crowding on DNA condensation. An experimental and theoretical approach

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ABSTRACT

Genome packaging in prokaryotic cells is believed to be carried out by a combination of DNA-binding proteins, crowding effects, and DNA supercoiling. Much is known about DNA–protein interaction as well as the condensation of DNA due to excluded volume effects however, and to our knowledge, few studies have targeted the potential synergistic role of DNA-binding proteins and crowding agents on DNA condensation.

This work aims at assessing the effect of crowding, induced by polyethylene glycol (PEG), on DNA–H-NS binding, with H-NS a histone-like nucleoid structuring protein that is believed to play a crucial role in gene regulation. A non-monotonic behavior was found upon the addition of PEG to DNA–H-NS complexes; 100 mg mL^{-1} of PEG leads to the expansion of the DNA, while 200 mg mL^{-1} of PEG to a stronger DNA condensation, when compared to complexes in the absence of PEG. Such behavior was found at intermediate concentrations of H-NS. Spermine (Spm) was additionally studied, to assess the importance of the specificity of the DNA-binding agent. The synergistic effect of Spm and PEG on DNA condensation was very clear and especially large for the higher studied concentration of PEG. The effect of ionic strength was additionally looked upon. Two regimes were found; at low Spm concentrations, DNA ψ-condensation dominates and systems with higher ionic strength show a larger DNA condensation. At large Spm concentrations, on the other hand, a larger ionic strength leads to a more moderate DNA condensation. Synergism effects were found to be larger at low DNA–Spm concentrations and high ionic strengths.

Results from Monte Carlo simulations, using a very simple model to mimic the experimental studies, also indicate a synergistic effect of the DNA-binding and crowding agents on DNA condensation for intermediate concentrations of the DNA-binding agents. Since H-NS proteins are known to self-assemble in the presence of DNA, an attractive potential was also used in the protein model. It was found that such self-association is needed to induce DNA condensation and that strong synergism occurred solely for intermediate attractive potentials. It is suggested that crowding agents act to reduce the critical association concentration of the proteins.

1. Introduction

The structural and organizational design of genomic DNA of prokaryotes is very different from that of eukaryotic genome. Eukaryotic genomic DNA is organized in the form of chromatin and localized inside a membrane bound nucleus, whereas in most bacteria, genomic DNA is packed into compact structures called nucleoid [1]. For example, genomic DNA of *Escherichia coli* is around 4.6×10^6 bp (base pairs) in size and 1.4 mm long having to fit inside the available nuclear axis of 2–4 μm. In both cases DNA condensation is, at least partially, driven by a specific set of DNA-binding proteins. In eukaryotes these proteins are called histones. DNA wraps around the histones octamer, forming complexes called nucleosomes, which, in turn associate into 30 nm-thick chromatin fibers [2]. Bacterial cells, on the other hand, do not possess one single type of DNA-binding proteins. Instead they have a range of proteins called nucleoid associated proteins (NAPs), which are often referred to as histone-like proteins, since they perform the same DNA condensation role and transcription control. Out of the 12 species of NAPs that have been identified in *E. coli* cell few of them are very well characterized: H-NS, FIS, Dps, HU, Lrp, and IHF [3]. These proteins bind to DNA in a sequence independent manner (although some prefer to bind to AT-rich regions due to the larger flexibility of these tracks [4]) and induce conformational and topological changes in the DNA strands. Although their participation in cellular activities like replication, recombination, and regulation of transcription is reasonably established [5,6]; their role on DNA condensation is less clear. Histone-like nucleoid structuring protein (H-NS) predominantly occurs in exponentially growing cells and it binds to DNA close to RNA polymerase site [7]. Nucleoid condensation by H-NS has been studied using single molecule techniques such as AFM [8] and optical tweezers [9]. At lower concentration, H-NS exists as a dimer which binds to the DNA and forms

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bridges between distal DNA segments that result in DNA–protein–DNA complexes [9]. It is also to some extent clear that H-NS oligomerizes along the DNA strand enhancing its condensation [10,11].

Other main contributors for DNA condensation in bacterial cell are enzymatic supercoiling of DNA, and the metabolically active cytoplasm. The bacterial cellular space is filled with a large amount of molecules (300–400 g/L) [12] that affect the reaction rates of biological processes and macromolecular interactions, due to crowding effects. It is often assumed that crowding molecules do not interact directly with the biomolecules in the cell but are, due to their excluded volume, believed to enhance interactions between macromolecules by two to three orders of magnitude [13,14]. Crowding effects in bacteria, mostly given by accumulated metabolic products like globular proteins and RNA [15], play a crucial role in many cellular processes like protein folding, DNA replication, transcription, and condensation, by influencing the thermodynamic activity, conformational entropy and free energies of associating molecules [16]. The role of crowding on DNA condensation in bacterial cells was first proposed by Murphy and Zimmerman [17], who suggested that nucleoid structures are stabilized by the synergistic role of protein binding and macromolecular crowding agents. They have shown, using a centrifugation assay followed by gel electrophoresis, that crowding agents (albumin or polyethylene glycol (PEG) 8000) and DNA-binding agents (DNA-bending protein HU or spermidine) jointly reduce the amounts of each other required for DNA condensation. It has been also suggested that this synergism is a consequence of the larger diameter and lower charge density of the protein–DNA filaments as compared to the naked DNA, and so, it should be general [18]. This point was illustrated by showing the synergism of another DNA-bending protein, Sso7d, and PEG on DNA condensation, using the same experimental procedures and theoretical arguments [18].

In this study we have looked into the synergistic effect of DNA-bridging protein (H-NS) and molecular crowding (PEG) on DNA condensation using a dye exclusion assay. We have assessed the importance of binding specificity and electrostatic effects by replacing the H-NS with spermine (Spm), and varying the salt concentration, respectively. Monte Carlo simulations were also performed using very simple models to describe a bacterial cell.

2. Experimental section

2.1. Materials

Salmon sperm DNA of ≤2000 bp received as 10 mg mL^{-1} solution was purchased from Invitrogen and used as received. Spermine (≥97%) (Spm) and polyethylene glycol (PEG) of molecular weight of 3000 were purchased from Sigma-Aldrich and used as received. The fluorescent dye Gelstar (493/527) 10,000× stock solution in DMSO was supplied by Life technologies. All stock solutions were prepared with Milli-Q deionized water (18.2 M Ω/cm resistivity at 25 °C).

2.2. Over-expression and purification of H-NS

P^{SSA2} plasmid (with 6x-His tag at C terminal) expressing *Salmonella typhimurium* H-NS protein was a generous gift from William Wiley Navarre of the Dept. of Molecular Genetics, University of Toronto, Canada. This plasmid was used to transform into ER2566 strain, grown overnight at 37 °C on LB agar plates supplemented with 100 μg mL^{-1} of ampicillin. Transformed cells were cultured at 37 °C until an optical density of 0.6 at 600 nm was reached. H-NS expression was induced by the addition of IPTG (0.1 mM final concentration) and leaving the cells overnight at 18 °C. Protein purification was done as described earlier [19]. In short, the cells were harvested by centrifugation and H-NS was purified using Qiagen Ni-NTA fast start kit. A second purification step was included; the eluate from the kit method was loaded onto a Hi-trapQ (GE healthcare) anion exchange chromatography. The anion exchange chromatography was initially equilibrated with buffer A (10 mM Tris [pH 8.0], 1 mM dithiothreitol [DTT]), and H-NS was eluted by applying a gradient of 0 to 100% buffer B (10 mM Tris [pH 8.0], 1 M NaCl, 1 mM DTT). Purified H-NS protein was buffer exchanged to 20 mM Tris pH 7.2, 300 mM KCl and 10% glycerol and stored at −20 °C. The H-NS purity was checked by SDS PAGE and confirmed using MALDI mass spectrometry. Protein concentration was quantified using the Bradford assay with bovine serum albumin as relative standard curve.

2.3. Sample preparation

(DNA–Spm)–PEG, (DNA–H-NS)–PEG complexes were prepared by first adding a fixed volume (10 μL) of DNA (final concentration of 2 μg mL^{-1}) to equal volumes of Gelstar equilibrated for at least 30 min at room temperature, followed by addition of 80 μL H-NS or Spm solutions of varying concentrations in 20 mM Tris (pH-7.4) and 300 mM KCl, and gently mixing with pipette tip. The reaction tubes were equilibrated at room temperature for about 1 h followed by addition of PEG (final concentrations of 100 mg mL^{-1} or 200 mg mL^{-1}), and further equilibrated at room temperature for 1 h prior to analysis.

2.4. Steady state fluorescence spectroscopy

Steady state fluorescence spectra were recorded using the Tecan Infinite 200-PRO multifunctional plate reader. The fluorophore used for DNA was Gelstar nucleic acid stain (Lonza), which has an emission maximum (λ_{em}) at 527 nm and an excitation maximum (λ_{ex}) at 493 nm in the presence of DNA. In order to optimize the quality of the measurement, the 10,000× concentrated stock solution of Gelstar was diluted to 10× as final working concentration.

2.5. Determination of the radius of gyration of DNA

Size-exclusion chromatography (SEC) with multi-angle static light scattering (MALS) was used to determine the radius of gyration, R_G, of the Salmon sperm DNA. In brief, measurements were carried out at ambient temperature on an HPLC system consisting of a solvent reservoir (mobile-phase: 20 mM Tris, 300 mM KCl, pH 7), on-line degasser, HPLA isocratic pump, autoinjector, precolumn, and column (TSK G-6000PWXL). The column outlet was connected to a Dawn HeleosII multiangle laser light scattering photometer (Wyatt, USA) (λ_0 = 660 nm) followed by Optilab T-Rex differential refractometer, with a flow rate of 0.5 mL min^{-1}. The injection volume was 100 μL and the sample concentration was 0.5 mg mL^{-1}. Data from the light scattering and the differential refractometers were collected and processed using Astra 6.1 software (Wyatt, USA), using a refractive index increment $(dn/dc)_\mu$ of 0.134 mL g^{-1} [20].

The R_G of DNA was found to be 78.1 (±0.3) nm. The R_G of the PEG3,000 molecules was below the range of the instrument but could be found in the literature: 2.26 nm [21]. It is interesting to note that, for the lowest PEG concentration used (100 mg mL^{-1}) and taking into account the described R_G, the PEG molecules occupy nearly the entire volume of the solution, with a volume fraction of $\phi = 0.97$. This indicates that, under the studied conditions, the PEG solutions are close to and above their overlap concentration. It should, nevertheless, be mentioned that the volume occupied by the PEG (polymer coil) contains mostly solvent.

3. Monte Carlo simulations

3.1. Model

A simple model was adopted to describe a bacterial cell. A 120 monomer-long polyanion (model DNA) is described as a sequence of negatively charged hard spheres (monomers) connected by harmonic bonds with the chain flexibility regulated by angular force terms.

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