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Interactions between DNA and Lysozyme

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Abstract

Various techniques including AFM, ellipsometry, surface tensiometry, surface dilational rheology, and infrared reflection-absorption spectroscopy (IRRAS) were employed to probe the interactions between DNA and lysozyme within the surface layer. In the experiments, a concentrated DNA solution was introduced into an aqueous subphase beneath a dispersed lysozyme layer. Notably, when compared to DNA's interactions with a monolayer of a cationic synthetic polyelectrolyte, the optical properties of the surface layer exhibited rapid changes following DNA injection, while the dynamic dilational surface elasticity remained relatively stable. This observation suggests the absence of a continuous network of DNA/lysozyme complexes. The swift increase in optical signals following DNA injection behind a lysozyme layer implies that DNA penetration is predominantly governed by diffusion. Furthermore, AFM images vividly illustrate the formation of elongated strands within the surface layer under low surface pressures. In contrast, increased surface compression leads to the emergence of folds and ridges, rather than the formation of a network of DNA/lysozyme aggregates. These results suggest that weaker interactions between lysozyme and duplex DNA, along with the stabilization of unpaired nucleotide loops at high local lysozyme concentrations in the surface layer, contribute to the generation of more disordered aggregates.

Keywords: DNA • Lysozyme • Adsorption kinetics

Introduction

The fundamental interplay between nucleic acids and proteins underpins all known life forms on Earth, and it has been a subject of relentless inquiry since the early 20th century. The orchestration of gene expression in living organisms hinges upon the formation of DNA complexes involving an array of proteins. At approximately neutral pH levels, DNA takes on a polyanionic character, engaging in electrostatic interactions with positively charged proteins [1]. One prime example of such interactions is the formation of chromatin, a densely packed complex comprising DNA and histone proteins. Concurrently, DNA can also engage with negatively charged proteins like serum albumins, primarily owing to the uneven distribution of charges on the surface of protein globules.

A focal point of extensive research into DNA-protein interactions is the development of DNA/protein complexes as solid substrates. The significance of protein binding to DNA strands serves both fundamental scientific inquiry and potential applications in the realm of nanotechnology. Of particular interest are the network architectures formed by DNA/protein complexes. For instance, DNA/histone complexes can materialize upon adsorption onto a mica surface, with recent studies suggesting potential antibacterial properties depending on their composition. However, it remains unclear whether such structures can be generated at the liquid-fluid interface. Using Atomic Force Microscopy (AFM), investigated a spread layer of the Cytochrome c/DNA complex, detecting fibrous aggregates larger than DNA strands that tended to coalesce into larger rods with prolonged incubation. Nonetheless, the formation of a distinct network of these aggregates remained unobserved [2].

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Lysozyme, a well-known protein endowed with antibacterial properties, serves as an excellent model for exploring DNA-protein interactions at the solution-air interface. This basic protein has an isoelectric point around pH 11, and electrostatic interactions are recognized as pivotal in the formation of DNA-lysozyme complexes. The molar ratio between these components significantly influences the morphology of DNA-lysozyme aggregates. Lundberg et al. reported the formation of flexible, worm-like assemblies of DNA and lysozyme at low lysozyme-to-DNA molar ratios. More recently, Zhang et al. demonstrated that varying protein concentration can lead to the creation of "overcharged" and "undercharged" complexes with distinct shapes when lysozyme globules bind to DNA. Morimoto et al. explored the impact of high lysozyme concentrations in the presence of macromolecular crowding, revealing that, at elevated concentrations, lysozyme does not stabilize DNA duplexes but instead promotes the formation of loops primarily composed of unpaired nucleotides [3].

Recent developments have showcased the utility of surface rheology in investigating the formation of DNA-containing nanostructures at the solution-air interface. This approach has enabled researchers to scrutinize the penetration of DNA into a monolayer composed of the synthetic polyelectrolyte poly(N,N-diallyl-N-hexyl-N-methylammonium) chloride (PDAHMAC), shedding light on the mechanisms underlying the creation of a structured DNA/polyelectrolyte network at the liquid-gas interface. In this study, we employ a multifaceted methodology to investigate the penetration of DNA into a lysozyme layer dispersed over an aqueous sub-phase. Our primary objectives are to ascertain the potential for network formation and to elucidate the characteristics of DNA/protein interactions at the liquid-gas interface. We aim to quantify the aggregation of DNA/lysozyme within the crowded molecular environment of the lysozyme spread layer [4].

Description

In specific cases, such as those involving DNA in conjunction with surfactants and polyelectrolytes, the kinetics of surface pressure and surface elasticity can yield valuable insights into the conformations of macromolecules within the surface layer. However, the DNA/lysozyme system presents a more intricate challenge. When a concentrated lysozyme solution is spread over a buffer solution, it results in the formation of a surface layer with relatively high dynamic elasticity, closely resembling the data observed for lysozyme

adsorption layers. Interestingly, unlike the injection of DNA beneath a layer of synthetic cationic polyelectrolyte, introducing DNA into the solution beneath the lysozyme layer does not induce significant changes in surface elasticity. Even after a 15-hour period following DNA injection, all alterations fall within the expected margin of error. This pattern also extends to the kinetic dependencies of surface pressure. If the initial surface pressure of the lysozyme layer is set at 10 mN/m, there is minimal increase in surface pressure observed 15 hours after DNA injection.

Nonetheless, the penetration of DNA into the lysozyme layer can be discerned through compression isotherms. After an hour of lysozyme spreading, a nearly linear rise in surface pressure is observed due to interactions among the compact lysozyme globules, without evident phase transition regions. The isotherm maintains its shape even after one hour of incubation, DNA injection, and lysozyme distribution over the buffered subphase. Furthermore, this process causes a noticeable shift in the isotherm towards larger surface areas, a well-established indication of DNA penetration into the distributed layers of varying compositions [5].

Ellipsometric measurements also provide evidence of DNA infiltration into the lysozyme layer. The "s" value significantly increases upon application of the lysozyme solution to the buffered subphase. Subsequently, a stable and densely packed film is formed, as demonstrated by a subsequent 2-fold surface compression that raises the "s" value by 3 degrees without any other discernible changes. Additional 4- and 8-fold compressions lead to rapid relaxation of the "s" values, likely due to lysozyme breakdown in the subphase. However, the "s" value does not return to its pre-compression level and reaches a maximum of approximately 5.3 degrees at an 8-fold compression.

Conclusion

To the best of our knowledge, this marks the inaugural exploration of DNA infiltration into a lysozyme layer dispersed over an aqueous subphase. While the dynamic surface elasticity exhibited only marginal changes in this particular instance, the combined application of ellipsometry and IRRAS revealed an escalation in the surface concentration of DNA molecules following their introduction into the subphase. Interestingly, in contrast to the scenario involving DNA penetration into a synthetic cationic polyelectrolyte layer, the DNA/lysozyme complexes maintain a distinct separation within the surface layer, despite the formation of DNA/lysozyme complexes within that very layer. Unlike DNA/PDAHMAC systems, the interaction between a lysozyme layer and DNA molecules transpires seamlessly without the need for a nucleation stage. These deviations in the mixed DNA/lysozyme layer characteristics likely stem from two fundamental factors. First, the interactions between the lysozyme layer and DNA are comparatively weaker than those occurring when DNA is introduced into PDAHMAC layers. Second, within the surface layer, lysozyme predominantly engages with non-canonical DNA structures, stabilizing them and giving rise to the formation of more disorderly aggregates.

Acknowledgement

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Conflict of Interest

None.

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