#### ISSN: 2472-1026

# **Connections of DNA with Lysozyme**

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### Abstract

AFM, ellipsometry, surface tensiometry, surface dilational rheology and infrared reflection-absorption spectroscopy (IRRAS) were used to investigate the interactions of DNA with lysozyme in the surface layer (AFM). Under a dispersed lysozyme layer, an aqueous subphase was injected with a concentrated DNA solution. In contrast to DNA interactions with a monolayer of a cationic synthetic polyelectrolyte, where the surface layer's optical properties changed quickly after DNA injection, the dynamic dilational surface elasticity almost did not change. This suggests that no continuous network of DNA/lysozyme complexes formed. The relatively quick increase in optical signals following a DNA injection behind a lysozyme layer suggests that diffusion regulates DNA penetration. The AFM images demonstrate the development of lengthy strands in the surface layer at low surface pressures. In contrast to a mixed layer of DNA and synthetic polyelectrolytes, increased surface compression results in the emergence of folds and ridges rather than a network of DNA/lysozyme aggregates. It is likely that weaker interactions between lysozyme and duplex DNA and the stabilisation of loops of unpaired nucleotides at high local lysozyme concentrations in the surface layer are the causes of the creation of more disordered aggregates.

Keywords: DNA · Lysozyme · Adsorption kinetics · Dilational surface rheology · Langmuir monolayers

# Introduction

All known forms of life on Earth depend on the interaction of nucleic acids with proteins, which has been the subject of ongoing research since the first part of the 20th century. Without the creation of DNA complexes involving a variety of proteins, the complex process of gene expression in living things is not conceivable. At pH levels around 7, DNA becomes a polyanion and can interact electrostatically with positively charged proteins [1]. The development of chromatin, a tightly packed complex of DNA with histones, is a typical illustration of such interactions. DNA can also interact with negatively charged proteins, such as serum albumins, at the same time, primarily as a result of the uneven distribution of charges on a protein globule's surface.

The development of DNA/protein complexes as solid substrates is a significant focus of the substantial research on the interaction of DNA with diverse proteins. The significance of protein assembly onto DNA strands for both fundamental research and possible applications in nanotechnology is what drives these investigations. DNA/protein complex network architectures are of particular interest. As an illustration, DNA/histone complexes may develop after adsorption onto the mica surface. Recent research has shown that, depending on their composition, these networks may have antibacterial capabilities. However, it is unknown if such structures can develop at the liquid-fluid interface. AFM was used by Paul et al. to examine a spread layer of the Cytochrome c/DNA complex and identify the presence of fibrous aggregates (AFM). These aggregates were larger than -DNA and tended to combine into larger rods as incubation durations were extended. A distinct network of the aggregates has not, however, been seen at the same time.

A well-known protein with antibacterial capabilities is called lysozyme. A

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**Received:** 05 September, 2022, Manuscript No. JFM-22-84948; **Editor assigned:** 07 September, 2022, PreQC No. P-84948; **Reviewed:** 19 September, 2022, QC No. Q-84948; **Revised:** 24 September, 2022, Manuscript No. R-84948; **Published:** 30 September, 2022, DOI: 10.37421/2472-1026.2022.7.179

good model for studying DNA-protein interactions at the solution-air interface is this basic protein, which has an isoelectric point at roughly pH 11. It is widely known that electrostatic interactions play a key role in the creation of DNA-lysozyme complexes. The molar ratio between the components has a significant impact on the shape of DNA-lysozyme aggregates. At low lysozyme to DNA molar ratios, Lundberg et al. found that flexible worm-like assemblies of DNA and lysozyme formed. Recently, Zhang et al. showed that electrostatic interactions controlled by the protein concentration can cause the binding of lysozyme globules to DNA to result in the creation of both "over-" and "undercharged" complexes with a distinct shape. Morimoto et al. just recently investigated the impact of high lysozyme concentrations in the presence of macromolecular crowding. Their research shown that, at high concentrations, DNA duplexes are not stabilised by lysozyme, but rather, loops made primarily of unpaired nucleotides.

Recently, it was demonstrated that surface rheology can be used to look into how DNA-containing nanostructures form at the solution-air interface. This method allowed researchers to examine how DNA entered a monolayer of the synthetic polyelectrolyte poly(N,N-diallyI-N-hexyI-N-methylammonium) chloride (PDAHMAC) and to understand the mechanism underlying the creation of a regular DNA/polyelectrolyte network at the liquid-gas interface. The same multi-technique method is used in this study to examine DNA penetration into a lysozyme layer that has been distributed over an aqueous sub-phase. The major goals are to confirm the likelihood of a network creation and to clarify the characteristics of DNA/protein interactions at the liquid-gas interface. We specifically want to quantify the DNA/lysozyme aggregation in the lysozyme spread layer in a crowded molecular environment.

## Description

In particular, in the situations of DNA/surfactant and DNA/polyelectrolyte systems, the kinetic correlations of surface pressure and surface elasticity can offer significant information on conformations of macromolecules in the surface layer. The DNA/lysozyme system example turned out to be more challenging. A layer with relatively high dynamic elasticity is created when a concentrated lysozyme solution is spread across the top of a buffer solution, closely resembling the data of lysozyme adsorption layers. Unlike DNA injection under a layer of a synthetic cationic polyelectrolyte, the subsequent injection of DNA into the solution beneath the protein layer does not cause appreciable changes in the surface elasticity. Even 15 hours after DNA injection, every alteration

was within the allowed margin of error. Kinetic dependencies of the surface pressure follow a similar pattern. If the initial surface pressure of the lysozyme layer is 10 mN/m, there is barely any rise in surface pressure 15 hours after the DNA injection.

Nevertheless, compression isotherms can be used to identify DNA penetration into the lysozyme layer. After the lysozyme had spread for an hour, a nearly linear rise in surface pressure was seen as a result of the interaction of hard lysozyme globules, but there were no obvious regions of phase transition. The isotherm's form remained unchanged after 1 hour of incubation, DNA injection and lysozyme spreading over the surface of a buffer solution. In addition, it causes the isotherm to noticeably shift to greater surface areas, which is a well-known sign that DNA has penetrated the distributed layers of various compositions [2].

Additionally, DNA penetration into the lysozyme layer is indicated by the ellipsometric measurements. The s value significantly rises when a lysozyme solution is applied to the buffered subphase. A stable, tightly packed film has formed, as evidenced by the subsequent 2-fold surface compression, which raises this value by 3 degrees without experiencing any other obvious alterations. The additional 4- and 8-fold compressions cause the s values to quickly relax, most likely as a result of lysozyme breakdown in the subphase. However, s does not achieve the value before the corresponding compressions and only reaches a maximum of about 5.3 degrees at an 8-fold compression.

## Discussion

It is well known that persistence length, which describes the stiffness of the polyelectrolyte chain, influences the attraction of DNA to certain polyions. In 0.1 M NaCl aqueous solution, DNA has a persistence length of about 50 nm, making it one among the stiffest polymers. At pH 7, lysozyme globules have a positive net charge of +8 and are ellipsoidal in shape with approximate diameters of 3.3 5.5 3.3 nm. The globules can easily bind to DNA strands in a "beads on a string" way since they are smaller than DNA molecules. At specific protein concentrations, such binding can cause the collapse of DNA strands and the formation of compact complexes. Long DNA threads are visible at the liquid surface according to the AFM findings from this investigation. The threads appear to be partially encased in protein molecules. The surface layer's high local protein content can result in the production of "overcharged" DNA-lysozyme complexes. In this situation, DNA compaction, which is seen in the bulk phase, can be avoided by the electrostatic attraction between

protein molecules coupled to DNA strands. Although DNA can interact with lysozyme at the solution-air interface, as shown by compression isotherms, AFM, ellipsometry and IRRAS findings, the associated changes of the dynamic surface properties are not far from the error limits [3-5].

# Conclusion

To the best of our knowledge, this is the first time DNA penetration into a lysozyme layer dispersed across an aqueous subphase has been investigated. While the dynamic surface elasticity only slightly altered in this example, the use of ellipsometry and IRRAS helped to identify an increase in the surface concentration of DNA molecules following DNA injection into a subphase. Contrary to the situation of DNA penetration into a layer of synthetic cationic polyelectrolyte, the DNA/lysozyme complexes are segregated in the surface layer despite the production of DNA/PDAHMAC systems, the interaction of a lysozyme layer with DNA molecules occurs continuously without the nucleation stage. There are probably two basic causes for the anomalies of mixed DNA/lysozyme layer features that have been found. First, lysozyme layer interactions with DNA are weaker than when DNA is inserted into PDAHMAC layers. Second, at the surface layer, lysozyme interacts mostly with non-canonical DNA structures, stabilising them and causing the formation of more disordered aggregates.

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How to cite this article: Noskov, Nikolay. "Connections of DNA with Lysozyme." J Forensic Med 7 (2022): 179.