

Impact of Lactoferrin and Lysozyme on Particle Diffusion and Microbial Transport in Mucus

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Introduction

Mucus constitutes a mesh-like hydrogel that pathogens must overcome to invade the underlying epithelium. The anti-microbial proteins, lactoferrin (LF) and lysozyme (LYS), are introduced during mucosal secretions and impact the microbiome by sequestering iron needed for bacterial growth and by lysing the cell wall. These proteins easily form ionic complexes¹ and thus may have the potential to undergo strong electrostatic interactions with mucin, the main structural component of mucus. This work tested the hypothesis that these proteins alter mucus barrier properties. By investigating the impact of LF and LYS on particle and microbial transport in mucus, we can enhance understanding of the function of these proteins in mucosal immunity and explore their potential to be used as treatments to modulate the mucus barrier to pathogens and particulates.

Materials and Methods

The impact of LF and LYS on particle diffusion and microbe velocity in mucus was investigated by multiple particle tracking. Three test protein solutions were prepared: LF (7.0 g/L), LYS (3.0 g/L), and a combination of LF and LYS (7.0 g/L, 3.0 g/L respectively) in maleate buffer (MB) at pH 6.5. MB served as a control solution. Fluorescent particles (FluoSpheres®) or *E. Coli-GFP* were added to the protein solutions and incubated for 5 minutes before exposure of the mixture to intestinal mucus. Particles (200 nm) tagged with carboxyl, amine and polyethylene glycol (PEG) functional groups were used to investigate the impact of surface charge (negative, positive, and neutral, respectively) on particle diffusion. The tracking of particles and microbes in mucus was accomplished with twenty-second videos captured using an Olympus DP70 digital color camera and Olympus DP imaging software, with at least 100 microbes or particles analyzed in each experiment. A modified MATLAB code² was used to track and calculate the mean-squared displacement ($\langle \text{MSD} \rangle$). Confocal microscopy was utilized to visualize bacteria and particle dispersion in mucus. A T-test analysis was used to identify the significance of the data obtained from the samples with respect to that collected from the controls.

Results and Discussion

The addition of LF significantly reduced particle diffusion (Fig 1A) but did not notably alter microbial transport in mucus (Fig 1B). Analysis of confocal microscopy images showed similar dispersions of particles and microbes in mucus with the presence and the absence of LF. These results suggest that while LF is reported to bind to acidic macromolecules (i.e. mucin), the interaction may not impact mucus microstructure, but may affect electrostatic interactions between mucin fibers and particles. The addition of LYS significantly reduced transport of charged particles, neutral particles, and microbes (Fig 1A). LYS notably reduced microbe transport in mucus by a factor of 5.4 (Fig 1B). Analysis of confocal microscopy images revealed that microbes and particles were confined to the region where the particle solution was introduced into the mucus. These results may be due to the strong ionic association¹ between LYS and mucin fibers, which may alter mucin cross-linking and mucus pore size, thus reducing transport and dispersion. The combination of LYS and LF significantly reduced diffusion of charged particles, resulting in diffusion rates similar to those exhibited by particles in the presence of LYS alone, while results obtained from neutral particle diffusion were similar to diffusion in the presence of LF alone. Microbial

transport results were similar to those obtained from controls (Fig 1). These results indicate that the potential effect of LYS on mucin cross-linking and mucus pore size is reduced in the presence of LF, thus, transport in the presence of both LYS and LF may be primarily affected by electrostatic interactions between the proteins and the particles.

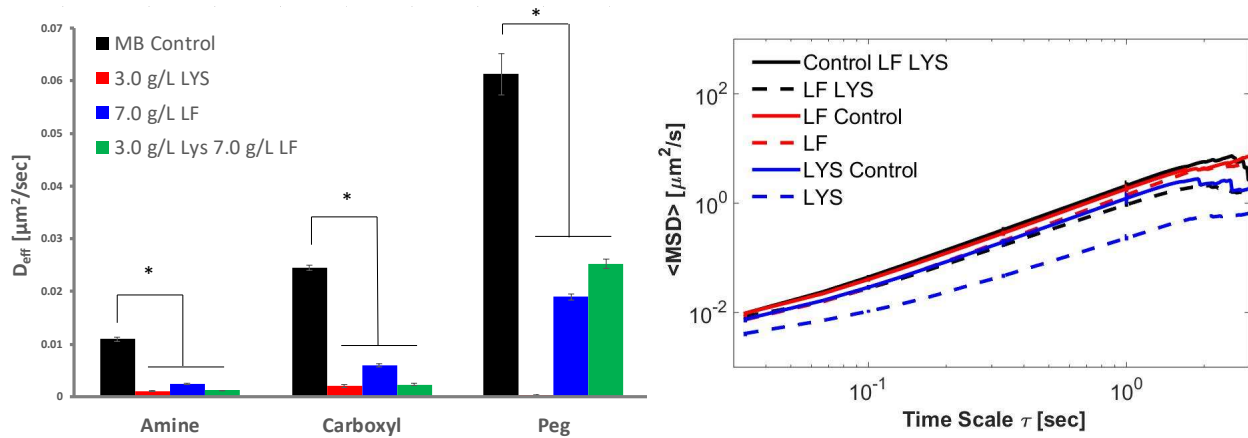


Fig 1. Particle Diffusion and Microbial Transport in Gastrointestinal Mucus A) Ensemble D_{eff} average at 3 seconds with standard errors based on 3 independent experiments. Ensemble $\langle \text{MSD} \rangle$ averages with standard errors based on 3 independent experiments, with greater than 100 particles and microbes in each experiment. * $p < 0.5$

Conclusions

The addition of LF and LYS was shown to have a significant impact on the particle diffusion through mucus; this impact is noted when proteins are added independently and in combination. Microbe transport is notably reduced in the presence of LYS but not in the presence of LF, potentially due to stronger ionic associations between LYS and mucins that is hypothesized to impact the mucus structure. The significant impact of LYS, LF and, the combination of LF and LYS on particle diffusion may result, in large part, from electrostatic interactions between particles and the surrounding mucin fibers, LF, and LYS. These results indicate that further investigation into the effects of other mucosal secreted proteins and their effect on mucus barrier properties is warranted. The information from this study could motivate the development of therapeutic treatments to modulate the mucus lining to prevent infection or treat disease.

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References

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