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Investigating Buffer Effects on Lysozyme Adsorption to Borosilicate Glass

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SUMMARY

Protein adsorption refers to the accumulation and adherence of a protein to the surface of a solid, but without surface penetration occurring. Proteins can adsorb to a variety of interfaces that are used in the manufacture, formulation, and storage of protein medicines. This can have unintended consequences such as a loss of expensive protein product and aggregate formation. This study investigates the role of buffer composition, pH, and protein concentration on the adsorption of lysozyme to borosilicate glass. Using reverse-phase HPLC and differential scanning fluorimetry, we quantified the mass of adsorbed lysozyme and assessed the stability of lysozyme in each buffer system. The highest amount of adsorbed lysozyme occurred in the sodium phosphate and histidine-HCl buffers at pH 7.4 and stability analysis showed that lysozyme had the lowest melt temperature in these buffers.

INTRODUCTION

Protein adsorption is a complex phenomenon that involves various types of protein-water-surface interactions and is affected by several factors namely solution environment, interfacial properties, and protein properties.[1] One of the critical factors that influences protein adsorption behaviour is protein stability.[2] Proteins with low structural stability can adsorb to interfaces in higher quantities and can display altered conformations and functionality even after desorption.[2] Protein formulation and stability are intrinsically linked as formulations are designed to stabilize protein structure. However, interactions between the protein, buffering media and solid interfaces have adverse effects on protein adsorption behaviour and can result in the loss of therapeutic cargo to the material surface which can lead to the formation of aggregates in solution.[3] The influence of buffer composition and pH on lysozyme stability and adsorption is highlighted in this study.

MATERIALS AND METHODS

Adsorption and Desorption Procedure: Solutions of lysozyme in different buffering media (Na-phosphate, histidine-HCl, Na-citrate, glycine-HCl) were prepared at 1 mg/mL or 5 mg/mL and at two ionic strengths (10 mM buffer or buffer with 140 mM NaCl) using pH 3.6, 5.5 and 7.4. Borosilicate substrates (Fortuna Optima®) were filled with 3 mL of lysozyme solution, sealed and incubated for 1 h at room temperature. Desorption of lysozyme was achieved using 1 mL of phosphate buffer saline pH 7.4 with Tween 20 0.05 %v/v using vortexing at 2200 rpm for 30 s.

Lysozyme Quantitation: Desorbed lysozyme was quantified using reverse-phase HPLC (RP-HPLC) using an Agilent 1200 series and a Poroshell 300SB-C8 column at 70°C with a 215 nm detection wavelength.

Thermal Stability Analysis: Differential scanning fluorimetry (DSF) was performed using a CFX96TM RT-qPCR instrument and SYPRO Orange Dye. Fluorescence was measured (Ex 597, Em 615 nm)

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during a temperature scan from 20 - 90°C, in 0.5°C steps with 10 s hold time.

RESULTS AND DISCUSSION

Quantification of Adsorbed Lysozyme by RP-HPLC

The mass of adsorbed lysozyme from each solution was quantified to ascertain the influence of buffer composition and pH on lysozyme's adsorption behaviour. Lysozyme at 1 mg/mL was investigated initially with and without 140 mM NaCl, ***Fig. 1***. The highest amount of adsorbed lysozyme was detected from the sodium phosphate buffer at pH 7.4.

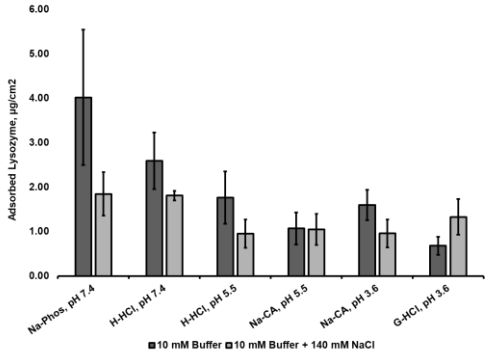

Fig. 1. *Effect of buffer composition on lysozyme adsorption to borosilicate glass using 1 mg/ml. Mean ± SD, n=6.*

Lysozyme solutions at 5 mg/mL with and without NaCl, were then investigated, ***Fig. 2***. Again, the highest amount of adsorbed lysozyme was observed for the sodium phosphate buffer at pH 7.4.

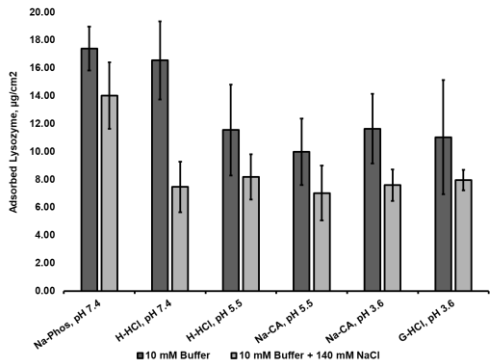

Fig. 2. *Effect of buffer composition on lysozyme adsorption to borosilicate glass using 5 mg/ml. Mean ± SD, n=6.*

Determination of protein stability using DSF

To determine if the adsorption behaviour could be attributed to stability, the impact of buffer choice on lysozyme melt temperature/unfolding was studied using DSF, ***Fig. 3***. Lysozyme's thermal stability in sodium phosphate and histidine-HCl buffers at pH 7.4 was lower with respect to the aqueous control.

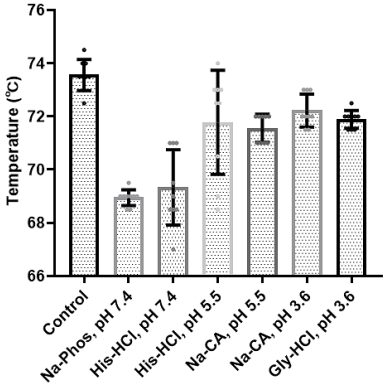

Fig. 3. *Influence of buffer choice on lysozyme thermal stability determined using DSF. Mean ± SD, n=3.*

CONCLUSIONS

The largest mass of adsorbed lysozyme was from the sodium phosphate and histidine-HCl buffers at pH 7.4. The thermal stability was lowered for these two buffers with respect to the control and other buffering systems investigated. These results support the theory that there is an interplay between protein thermodynamic stability, unfolding behaviour and adsorption.

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