Probing the Impact of Protein/Peptide Adsorption onto Solid-Substrates on Functional Behavior and Stability

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Introduction

Protein adsorption has desirable applications in the biopharmaceutical industry where separation, purification and filtration are concerned. However, non-specific adsorption has unintended consequences such as loss of expensive protein medicines which is particularly inconvenient during formulation, storage, and delivery.[1]

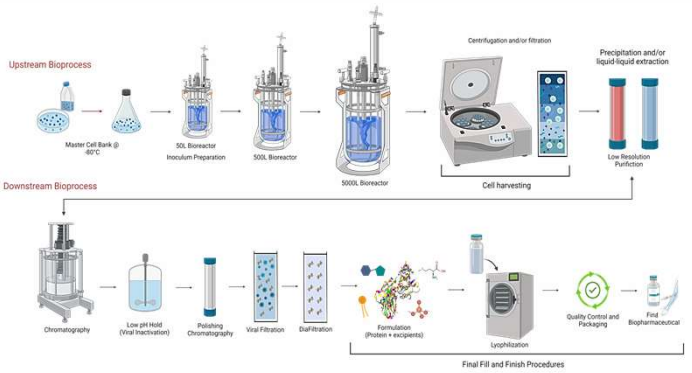

Figure 1: Bioprocess Flow Diagram[2]

Aims & Objectives

Aim: To explore protein-surface interactions and excipient influence on adsorption behaviour

Objectives:

- To characterize (quantity, structure, activity) adsorbed proteins from different single-use interfaces
- Examine formulation effects (buffer choice, pH)
- Outline the role of protein-surface interactions in adsorption processes

Methods

- RP-HPLC – Lysozyme Quantification
- CD Spectroscopy – Lysozyme Secondary Structure
- Isothermal Titration Calorimetry – Lysozyme Binding
- Atomic Force Microscopy – Lysozyme Surface Interactions

Results & Discussion

Lysozyme Characterization

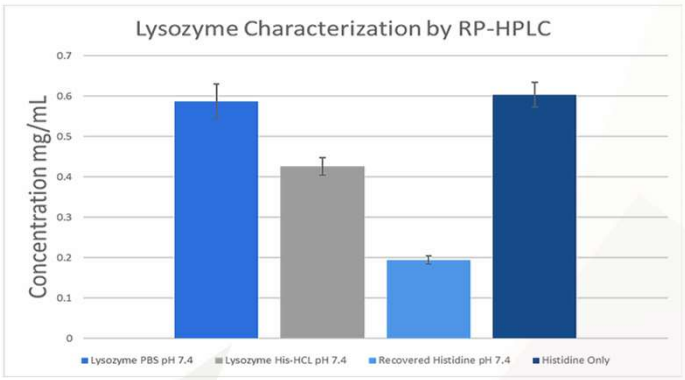

Figure 2: The largest amount of lysozyme adsorbed from phosphate buffer. The concentration of adsorbed lysozyme decreased when adsorbing from histidine buffer. The reduction in lysozyme adsorption occurred because histidine also adsorbed to the surface.

Lysozyme Structural Analysis (Circular Dichroism Spectroscopy)

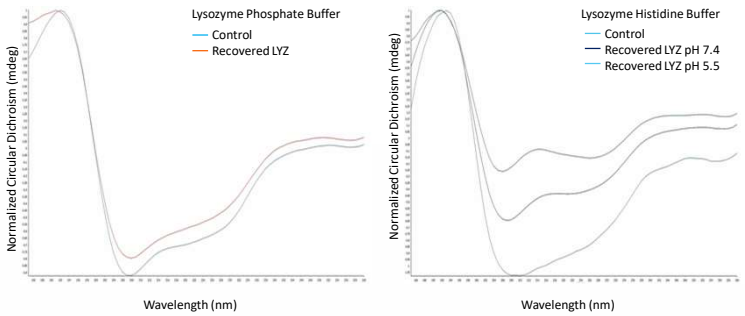

Figure 3: CD spectroscopy data indicates that adsorbed lysozyme displays slight changes to secondary structure upon recovery from the surface of borosilicate glass. Adsorbed lysozyme from each buffer system exhibits different spectral properties to their respective controls suggesting that adsorption can lead to irreversible structural reorientation.

Lysozyme-Buffer Interactions (Isothermal Titration Calorimetry)

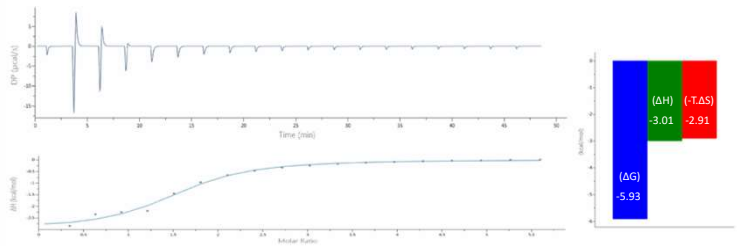

Figure 4: ITC indicated that lysozyme and His-HCL pH 7.4 were interacting in solution through an exothermic process where the binding interactions were spontaneous hydrogen and hydrophobic bonds. The area under each peak integrated and plotted against the molar ratio of titrant to titrand were fit to a one-site independent binding model. The enthalpy (ΔH) of binding is -3.01 kcal/mol and stoichiometry of binding is 1:1.5 (n = 1.51).

Lysozyme-Surface Interactions (Atomic Force Microscopy)

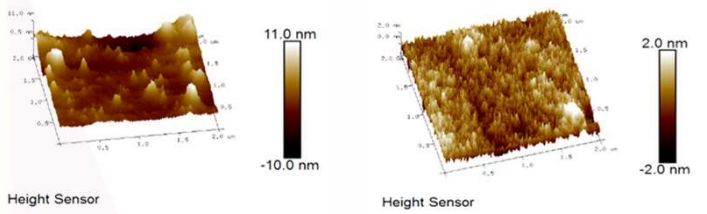

Figure 5: Lysozyme from PBS pH 7.4 consists of large irregular shaped peaks with variability in heights. Lysozyme from Histidine-HCL pH 7.4 exhibits a dense structure, indicating a high degree of surface coverage.

Conclusions

These results suggest that buffer choice has a strong effect on the adsorption profile of lysozyme to borosilicate. Histidine buffer has a moderate ability at reducing adsorption which is attributed to histidine binding to lysozyme. CD Spectroscopy revealed that adsorption can cause a slight change in secondary structure and AFM highlighted that buffer choice can influence protein-surface interactions.

References

1. *Nakanishi, K., Sakiyama, T. & Imamura, K. On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. J. Biosci. Bioeng.*
2. *Jozala AF, et al. Biopharmaceuticals from microorganisms: from production to purification. Braz J Microbiol.*

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