THE COLLEGE OF WOOSTER

Kylie M. Schmitz
Advised by: Paul L. Edmiston

Biochemistry & Molecular Biology Program
The College of Wooster

Structural Stability of Bovine Serum Albumin upon Adsorption to SOMS Measured by FT-IR

BSA adsorbed to SOMS maintains stability in temperatures up to 99 °C, solutions with up to 50% ethanol, and guanidine hydrochloride solutions with molarity up to 6 M.

Abstract

Swellable organically modified silica (SOMS) is a porous material that has been shown to non-specifically adsorb proteins. The goal of this project was to study whether bovine serum albumin (BSA) maintains a folded state upon adsorption to SOMS. Measurements were taken for BSA loading and folded state upon adsorption, as well as structural stability in denaturing conditions. Sequential adsorption experiments revealed that SOMS irreversibly adsorbs >380 mg of BSA per gram of SOMS. Thermal and chemical stability experiments showed that BSA adsorbed to SOMS maintained stability in temperatures up to 99 °C, solutions with up to 50% EtOH-d6, and guanidine HCl solutions with molarity up to 6 M.

Key Questions

1. How do proteins adsorb to surfaces and why is the reaction favorable?
2. How does surface chemistry affect protein adsorption?
3. How does adsorption affect protein structure, including conformation, hydration state, and structural stability?
4. Does adsorption have an impact on the biological function of the protein?

Background

- All surfaces in contact with biological solutions adsorb proteins with mechanisms that are not fully understood.
- Factors that may affect adsorption include protein affinity and reversibility, orientation, denaturation, surface chemistry, specific interactions, and competitive binding (Fig. 1).
- Protein adsorption is important for enzyme immobilization, as well as environmental decontamination, synthesis of food additives, and biodiesel synthesis.
- While non-porous materials are capable of adsorption, porous materials provide a three-dimensional matrix of surfaces, which allows for increased adsorption capacity and makes measurements more feasible.

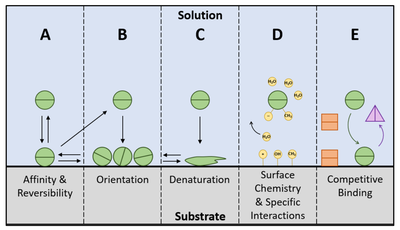


Figure 1: *Properties of protein adsorption to solid surfaces and their proposed mechanisms.*

Specific Aims

1. Adsorb BSA to the surface of SOMS at different degrees of loading
2. Measure the infrared spectrum of adsorbed BSA to determine the folding state as a function of protein loading
3. Measure the structural stability of BSA adsorbed to SOMS using thermal and chemical denaturation

Swellable Organically Modified Silica (SOMS)

- Mesoporous material with surface area of 450 m^2/g
- Hydrophobic due to functional groups on BTEB (Fig. 2)
- Rapid and reversible pore expansion (Fig. 3)
- Spontaneously adsorbs over 400 mg/g of protein

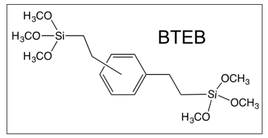


Figure 2: *Chemical structure of BTEB showing hydrophobic functional groups.*

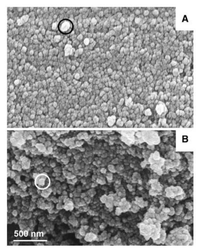
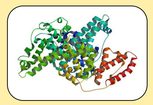


Figure 3: *Scanning electron micrographs of SOMS while unswollen (a) and swollen with 0.6 TMA (b).*

Edmiston et al., *Adsorption*, **2018**, *24*, 53-63

Why BSA?

- Broadly Studied
- Can be fluorescently labeled
- Minimal protein-protein affinity, preventing aggregation
- Critical for medical applications



BSA Loading and Adsorbed Structure

Methods

- SOMS particles were finely ground (50-100 µm particle size) and swollen with EtOH (~1mL)
- Two consecutive PBS buffer (8 mL) rinses were performed to remove EtOH from pores
- BSA solution was added (8 mL, 1 mg/mL) with equilibration time of 2 days
- BSA adsorption was repeated twice, four times, or six times, with three samples at each loading amount, then measured using UV-Vis spectrometry

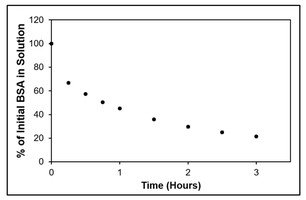


Figure 4: *Depletion of BSA in solution upon adsorption to SOMS over the course of three hours after initial BSA addition.*

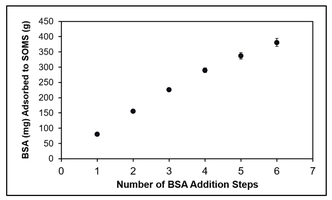


Figure 5: *BSA adsorption to SOMS after multiple additions of protein in solution.*

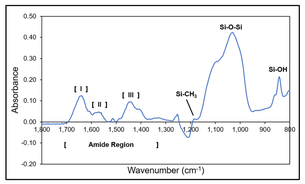


Figure 6: *IR spectrum of BSA showing all amide regions and SOMS peaks.*

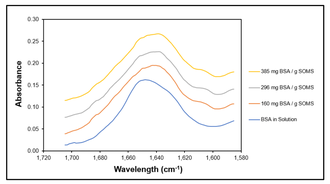


Figure 7: *IR spectra of BSA before and after adsorption to SOMS in three different loading quantities.*

Chemical Stability of Adsorbed BSA: Deuterated Ethanol (ethanol-d6)

Methods

- BSA-SOMS samples were exposed to 8 solutions (500 µL) with EtOH-d6 concentrations of 0%, 5%, 10%, 20%, 30%, 50%, 70%, and 90% (v/v)
- Control samples were prepared using 50 mg/mL BSA in the EtOH-d6 / D_2O solutions listed above
- Samples were measured using IR spectroscopy

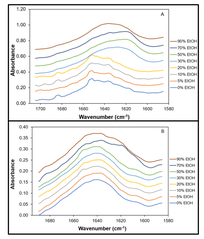


Figure 8: *IR spectra in the amide region for BSA in solution (a) and BSA adsorbed to SOMS (~367 mg BSA / g of SOMS) (b) in varying EtOH-d6 concentrations.*

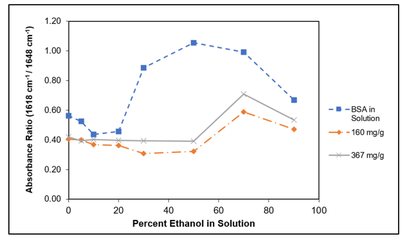
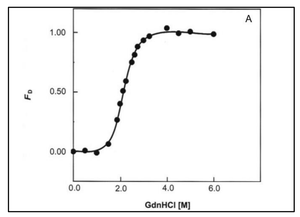


Figure 9: *Ratio between IR absorbance at 1618 cm^{-1} and 1648 cm^{-1} for BSA adsorbed to SOMS and in solution after exposure to EtOH-d6.*

Chemical Stability of Adsorbed BSA: Guanidine Hydrochloride

Methods

- BSA-SOMS samples were exposed to 6 solutions (25 mL) with guanidine HCl and PBS (0 M, 1.6 M, 2.4 M, 3.2 M, 4.8 M, 6 M)
- Samples were equilibrated for 24 hours, then supernatants were exchanged with PBS (8 mL), then D_2O (4 mL) (24-hour equilibration for each)
- Samples were measured using IR spectroscopy and results were compared to the literature



Halim et al., *Journal of Biochemistry*, **2008**, *144*, 33-38

Figure 10: *Normalized denaturation curve for guanidine HCl denaturation of BSA in 0.1 M Tris-HCl buffer, pH 8.0, and 25 °C. Intrinsic fluorescence measurements were made at 340 nm on excitation at 280 nm.*

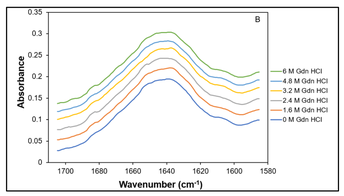


Figure 11: *IR spectra in the amide region for BSA adsorbed to SOMS (~385 mg BSA / g of SOMS) in varying guanidine HCl concentrations.*

Thermal Stability of Adsorbed BSA

Methods

- A heating block was set up to hold multiple BSA-SOMS samples and one water sample with a thermocouple to monitor the temperature
- BSA-SOMS samples were gradually heated in the heating block
- Measurements were taken using IR spectroscopy at ~5-degree increments between 50 °C and 99 °C (5-15 minutes of equilibration time)

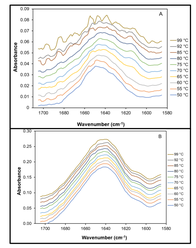


Figure 12: *FT-IR spectra in the amide region for BSA in solution (a) and BSA adsorbed to SOMS (~347 mg BSA / g of SOMS) (b) in varying temperatures.*

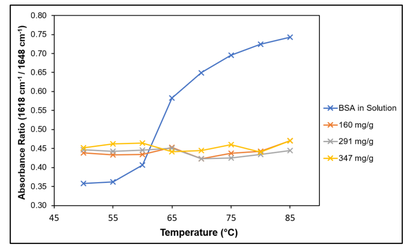


Figure 13: *Ratio between IR absorbance at 1618 cm^{-1} and 1648 cm^{-1} for thermal denaturation samples.*

Conclusions

BSA adsorbs strongly and irreversibly to SOMS, resisting desorption from EtOH-d6 and guanidine HCl exposure. Kinetics experiments and the calculated surface coverage of adsorbed BSA (44%) suggest monolayer adsorption, with the mesoporous structure of SOMS causing interactions to occur with multiple faces of BSA molecules (Fig. 14).

Overall, SOMS was found to have a protective effect on BSA molecules, preventing them from unfolding in thermal and chemical denaturing conditions. BSA adsorbed to SOMS maintained stability in temperatures up to 99 °C, solutions with up to 50% EtOH-d6, and guanidine HCl solutions with molarity up to 6 M.

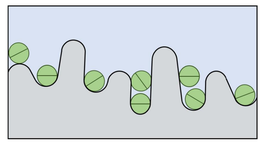


Figure 14: *Possible protein-surface interactions, including contact with more than one surface of BSA molecules due to the mesoporous structure of SOMS.*

Future Work

- Future work should further investigate the mechanism of protein adsorption to SOMS and the protein-surface interactions involved.
- The limits of protein stability should be studied more extensively to fully understand how SOMS protects proteins from denaturation.
- Future projects should explore the mechanism of strong protein adsorption and whether adsorbed biomolecules maintain bioactivity (enzymatic activity) in denaturing conditions.

Acknowledgements

The College of Wooster Independent Study Funding: **Department of Chemistry**