Immobilization of Transferrin in Swellable Organically Modified Silica for Remediation of Heavy Metal Ions

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Background

- Due to climate change and intensification of agriculture and development, access to clean water is an issue to millions of people globally.
- While water pollution exists in multiple forms, pollution from heavy metal ions can cause a lot of diseases and be fatal. Heavy metal ions are often introduced through metallurgy, rubber industries, and oil refineries depositing their waste into the environment. Such metal ions can accumulate in the body and disrupt metabolic systems, which ultimately lead to conditions such as softening of bones, nervous system problems or cancer.
- Methods used to remove heavy metal ions from water such as flotation, sand filtration or coagulation can often produce byproducts or require prior treatment which can be expensive for developing countries. Proteins can be used to extract metal ions as an alternative because they are sustainable, selective, have high capacity for binding and produces no waste.
- While immobilized proteins have been used to extract heavy metal ions before, research has not yet been done for the purposes of purifying water. SOMS (swellable organically modified silica) can adsorb proteins when expanded, and transferrin can be used as a model protein to be immobilized in SOMS as it can bind to metal ions.

Swellable Organically Modified Silica (SOMS)

Figure 1: Expansion of SOMS with the addition of organic material.

- SOMS is made from the polycondensation of bis(trimethoxysilylethyl)-benzene (BTEB), comprised of crosslinked interconnected organosilicate structures which produces a mesoporous material. It swells upon adsorption of organic liquid which makes the matrix expand three times its original size.
- Hypothesized : Organic molecules adsorb to the interior pore surfaces in SOMS, disrupt pore wall contacts, expands the material and creates the pores. Swelling gives rise to pore volume which has sufficient space to allow protein diffusion.
- Experiments have shown SOMS can adsorb protein without disrupting the biofunctionality of proteins, and small molecules can diffuse into adsorbed proteins.

Structure of Transferrin

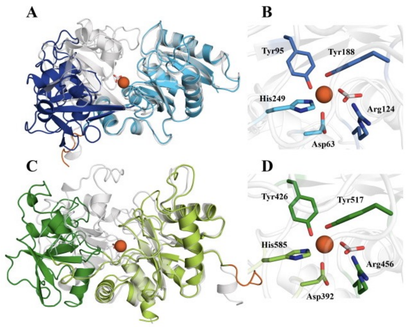

Figure 2: Structure of transferrin. A & C show the movement within the domains when it goes from open (grey) to close (colored). B & C show the residues that are involved in the binding of iron to transferrin. Each domain binds one iron, and overall two ions in one protein.

- Transferrin has two homologous lobes, divided into subdomains N1 and N2, and C1 and C2.
- Hinge region connects the two domains which contains rhe Fe^{3+} binding site.
- Upon binding of Fe^{3+} and bicarbonate ion, transferrin undergoes conformational changes from open to close. The N domain rotates 59 degrees, and C domain rotates 51 degrees.
- The close to open conformation still not understood, but it is hypothesized that it is sensitive to low pH as the Lys-206 and Lys-296 are protonated which causes repulsion.

Research Question & Specific Aims

Can transferrin immobilized in SOMS bind to Fe^{3+}?

- Measure transferrin and BSA adsorption into expanded SOMS using UV-Vis spectrophotometry to understand SOMS' protein binding capacity.
- Add iron solution to the samples which was measured spectrophotometrically using 1,10 – phenanthroline. The stoichiometry of iron binding to transferrin or SOMS was measured.
- FT-IR was used to measure the secondary conformational changes of transferrin when bound to iron.
- Understand the interactions iron uses to bind to SOMS and immobilized proteins using desorption tests with KCl, DI water, phosphate buffer and EDTA.

Adsorption of transferrin into SOMS

Table 1 : Percentage proteins adsorbed into SOMS from solution after 24 hr equilibrium.

Protein	Protein Adsorption %	Protein mg adsorbed/g of SOMS
Transferrin (x1)*	87	69
Transferrin (x2)	86	138
Transferrin (x5)	87	344
SOMS + BSA (x1)	96	77

BSA was adsorbed more than transferrin, due to size. Transferrin is 80 kDA and BSA is 66 kDA which allowed it to be adsorbed faster.
* Transferrin (x1) denotes transferrin was adsorbed into SOMS once, while transferrin (x2) denotes transferrin was adsorbed twie cumulartively, and so on.

Adsorption of Fe^{3+} into immobilized transferrin

Table 2: Average cumulative Fe^{3+} adsorption per 100mg of SOMS (µg/mg) for each sample as $FeCl_3$.

Sample	Av. Fe^{3+} adsorbed ug/g of SOMS	Ratio of Fe^{3+}/transferrin
SOMS	885± 13	N/A
Transferrin (x1)	289± 11	2.38
Transferrin (x2)	292± 11	1.54
Transferrin (x5)	437± 8	0.911
SOMS + BSA	492±18	N/A

SOMS adsorbed more Fe^{3+} compared to the immobilized protein samples. Transferrin is unable to bind any Fe^{3+}, and it is assumed SOMS binds iron in all the samples, and the presence of proteins rather impede the binding of SOMS to iron.

Desorption of Fe^{3+} from immobilized transferrin

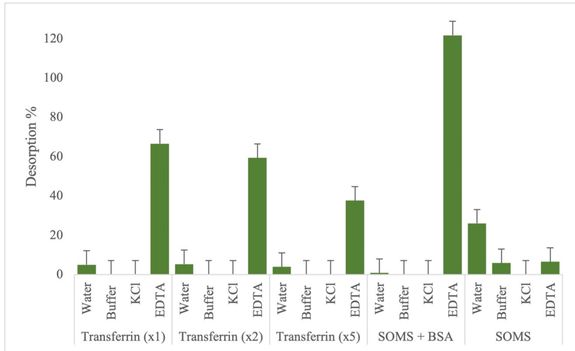

Figure 3 : Desorption of iron from the transferrin samples and SOMS.

For transferrin samples, most of the iron was removed by EDTA which means significant amount of the iron was bound to transferrin through hydrogen bonds. For BSA, 120% was removed by EDTA, most likely due to an error. For SOMS, 40% of the adsorbed iron was desorbed. Water removed iron most effectively compared to KCl, buffer and EDTA in SOMS. The adsorbed iron was bound through weak electrostatic forces which was easily broken by water in SOMS.

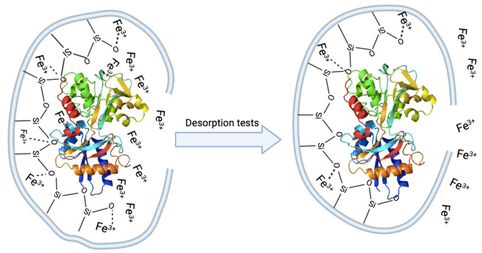

Figure 4: Scheme of how iron may be binding to SOMS with immobilized protein inside. Once the desorbing agents are added, the ions leave the expanded SOMS pockets, while some stay imbedded within the pockets.

FT-IR Spectroscopy

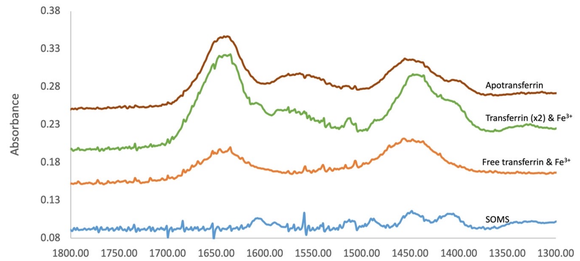

Figure 35:The IR spectra in the amide region for apotransferrin, free transferrin with iron bound, immobilized transferrin with iron bound, and SOMS.

FT-IR showed that the peaks 1415 cm^{-1} and 1560 cm^{-1} for free transferrin with iron bound was absent, but still present for the immobilized transferrin with iron bound. Immobilized transferrin is unable to undergo the conformational changes in the N and C domains which causes 59 and 51 degrees of rotation respectively. As a result, immobilized transferrin cannot bind to iron as it cannot undergo the required conformational changes.

Conclusions

- SOMS strongly binds transferrin, despite it being a large protein.
- Iron ions do not bind to immobilized transferrin, but rather to SOMS which is hydrophobic. The presence of proteins impeded the binding of iron to SOMS.
- The FT-IR detected the lack of peaks in the amide regions for free transferrin with iron bound, while the immobilized transferrin had the peaks present. Transferrin cannot bind to iron when immobilized as it cannot undergo the conformational changes.

Future Work

SOMS' binding to Fe^{3+} needs to be better understood
The absence of the amide peaks in transferrin bound to iron should be further investigated. Transferrin immobilized in SOMS can be used to study how the protein's conformational changes are altered due to the restriction in movement.