Metal Binding and Disulfide Bond Breakage Can Be Decoupled in an Engineered Metalloprotein

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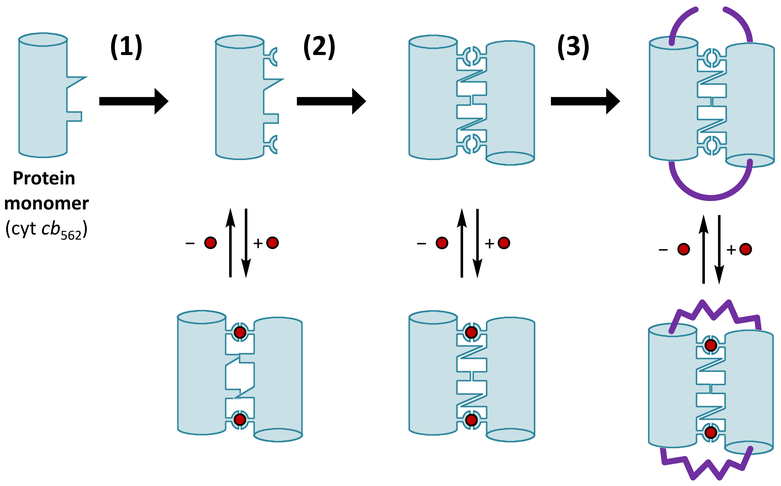
Abstract

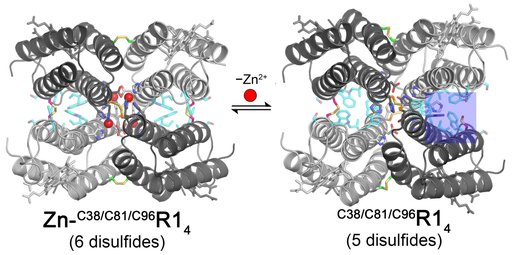
Proteins are molecules in cells that carry out many important tasks, including carrying oxygen in our blood, converting food to energy, and communicating information about a cell's environment to the inside of a cell. Since proteins serve many functions we are interested in engineering proteins that have new properties. Previously, we engineered a protein, $^{C38/C81/C96}$R1, that forms a tetramer which binds Zn and which contains six disulfide bonds. Surprisingly, when the Zn is removed, one C38/C38 disulfide bond breaks.

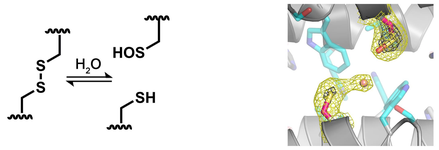
We are working with the ^{C38}RIDC1 protein and hope to find that, unlike $^{C38/C81/C96}$R1, it can keep a disulfide bond when Zn is removed. In order to study this, we must purify our protein from *E. coli* cell culture. Then we can combine our protein with chelators, reductants, and our Zn metal to determine if ^{C38}R1 can bind to zinc and create a disulfide bond without the disulfide bond formation being metal dependent.

Introduction & Background



1. Add metal binding amino acids to make metal-dependent protein assemblies.
2. Add hydrophobic interfaces that allow protein to self assemble.
3. Add disulfide bonds can break or relax when the metal is removed.





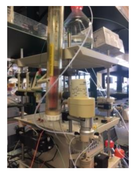
The previously studied $^{C38/C81/C96}$R1 contains six disulfide bonds, one of which, C38/C38, breaks when the Zn is removed from the tetramer. There are two possible reasons for this behavior:

1. The protein environment can affect whether the disulfide bond remains or breaks.
2. It is a fragile bond that will always break.

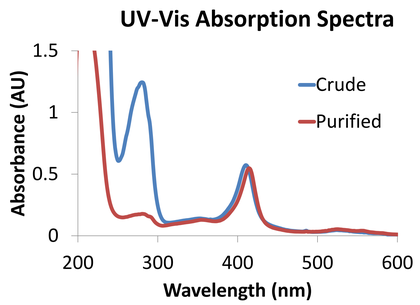
We wanted to determine which possibility is true by studying ^{C38}R1, which we expect can form a C38/C38 disulfide bond, and which has no other disulfide bonds.

Results Part I (^{C38}R1 Purification)

^{C38}R1 Cell Pellet

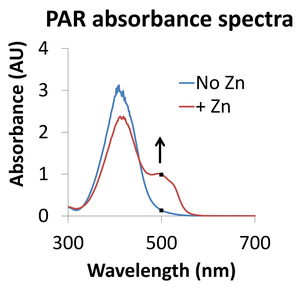


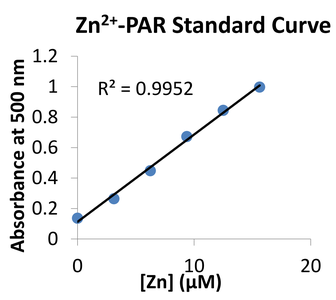
Purified ^{C38}R1

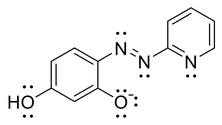


- Express protein in *E. coli* cell culture
- Sonicate cell pellet
- Run protein through CM-sepharose column (cation exchanger) in acetate (acid buffer)
- Run protein through Q-column (anion exchanger) in phosphate (basic buffer)
- Run through S75 column (size-exclusion) on the FPLC
- Concentrated purified protein

Results Part II (PAR Assay)







PAR

Sample	Zn/Protein
Apo ^{C38}R1	~0
Zn-^{C38}R1	0.12

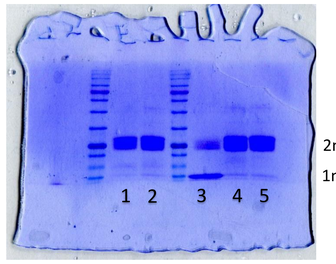
Zn does not absorb visible light, but we can bind it to PAR so that we can see the absorbance of the Zn-PAR complex which allows us to see the amount of Zn in a sample.

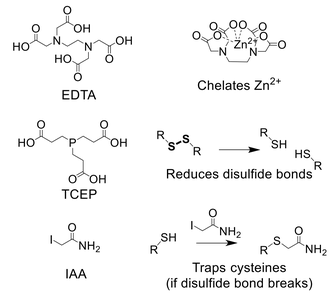
For the Zn-Par Standard Curve we incrementally added Zn to our PAR solution. This gives us a way to convert between absorbance at 500 nm (A_{500}) and the concentration of Zn ([Zn]).

The difference between our protein and par absorbance and PAR/EDTA/protein absorbance give us the absorbance of zinc was lower than expected at 0.12 Zn per protein. We expect that ^{C38}R1 can bind 1 Zn per protein.

Results Part III (Gel + Protein treatments)

SDS-PAGE Gel





Five sample treatments are shown in the gel above:

1. Isolated protein: The isolated protein is simply purified protein
2. Apo protein: protein is chelated then ran through a column to separate the protein from the metal-bound chelator
3. TCEP + Protein: TCEP is a reductant whose job is to show the breakage of the disulfide bond. The monomer band indicates that the sample without a reductant formed a disulfide bond.
4. Zn + Protein: the disulfide bond forms in the presence of Zn which acts like the $^{C38/C81/C96}$R1
5. Zn + IAA + EDTA + Protein: Adding IAA and EDTA to the Zn + protein sample shows that the disulfide bond does not break, despite the fact that chelators remove the metal. Lastly, IAA would capture any cysteines if the disulfide bond broke, which was not observed.

The above results show the ^{C38}R1 protein behaves differently than the $^{C38/C81/C96}$R1 protein because the ^{C38}R1 protein gel only shows dimers, and not a mix of monomers and dimers. This indicates that the disulfide bond never breaks in the ^{C38}R1 protein.

Conclusions and Future Directions

Conclusions

- The C38R1 protein can bind Zn and can create a disulfide bond
- The disulfide bond is not dependent on Zn
- The breakage of the C38/C38 disulfide in C38/C81/C96R1 is probably due to the protein environment and not a property of the disulfide bond by itself.

Future Directions

- Determine x-ray crystal structure C38R1 (apo and Zn-bound)
- Repeat PAR assay to determine why Zn/protein was so low

References

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