Investigation of Tyrosine-Cysteine Crosslinks in a Model Protein

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Introduction

Proteins, made up of amino acid building blocks, undergo various modifications after translation. Protein-derived cofactors are a unique class of redox active posttranslational modifications formed in or by metalloproteins. These cofactors provide new reactivity to the generally unreactive amino-acid side-chains through covalent crosslinkages between amino acid side chains. One of these crosslinkages is bond formation between Tyrosine and Cysteine amino acids.

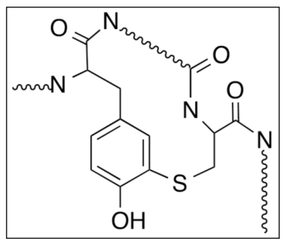


Figure 1. The tyrosine-cysteine crosslink

The covalent bond formed between these two amino acids has been recognized to have active functions inside its parent protein. In galactose oxidase, the Tyr-Cys crosslink functions as a co-oxidant in redox chemistry.1 In cysteine-dioxygenase, Tyr-Cys is proposed to contribute rigid hydrogen bond donation.2 The Tyr-Cys crosslink also has implications as an antioxidant in the protein, as seen in cytochrome c oxidase where His-Tyr, an analogous crosslink, reduces reactivity of the heme site.3 Small molecule antioxidants are well known to protect cells from damaging free-radical species and have thus been targeted to address issues of human health such as inflammation, cancer and aging.4 The potential for protein-localized antioxidants to serve a similar role remains an underdeveloped area of inquiry.

Mechanistic Study

Model Protein: BF4112

In light of this bond's significance, the mechanistic details of Tyr-Cys crosslink formation has been examined using an orphan protein from *Bacteroides fragilis*, BF4112, as a chemical model. BF4112 has a tyrosine and cysteine side chain geometrically predisposed for Tyr-Cys crosslink formation adjacent to a His2Glu coordinated metal binding site (Figure 2, PDB 3CEW).[5] BF4112 was selected as potential crosslink forming protein through a bioinformatic screening of the protein database and was confirmed to form a tyrosine-cysteine crosslink by proteomic analysis (Figure 3).[6] BF4112 is small (15 kDa) and has a single tryptophan residue, making it ideal for spectroscopic study.

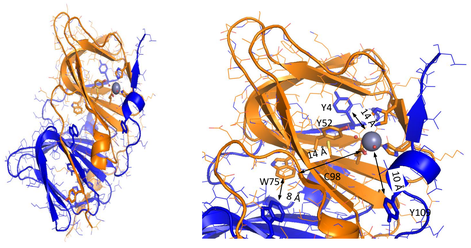


Figure 2. (Left) Crystal structure of apo BF4112. (Right) Close up of Y52, C98, and His_2Glu metal binding site in BF4112.

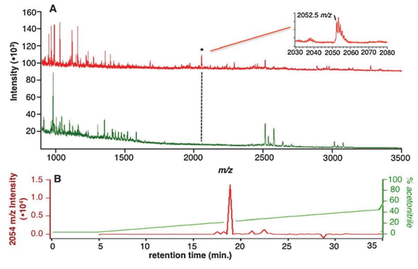


Figure 3. Liquid chromatography-mass spectral analysis of trypsin/Glu-C digests of CuI-bound BF4112. (A) MALDI-TOF mass spectrum of 19.0–19.3 min fraction is shown for oxygenated (red) and unoxygenated (green) samples; inset, expanded view of starred peak. (B) Reconstructed ion chromatogram of intensities at 2054 2 m/z, red, and elution gradient, green.

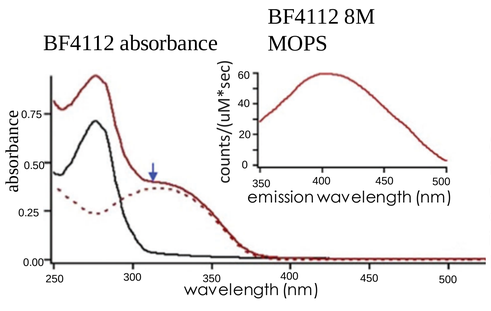
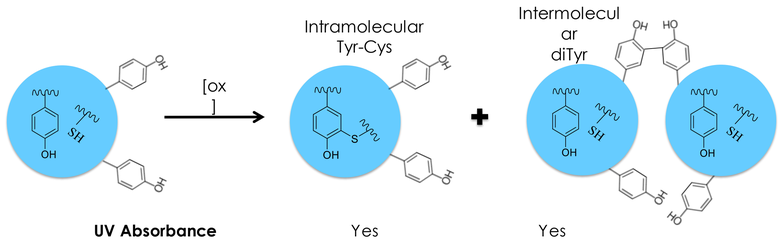


Figure 4. Absorbance data of crosslink formation. The black curve is uncrosslinked BF4112, the red curve crosslinked BF4112, and the dashed line is the difference. (Inset) Crosslinded BF4112 fluorescence in 8M MOPS.

Spectroscopic Signatures

$$Cu^{2+}(Tyr\ Cys) \xrightarrow{S_2O_6^{2-}} Cu^{+}(Tyr\ Cys) + O_2 + 2H^{+} \rightarrow Cu^{+}(Tyr\text{-}Cys) + H_2O_2$$



UV Absorbance	Yes	Yes
SDS PAGE	Fast mobility band	Dimer band
Fluorescence Emission	400nm max	400nm max

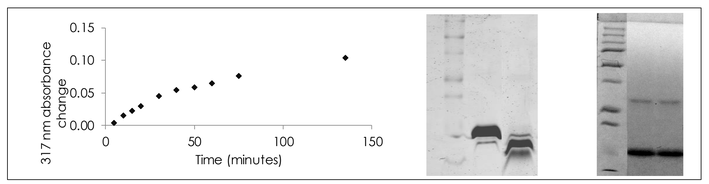


Figure 5. (Top) Oxidation products of BF4112. (Bottom) UV Absorbance, SDS PAGE fast mobility, and SDS PAGE dimer detection assays of BF4112 oxidation products: (Left) Increase in absorbance at 317 nm of reduced Cu-bound BF4112 after O_2 exposure (Middle) Ladder, apoBF4112, and Cu^{I} + O_2 treated BF4112 sample. The latter displays the altered mobility of the crosslinked product (Right) -OCl treated BF4112 displaying dimer bands.

Fluorescence Assay

A fluorescence assay visualizes the phenolate form of the tyrosine-cysteine crosslink at 317 nm. Spectral subtraction of a pH 7 solution from a pH 10 solution provides an emission spectrum void of tryptophan interference. This assay can then provide quantitative data on amount of tyrosine-cysteine crosslink in solution.

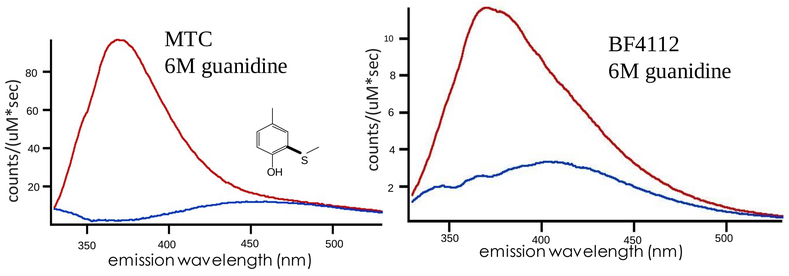


Figure 6. (Left) (Methylthio)cresol was used as a model compound for BF4112 fluorescence, as it has the same crosslink structure. (Right) BF4112 fluorescence over the same wavelength in pH10 (red) and pH 7 blue) in 6M guanidine.

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Results

Five different versions of BF4112 were tested using the fluorescence Assay. Wild type BF4112. Y52F in which the tyrosine involved in the crosslink has been removed. Y4F Y109F in which the two other crosslinks have been removed. C98S in which the cysteine involved in the crosslink has been removed. And wild type that had been treated with zinc instead of copper. A biotin enhanced sample from last year had a ration 2.6. This value is 100% crosslink and the yields of the other samples can be determined by comparing their slope ratios to the biotin sample.

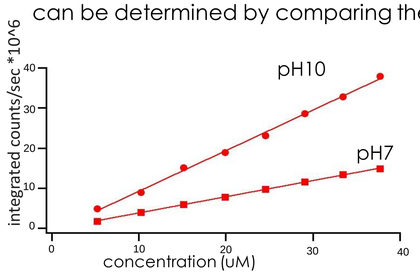

Figure 7. Fluorescence of wild type BF4112 in 6M guanidine with increasing concentration. The ratio of the pH 10 slope ($9.99*10^5$ counts/uM*s) and pH 7 slope ($4.06*10^5$ counts/uM*s) is 2.46 +/- 0.09 and is used for %yield Cys-Tyr determination.

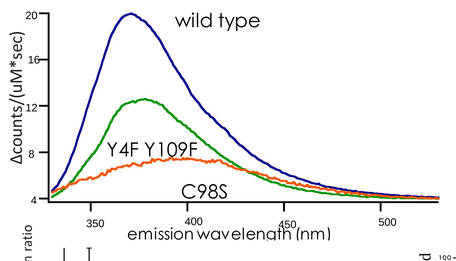

Figure 8. Fluorescence emission difference spectra (pH10-pH7) for crosslinking samples; wild type (blue), Y4FY109F (green), and C98S (orange). The C98S is significantly red shifted as for dityrosine.

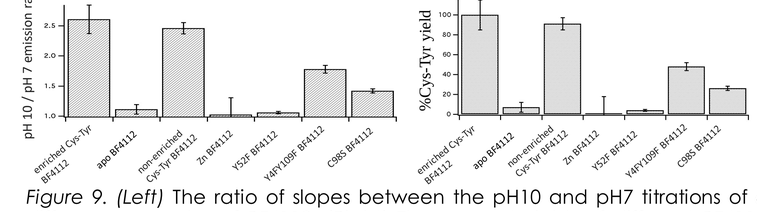

Figure 9. (Left) The ratio of slopes between the pH10 and pH7 titrations of 6 difference samples of BF4112. (Right) The percent yields of different BF4112 samples using the ratio of the pH10 to pH7 titration to quantify yield. A biotin enhanced sample is set at 100% with the other samples compared to it.

Conclusions

- Wild type BF4112 in copper has a 90% yield in crosslink
- Taking out the Y52F tyrosine prevents any crosslink from forming
- Apo and zinc does not work to form a cross link
- The fluorescence for the Y4F Y109F and C98S show that other tyrosines are being oxidized in the process
- Future directions are to explore using other transition metals, and to look into C98S and possible further.
- This is a fundamentally different mechanism than what has been reported for cys-tyre formation in galactose oxidase

References

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