Engineering Human Heteropolymer Ferritin Towards Understanding Subunits Self-Assembly

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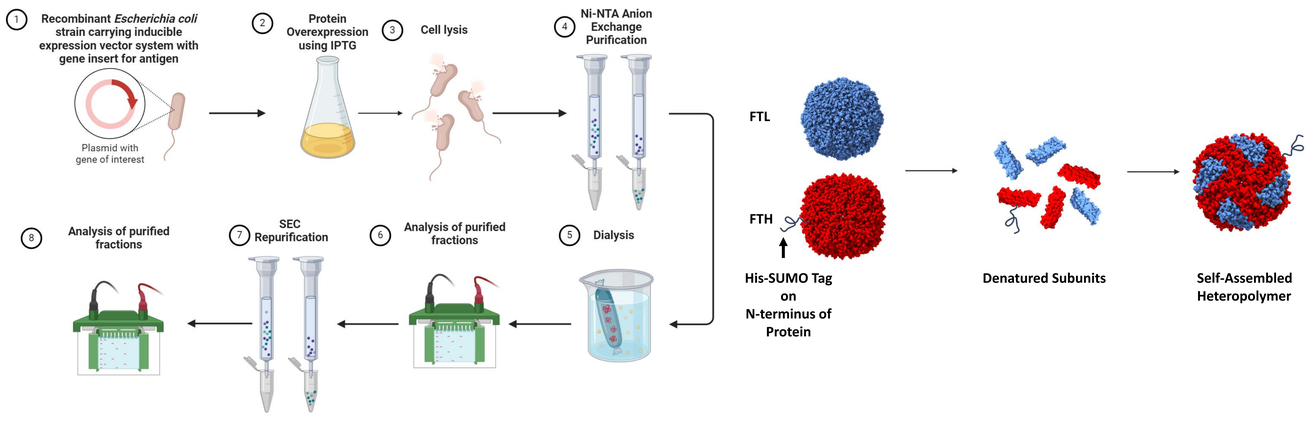
INTRODUCTION

- Ferritin stands as a fundamental player in cellular iron metabolism, serving as a crucial reservoir for iron storage and a regulator of iron homeostasis.
- Comprising a 24-subunit assembly, ferritin in mammals consists of Heavy (H) and Light (L) subunits, with variations in their ratios across different cellular contexts
- Although being synthesized from different genes, H and L subunits are able to co-assemble to form hybrid shells without the accumulation of H and L homopolymer
- Despite decades of study, much remains unknown about ferritin assembly, subunit heterogeneity and physiological functions, necessitating investigation into the association between H and L subunits in forming the 24-mer ferritin structure.

MATERIALS AND METHODS



Induction of SUMO-FTH expression utilized 0.5 mM IPTG, while PDS20-FTL was self-inducing. Incubation occurred in LB (FTL) and M9 (Sumo-FTH) media for 7 and 4 hours, respectively, at 37°C.

SUMO-FTH purification involved Ni-NTA histrap column chromatography, using a buffer exchange gradient elution. FTL purification employed size-exclusion chromatography on a Superose 12 GL Analytical Column followed by a 2nd repurification.

H-Sumo-L heteropolymer purification utilized Ni-NTA Column chromatography, followed by SEC repurification. All fractions were analyzed by 12% SDS-PAGE and 6% Native-Page.

The SUMO-FTH and Tag-free FTL proteins were combined in an 8 M urea buffer solution (10mg each) and gradually reduced to pH 2.0 using 1 M HCl to degrade homopolymers into individual subunits. Subsequently, pH was restored to 7.4 using 0.5M NaOH to facilitate reassembly of HSUMO and L proteins into a heteropolymer for further analysis.

RESULTS

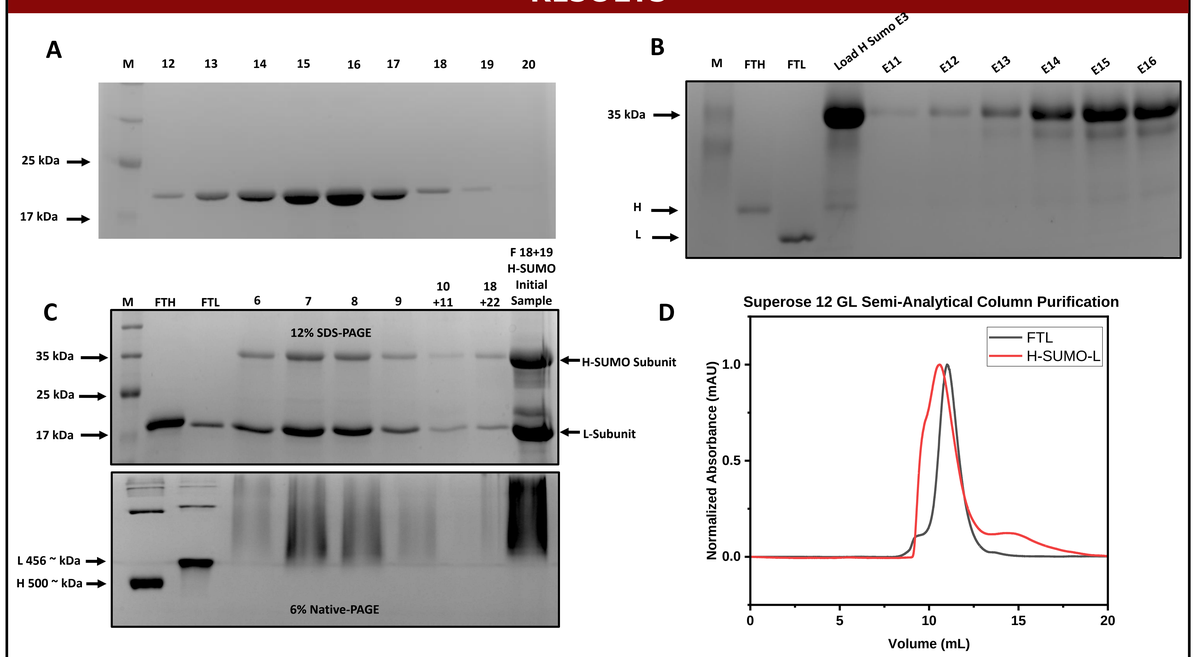


Figure 1. In vitro reassembly of H-SUMO-L heteropolymer ferritin. **A.** Ferritin L homopolymer fractions after SEC purification characterized by 12% SDS-PAGE. **B.** H-SUMO elution fractions, E, after Ni-NTA Purification and SEC repurification characterized by 12 % SDS-PAGE compared to standard Ferritin H and Ferritin L homopolymer, FTH and FTL respectively. **C.** In vitro reassembled H-SUMO-L heteropolymer ferritin fractions after purification on Ni-NTA column and SEC repurification characterized by 12% SPS-PAGE (Top) and 6% Native-PAGE (Bottom). **D.** SEC chromatogram of FTL and H-SUMO-L proteins normalized to FTL absorbance.

DISCUSSION

To confirm the formation of the heteropolymer, we employed purification techniques such as Ni-NTA chromatography and Size Exclusion Chromatography. These methods, coupled with SDS- and Native-PAGE analysis, provided valuable insights into the composition of the synthesized heteropolymer.

The Ferritin L-subunit typically weighs around 19 kDa, while the H-subunit weighs approximately 21 kDa. The SUMO-tag utilized in our study has a molecular weight of ~12 kDa but migrates similarly to ~15 kDa on SDS-Page due to its unique properties. We anticipated that the assembled heteropolymer on SDS-Page would exhibit bands for each SUMO-H and L subunits around 36 kDa and 19 kDa respectively, and these bands were observed accordingly **(Fig. 1C)**.

Native-PAGE analysis revealed a protein with a larger molecular weight compared to the weights of HSUMO and FTL proteins in their native state. Ferritin H Homopolymer (FTH) typically migrates to around 500 kDa, while Ferritin L Homopolymer (FTL) migrates to around 456 kDa. We would expect to see the heteropolymer protein run approximately 660 kDa. However, due to characteristics of the synthesized heteropolymer, a distinct band is not observed and there may be the potential of numerous aggregates formed, but further analysis is required.

Future Work

Optimization of procedural conditions to improve yield and protein stability.

Imaging of our synthesized heteropolymer ferritin sample using CRYO-EM. This will help us to better understand how the SUMO-H and L subunits co-localize and whether there is any preferential binding association between H-L heterodimers versus H-H and L-L homodimers.

REFERENCES

Srivastava, A. K.; Arosio, P.; Poli, M.; Bou-Abdallah, F. A Novel Approach for the Synthesis of Human Heteropolymer Ferritins of Different H to L Subunit Ratios. J. Mol. Biol. 2021, 433 (19), 167198. https://doi.org/10.1016/j.jmb.2021.167198.

Bou-Abdallah, F. The Iron Redox and Hydrolysis Chemistry of the Ferritins. Biochim. Biophys. Acta - Gen. Subj. 2010, 1800 (8), 719–731. https://doi.org/10.1016/j.bbagen.2010.03.021.

Harrison, P. M.; Arosio, P. The Ferritins: Molecular Properties, Iron Storage Function and Cellular Regulation. Biochim. Biophys. Acta 1996, 1275 (3), 161–203. https://doi.org/10.1016/0005-2728(96)00022-9.

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