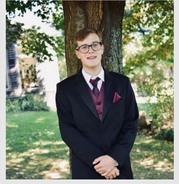

Gideon L. Smith

Mutant L-Chain Ferritins that Cause Neuroferritinopathy Alter Ferritin Functionality and Iron Permeability

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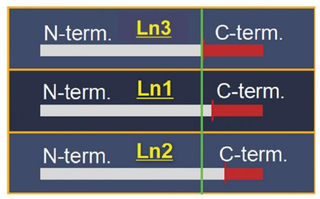
Background

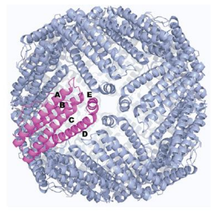
Ferritin is a ubiquitous protein with roles in iron storage, detoxication, and homeostasis. Ferritins are composed of 24 subunits of two different types, H (Heavy) and L (Light). The H-subunit contains ferroxidase centers for the oxidation of Fe(II) to Fe(III) and subsequent iron core formation. The L-subunit contains a high concentration of carboxyl groups for the site of iron nulceaion and mineralization. Neuroferrtinapathy (NF) is a genetic disease characterized by iron accumulation in the brain, caused by mutations of ferritin light-chain genes. Symptoms of NF include involuntary movement disorders and cognitive decline. Nine genetic mutations have been correlated with NF onset, three of which are discussed here: Ln3, Ln1, and Ln2, and one non-pathogenic mutation, L135P. The thermodynamic stability as well as the iron uptake, release, and capacity of these mutants are studied and compared to wild-type ferritin (WT).

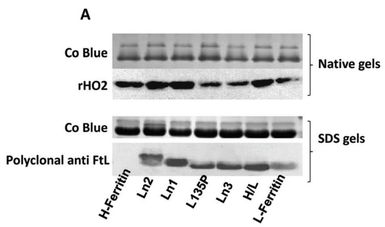
Materials and Methods

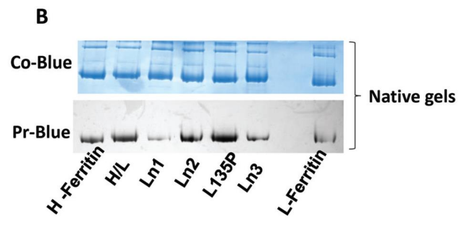
Heteropolymers cloned with plasmids in *E. Coli* bacteria.
Western blotting: Coomassie blue used for protein concentration, rHO2 antibody used for H, polyclonal anti FtL used for L, Prussian blue used for Fe concentration.
CE was used to characterize the H and L ratios of the ferritins using an Agilent 7100 CE system.
TA Instruments NanoDSC was used to measure the thermal stability of the proteins.
Varian Cary 50 UV-Vis spectrophotometer was used for absorbance kinetics.
FMN, NADH, and Ferrozine were used for Fe reduction kinetics.
Experiments run in 0.1 M Hepes Buffer, pH 7.

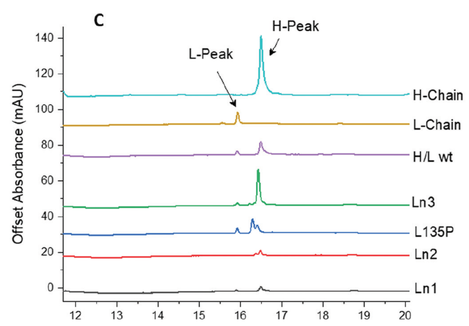
Results

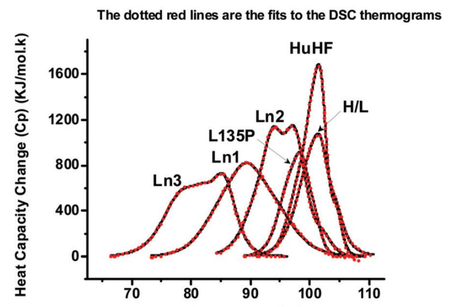


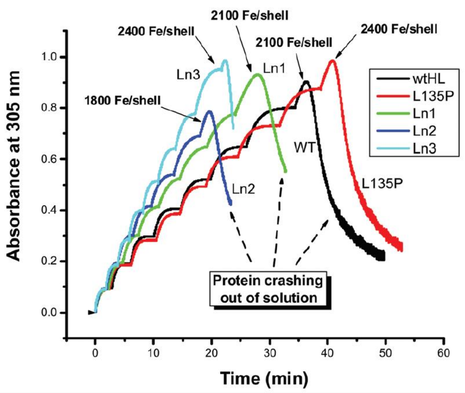


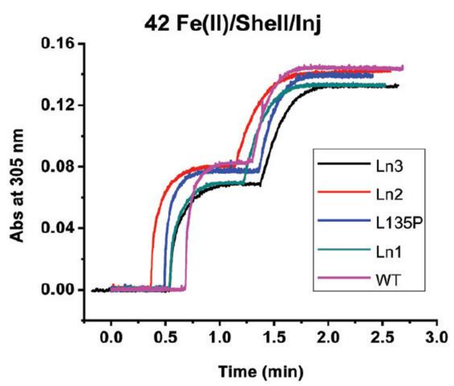


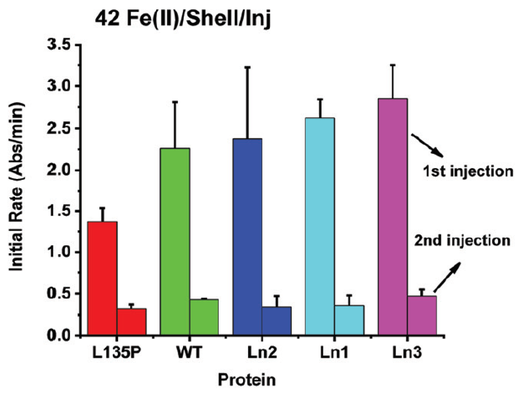


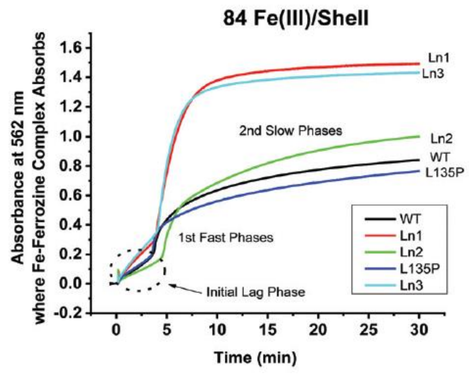


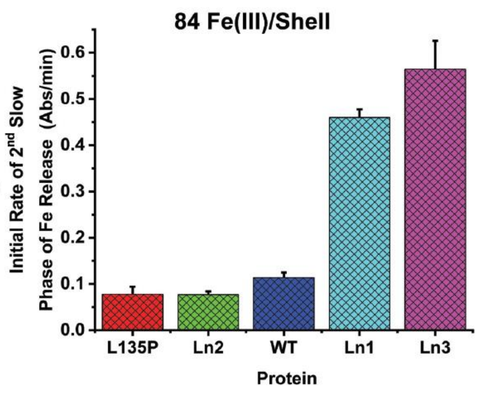












Discussion

The subunit distribution of each heteropolymer and iron content were assessed using western blotting and confirmed using CE to contain 10-15% (2.4-3.6) L and 85-90% (20.4-21.6) H. Heat stability of the proteins was shown in order Ln3 < Ln1 < Ln2 < L135P < HuHF = wt H/L, correlating directly with location of frameshift mutations. Double-peaks seen in thermograms indicates unfolding of secondary domains formed from mutations. Comparable iron storage was seen in all ferritin. Faster iron uptake (oxidation) rates were seen in all mutant samples as well as faster iron release (reduction) rates. Faster release/uptake rates and lower stability in mutations indicates significant structural affects and altered pore size in mutations.

Conclusions

- Heteropolymers characterized to be between 20-21 H subunits and 3-4 L.
- Determined a stability order of Ln3 < Ln1 < Ln2 < L135P < WT.
- Similar storage capacity seen in all proteins.
- Increased rate of uptake and release seen in mutant types.
- Alterations of the 4-fold channels due to altered L subunit structure leads to increased iron permeability and iron mismanagement.

Acknowledgments

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