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Structural and functional insights into aceruloplasminemia: a story of missing copper

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Abstract

Aceruloplasminemia is a rare autosomal recessive iron overload disease caused by mutations in the gene of the ferroxidase ceruloplasmin. Based on the X-ray structure of human ceruloplasmin, attempts were made to interpret the aceruloplaminemia mutations. For some cases of missense and nonsense mutations (H978Q, G631R, G969S, W1017X, R882X) we were able to provide plausible structural explanations of the mutants' dysfunction.

Introduction

Aceruloplasminemia is an inherited genetic disorder that is caused by defects in the ceruloplasmin gene. Aceruloplasminemia is characterized by diabetes, retinal degradation, and progressive neurological symptoms, including extrapyramidal syndrome, ataxia, and dementia. An impairment of the ceruloplasmin ferroxidase activity is consistent with a gradual accumulation of intracellular iron leading to systemic haemosiderosis [1-3]. Ceruloplasmin, a copper-containing glycoprotein with a molecular weight of 132 kDa, belongs to a family of multinuclear "blue" oxidases [4] and is composed of a single chain of 1046 amino acids with a carbohydrate content of 7–8%. The X-ray structure of human ceruloplasmin (hCP) reveals that the molecule is composed of six cupredoxin-type domains with large loop insertions

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[5,6]. Three of six integral copper atoms are mononuclear (T1-copper) and they localized in the even domains 2, 4, and 6. Three remaining coppers form a trinuclear copper cluster T2/T3 at the interface of domains 1 and 6, Fig.1.

Fig.1. X-ray structure of hCP (PDB ID: 2j5w). A view of the molecule along the pseudo-threefold axis, 6 integral copper atoms are shown in blue spheres.

Ceruloplasmin is initially synthesized as apo-ceruloplasmin and incorporates copper into the apo-protein in the Golgi body. The role of ceruloplasmin, formed as holo-ceruloplasmin, is a ferroxidase mediating ferrous ion oxidation and subsequent transfer to transferrin. In normal subjects, serum transferrin is acting as a shuttle to deliver iron to the bone marrow where it is picked up by hemoglobin. The mutant ceruloplasmin revealed three distinct pathological mechanisms (Fig.2) including mutations resulting in the retention of the protein in the endoplasmic reticulum, mutations resulting in abrogation of copper incorporation into apo-ceruloplasmin, and the mutants with impaired ferroxidase activity [2,3].

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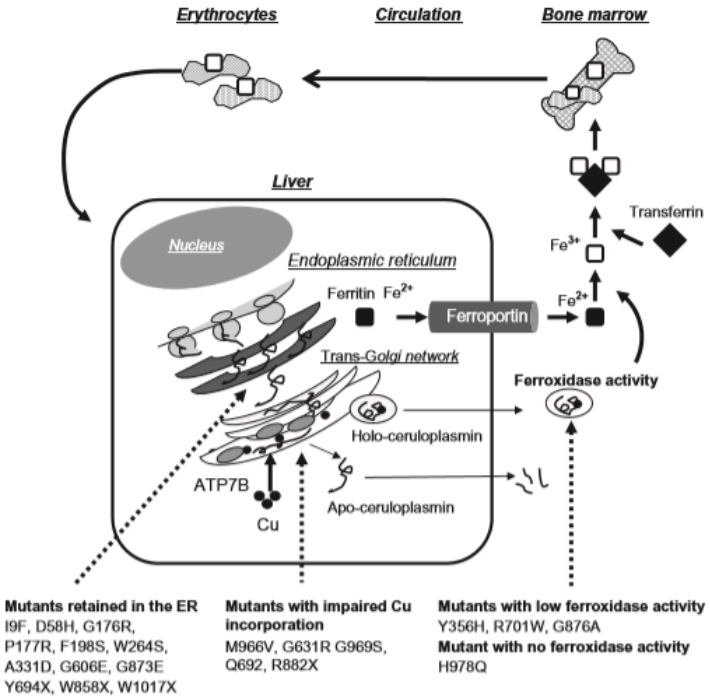

Fig.2. A model for the interaction between iron and copper homeostasis in normal subjects and in the aceruloplasminemia patients [3].

Results

In certain cases, mutations in the ceruloplasmin gene with aceruloplasminemia appear to be caused by an incomplete expression of the ceruloplasmin molecule with the polypeptide chain being prematurely terminated [8]. Our earlier work on the X-ray structure of hCP (PDB ID: 1kcw, [5]) provided structural explanations as to why, if the truncated molecules are actually secreted into the bloodstream, they will have their oxidase function severely impaired, Fig.3.

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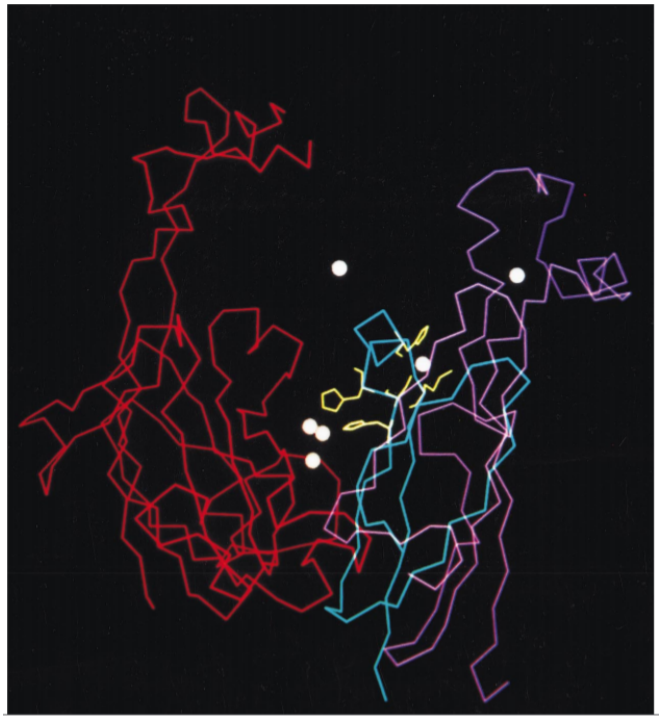

Fig.3. Traces of the a-carbon polypeptide backbone of domains 1 and 6 in the hCP structure. A portion of the polypeptide chain (residues 991 to 1046, colored blue) is missing in the truncated enzyme. The copper-binding ligands (yellow) that would be concomitantly missing comprise three ligands of the domain 6 mononuclear copper and two of the histidine ligands of the trinuclear cluster. In the absence of these ligands, domain 6 is unlikely to bind copper (white spheres) and this should have a profound effect on the oxidase efficiency of the enzyme [5].

Recently, we have investigated a few mutations of the wild-type hCP (PDB ID: 2j5w, [6]) including three missense, **H978, G631R, G969S**, and two nonsense mutants, **W1017X and R882X** (Fig.4). We used an in-house pipeline, Missense3D [9], for assessing missense variants. The core program of the pipeline, PHYRE2, applies the 'hidden Markov chain' algorithm [10] to predict the target mutant structure using the template provided by the user. Accurate side-chain rotamer conformation for a mutated residue is achieved using the program SCWRL4 [11].

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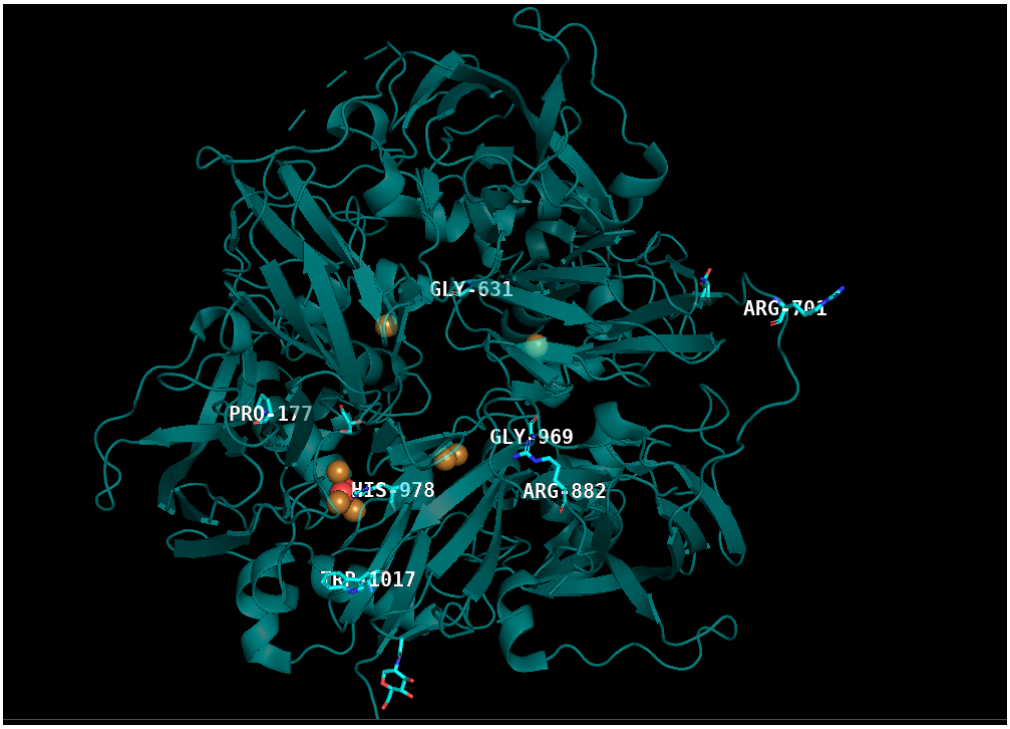

Fig.4. Various sites of mutations in the hCP structure, copper atoms are shown in light brown spheres.

1. H978Q

This substitution replaces a buried charged residue His with an uncharged residue Gln and it disrupts all side-chain / main-chain H-bonds formed by His978, a ligand of the T2-copper in the trinuclear cluster T2/T3 (Fig.5). Also, the substitution disrupts the wild-type salt bridge between the ND1 atom of H978 and the OD1 atom of D995. In addition, a new conformation of the imidazole ring of the second histidine ligand, H101, makes NE2 -Cu bond weaker compared to the wild-type bond.

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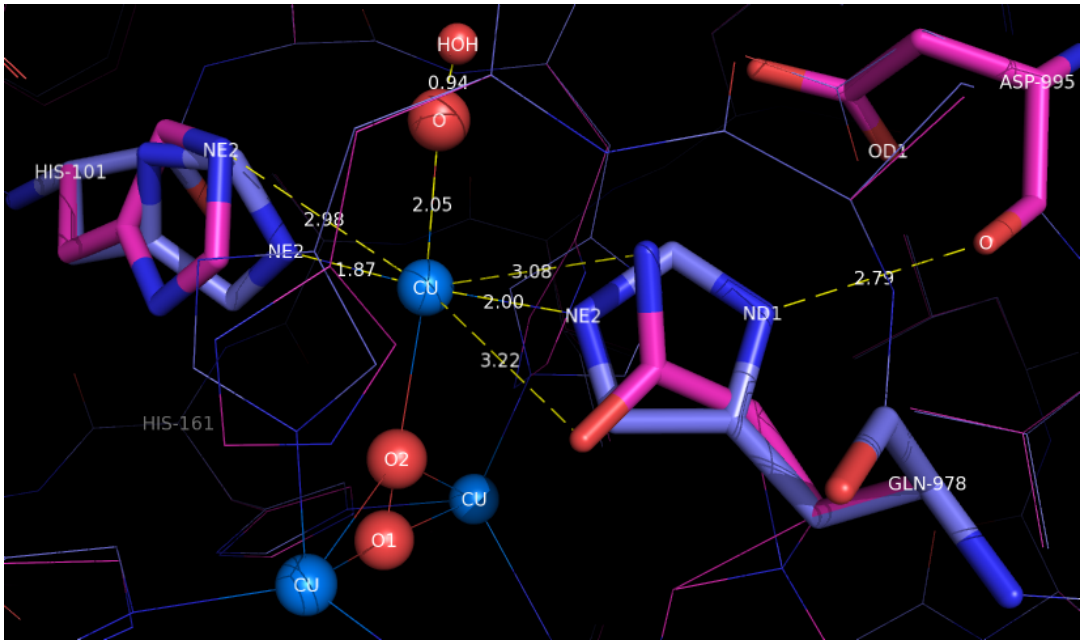

Fig.5. The trinuclear copper cluster of the H978Q mutant structure generated with PHYRE2 [9]. The heteroatoms were not included in the calculations. Wild-type hCP residues are depicted in grey-blue, residues of the mutant - in magenta.

As the result, T2-copper would be probably missing in the H978Q mutant structure. A model of this mutant created with the SWISS-MODEL software [12] confirmed our suggestion. All heteroatoms comprising three coppers of the trinuclear cluster and bound oxygen molecule were included in the template (hCP, PDB ID: 2j5w). Surprisingly, the resulting H978Q mutant structure *did not contain T2-copper and molecular oxygen*. The bond distances and calculated energies for the Gln978 side-chain interactions with copper did not satisfy the criteria of the program [12]. Thus, it generated a T2-depleted form of the molecule, Fig.6.

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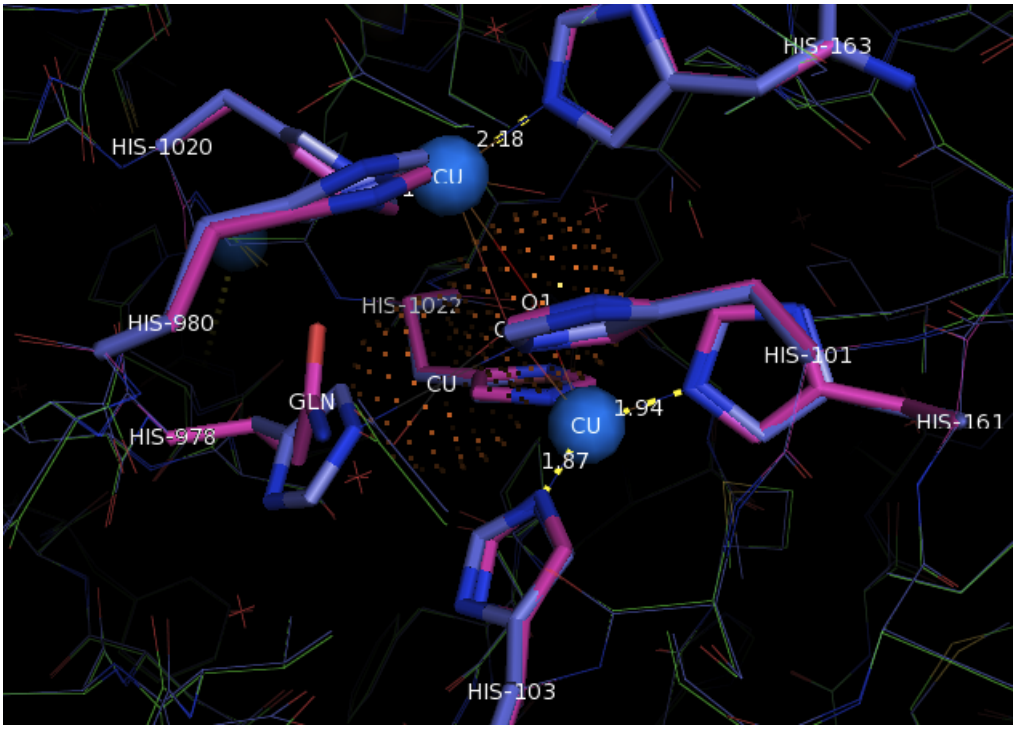

Fig.6. The trinuclear copper cluster of the H978Q mutant structure generated with SWISS-MODEL [12]. Missing T2-copper and molecular oxygen are shown in orange dots.

The absence of the T2-copper in the trinuclear center would disrupt the catalytic cycle of hCP. Indeed, the H978Q is reported to be a mutant with the impaired ferroxidase activity [3].

2. G631R

This substitution replaces a buried uncharged glycine with a charged arginine. The mutant's Arg631 forms a salt bridge with Asp635, one of the conserved residues of the ET pathway between the two mononuclear copper centers: Cu2-H324 …. E633 A634 D635 V636 …. H637-Cu4 [7]. A new salt bridge weakens the hydrogen bond between D635 and H637, a ligand of the T1-copper, and this may trigger the copper loss and/or ability to bind copper in domain 4.

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3. G969S

This substitution replaces a buried glycine with serine. Ser969 forms a new hydrogen bond with Asp973, one of the conserved residues of the ET pathway between the two mononuclear copper centers: Cu4-H685…. E971 I972 D973 L974 …. H975-Cu6 [7]. In turn, D973 makes an H-bond with the histidine H975, a ligand of the T1-copper. Thus, the interaction of D973 with S969 in the mutant structure would affect the copper-binding ability in domain 6.

4. W1017X and R882X

W1017X: a portion of the polypeptide chain, residues 1017 to 1046, is missing in the mutant molecule. **R882X**: the whole domain 6 will be truncated. These missing C-terminal segments contain the ligands of the mononuclear copper center and the trinuclear cluster of the 6th domain, and the ligands of a labile copper site. The loss of the binding ability of copper due to the absence of copper ligands in the 6th domain would have a dramatic effect on the oxidase activity of hCP. It is probable that the incomplete enzyme will adopt a different organization of the tertiary structure and may well exist in an open configuration, which would prevent the protein from secretion into the bloodstream.

Further details and figures for the **G631R, G969S, W1017X, and R882X** mutants are given here: http://slavazaitsev914078364.wordpress.com/aceruloplasminemia-mutants/

Concluding remarks

The mutated residues selected for our studies are either copper ligands or residues located in close proximity to the copper atoms of the active sites. Together with the nonsense mutations resulting in a truncated enzyme with the copper ligands partly or completely missing, this facilitates the analysis. The effect of mutation could be explained as the result of a copper loss.

Without 'help of missing copper', our attempts to interpret the other mutations of the aceruloplasminemia list have been unsuccessful. For instance, mutants **R701W and P177R** (Fig.4) remain mysterious.

R701W: arginine 701 is located on the surface of the hCP molecule in a large solvent-exposed loop connecting domains 4 and 5. No structural damage has been detected by Missense3D for the replacement Arg –>Trp. However, according to [2,3], this mutant's ferroxidase activity is seriously reduced. The authors [2] suggest that R701W mutation affects the interaction of ceruloplasmin with the copper-transporter ATP7B. **P177R**: buried proline 177

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lies in relative proximity to several neighboring hydrophobic residues and it resides within a repeated motif G(FLI)(LI)GP of the hCP pseudo-hexamer. It is not clear how the replacement of the uncharged proline by charged arginine in this mutant may lead to the protein unfolding [13].

Further experimental studies will be useful in completing the story of the ceruloplasmin mutants. A deeper understanding of the molecular mechanisms of aceruloplasminemia may provide novel therapeutic strategies in the treatment of this disease [1-3,14].

References

1. M. Kruger (2013), Int.Rev.Neurobiology, Ch. 8 ,110: 165-194.

2. M. diPatti, N. Maio, G. Rizzo, G. De Francesco, T. Persichini, M. Colasanti, F. Polticelli and G. Musci (2009), J. Biol. Chem., 284: 4545-4554.

3. S. Kono (2013), Int. Rev. Neurobiology, Ch.6, 110: 125-151.

4. A. Messerschmidt (1997), 'Multi-copper oxidases'. World Scientific, Singapore.

5. P. Lindley, G. Card, I. Zaitseva, V. Zaitsev, B. Reinhammer, E. Selin-Lindgren, K. Yoshida (1997), J. Biol. Inorg. Chem. 2: 454-463.

6. I. Bento, C. Peixoto, V. Zaitsev and P. Lindley (2007), Acta Cryst. D63: 240-248.

7. P. Lindley, G. Card, I. Zaitseva, V. Zaitsev (1999), In Perspectives of Bioinorganic Chemistry, 4: 51-82.

8. K. Yoshida, K. Furihata, S. Takeda, A. Nakamura, K. Yamamoto, H. Morita, S. Hiyamuta, S. Ikeda, N. Shimizu, N. Yanagisawa (1995), Nature Genetics. 9: 267–72.

9. S. Ittisoponpisan, S. A. Islam, T. Khanna, E. Alhuzimi, A. David and M. Sternberg (2019), J. Mol. Biol. 431: 2197-2212.

10. L. Kelley, S. Mezulis, C. Yates, M. Wass and M. Sternberg (2015), Nature Protocols, 10: 845-858.

11. G. Krivov, M. Shapovalov and R. Dunbrack Jr. (2009). Proteins, 77: 778–795.

12. A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, F. Heer, T. deBeer, C. Rempfer, L. Bordoli, R. Lepore, T. Swede (2018). Nucleic Acids Research, 46:W1, W296–W303.

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`https://doi.org/10.20935/AL3232`.

13. N. Hellman, S. Kono, H. Miyajima, and J.D. Gitlin (2002), J. Biol. Chem. 277: 1375-1380.

14. L. Vroegindeweij, A. Boon, J. Wilson and J. Langendonk (2020), Orphanet Journal of Rare Diseases, 15:105-112.

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