INVESTIGATION ON A MMACHC MUTANT FROM *cbl*C DISEASE

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Abstract
The cblC disease is an inborn disorder of the vitamin B12 (cobalamin, Cbl) metabolism characterized by methylmalonic aciduria and homocystinuria. The clinical consequences of this disease are devastating and, even when early treated with current therapies, the affected children manifest symptoms involving vision, growth, and learning. The illness is caused by mutations in the gene codifying for MMACHC, a 282aa protein that transports and, with the help of specific cofactors like glutathione (GSH), transforms the different Cbl forms. Although the crystal structure of the wild-type (WT) protein is available [1,2], many molecular features of MMACHC physiopathology remain to be understood and a systematic study on the effect of each specific mutation on the resulting protein is still lacking.

Here we present data on the biophysical characterization of wild type MMACHC, and two variants resulting from the pathological mutations found in CblC patients: the mutant R161Q and the truncated protein p.R132X resulting from the c.394C>T mutation that, along with c.271dupA and c.331C>T, is among the most common mutations in cblC. By using a biophysical approach including spectroscopy, Small Angle X-ray and Light Scattering we investigated protein stability, structural properties, ability to bind and transform the vitamin B12 and to assemble in a dimeric structure that, since the first pioneering study on the protein crystal structure [2] was considered to be the functional oligomeric form of the WT protein. Important indications on the behavior of the proteins resulted from the Molecular Dynamics (MD) simulations carried out on MMACHC-R132X in the presence of Cbl [3] and MMACHC-R161Q in the presence of Cbl and GSH. Instabilities in the interactions between the mutants and the cofactors (Cbl, GSH), revealed by MD simulations well correlate with experimental data on proteins structure and function, casting further light on the molecular basis of the impairment of enzymatic activity.
Overall our results reveal how a biophysical approach based on the complementarity of computational and experimental methods offer new insights in the study of specific effect of the pathological cblC mutations on MMACHC protein structure, stability and Cbl binding properties and help prospecting new routes for the cblC treatment.

References

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