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Pathogenic Mutations Differentially Affect the Catalytic Activities of the Human B_{12}-processing Chaperone CblC and Increase Futile Redox Cycling*

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Capsule

Background: CblC processes cobalamins entering a cell to a common intermediate.

Results: Pathogenic mutations at Arg-161 weaken glutathione binding to CblC and stabilize cob(II)alamin.

Conclusion: The R161Q/G mutations impair the dealkylation but not the decyanation activity of CblC.

Significance: Increased redox cycling by the CblC mutants explains the observed cellular oxidative stress associated with this disorder.

Abstract

Human CblC catalyzes the elimination of the upper axial ligand in cobalamin or B_{12} derivatives entering the cell from circulation. This processing step is critical for assimilation of dietary cobalamin into the active cofactor forms that support the B_{12}-dependent enzymes, methionine synthase and methylmalonyl-CoA mutase. Using a modified nitroreductase scaffold tailored to bind cobalamin and glutathione, CblC exhibits versatility in the mechanism by which it removes cyano *versus* alkyl ligands in cobalamin. In this study, we have characterized the effects of two pathogenic missense mutations at the same residue, R161G and R161Q, which are associated with early and late onset of the CblC disorder, respectively. We find that the R161Q and R161G CblC mutants display lower protein stability and decreased dealkylation but not decyanation activity, suggesting that cyanocobalamin might be therapeutically useful for patients carrying mutations at Arg-161. The mutant proteins also exhibit impaired glutathione binding. In the presence of physiologically relevant glutathione concentrations, stabilization of the cob(II)alamin derivative is observed, which occurs at the expense of increased oxidation of glutathione. Futile redox cycling, which is suppressed in wild-type human CblC, explains the reported increase in oxidative stress levels associated with the CblC disorder.

Adenosylcobalamin (AdoCbl) Chaperone Enzyme Kinetics Oxidation-Reduction (Redox) Trafficking Cofactor

Footnotes

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