Clinical missense mutations in MMACHC gene affect its conformational stability and vitamin B12-binding activity: The example of R161Q mutation.

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MMACHC is a crucial protein in the metabolism of vitamin B12 (cobalamin, Cbl) chemically transforming it into its active forms (AdoCbl and MeCbl), which are cofactors in important cellular reactions. Mutations in the gene encoding MMACHC are responsible for the metabolic disorder CblC methylmalonic aciduria and homocystinuria, cblC type (cblC), which, in most cases, affects children, causing neurocognitive and cardiovascular dysfunctions. Here, through the combined use of circular dichroism and differential scanning calorimetry, we demonstrate how a pathological mutation, R161Q, occurring far from the Cbl-binding pocket, affects the MMACHC thermal unfolding pathway and the populations of conformational intermediates. The binding properties to Cbl and the ability to form homodimers, triggered by the presence of the ligand, and assessed by native electrophoresis, light and small-angle X-ray scattering, were also impaired. Our study confirms a correlation between the energetics of thermal unfolding of MMACHC and its Cbl-binding properties, gaining knowledge into the molecular mechanisms underlying the function of MMACHC and providing insights for potential therapeutic interventions.