MUTATIONS OF UROPORPHYRINOGEN III SYNTHASE RELATED TO CONGENITAL ERYTHROPOIETIC PORPHYRYA ARE LOCATED IN DESTABILIZING PROTEIN REGIONS

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Our group have investigated the relationship between the congenital erythropoietic porphyria (CEP) and the deleterious mutations in the uroporphyrinogen III synthase (UROIIIS), the fourth enzyme in the heme group biosynthetic route. First, the 25 miss-sense mutations reported in the literature have been cloned, expressed and their enzymatic activities have been determined over pure protein as a relative measure of the wild type value. In general, most of the mutations retain much of the wild type activity, consistent with the recessive character of the disease. Mutations showing a significant decrease in activity correlate well with the putative residues involved in binding. Second, the enzyme stability has been rigorously examined by unfolding kinetics monitored by circular dichroism. UROIIIS is a thermolabile enzyme driven by irreversible unfolding and, therefore, the enzyme activity is a function of time. The same analysis carried out over the suite of mutants allowed identifying a helical region in the molecule essential to retain the folded conformation and ultimately the activity over time. In the middle of this helix there is cysteine 73, involved in one third of the CEP patients. Finally, an integrative analysis of the activity and kinetic data for all the mutants can largely explain the distinct severity of the disease found in CEP patients.

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