HUMAN ADENINE PHOSPHORIBOSYLTRANSFERASE (APRT) MUTATIONS: STRUCTURAL STUDIES AND CORRELATION WITH UROLITHIASIS

Pimenta Jr., A. A.[1], Rodrigues, E. M.[1], Silva, M.[1], Thiemann, O. H.[1]

[1] Centro de Biotecnologia Molecular Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo, São Paulo, Brazil.

The APRT from *Homo sapiens* (hAPRT) is a type I phosphoribosyltransferases and catalyze the conversion of adenine and PRPP to AMP, through a magnesium ion dependent reaction. This enzyme is a homodimer with 180 residues per subunit of approximately 20 kDa. Mutations in hAPRT are linked to serious kidney illness such as nephrolithiasis, interstitial nephritis, and chronic renal failure as a result of 2,8-dihydroxyadenine (DHA) precipitation in the renal interstitium. Eigth hAPRT point mutations are identified in patients with DHA-urolithiasis. To address the molecular basis of the disease, structural and kinetics studies of those mutants are being performed. The mutants, D65V, L110P, M136T, R67Q, R89Q, I112F and F173G, were obtained by site-directed mutagenesis on the recombinant hAPRT gene and cloned into pET-29a(+) vector. Expression and purification protocols have been established allowing for the production of the recombinant mutants. Crystallization screenings of the mutants are being carried out and a new enzymatic assay for hAPRT was established using the precipitation of the adenine with $LaCl_3$. Preliminary kinetics parameters were obtained for the native hAPRT (K_m = 6,1 ± 1,8 and V_{max} = (16,2 ± 1,3) x 10^{-5}). The kinetic parameters of the mutant hAPRTs are being carried out, as well as their crystallization.

Keywords: APRT, phosphoribosyltransferases, urolithiasis.

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