Structural basis of inhibition of the NUDIX family ORF141

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NUDIX hydrolases exist in a wide variety of species, ranging from humans to viruses. Initially, these enzymes were considered "housecleaning" enzymes based upon their ability to remove mutagenic nucleotides such as 8-oxo-dGTP. Since then, many more functions of NUDIX enzymes have been discovered and characterized such as: mRNA degradation, regulation of coenzyme A, and hydrolysis of nucleoside diphosphate sugars. Named after their shared ability, this superfamily catalyzes the hydrolysis of nucleoside diphosphate linked to a moiety X, hence the name "NUDIX". This superfamily is characterized by the presence of a sequence signature motif of 23 amino acids, G1N[5X]E7N[7X]R15NE16NXXE19NE20NXG22NU, known as the NUDIX box. This motif has been identified in over 20,000 species, all with differing genome sizes and number of NUDIX genes. The NUDIX signature sequence has three glutamates (E16NXX19NE20N) that are at the center of the β-strand-loop-helix-loop motif. Moreover, they bind the catalytic divalent cations that bridge the protein and the pyrophosphate of the substrate. Although the sequence signature motif allows for the identification of these enzymes into the superfamily, the classification of these enzymes into families requires information regarding the reaction they catalyze, preferred substrate, quaternary structure, and phenotype to formulate rules.

Previously, the NUDIX family represented by ORF141 was identified to be nucleoside triphosphatases with a preference for pyrimidine deoxy-nucleoside triphosphates, such as deoxyguanosine triphosphate (dGTP). Recent studies have discovered ORF141 to be an atypical NUDIX that prefers geranyl pyrophosphate, which lacks the typical nucleoside base of the archetypical NUDIX substrates.

To establish the structural determinants of substrate specificity for ORF141, we have determined the structure of apo ORF141 to 1.54 Å by x-ray crystallography. Interestingly, ORF141's NUDIX fold displays a hairpin insertion when compared to the minimal NUDIX fold observed in the NUDIX enzyme family with a preference for dGTP. We determined that bisphosphonates inhibit ORF141. To define the structural basis of inhibition, we determined the structure of ORF141 with three bisphosphonates: risedronate, [2-(n-pentylamino)ethane-1,1-diyl]bisphosphonic acid (BR6), and 2-(n-hexylamino) ethane-1,1-diyl]bisphosphonic acid (BR18). Each of the complex structures show that Mg2+ atoms mediate their binding to the glutamates of the NUDIX signature sequence. Interestingly, when bound to the bisphosphonates, ORF141's hairpin insertion displays a conformational change of up to 11 Å away from the active site and proportional to the length of the bisphosphonate's R derivative. For example, the 3-pyrindinylethyl group of the risedronate exerts less of a displacement in comparison to the longer hexylamine group of BR6.