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**The High-Resolution Crystal Structure of Eukaryotic Assimilatory Nitrate Reductase Mo Domain.**

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Nitrate reductases catalyze the first and rate-limiting step of nitrogen assimilation in autotrophs. This assimilatory pathway plays a key role in the global nitrogen cycle and about 90% of all reduced nitrogen found in living organisms is derived from nitrate assimilation. Eukaryotic nitrate reductase consists of four domains with three catalytic centers. The large N-terminal domain possesses a molybdenum center, the central domain contains a b-type cytochrome and the C-terminal domain binds flavine adenine dinucleotide (FAD). Nitrate reduction takes place at the molybdenum domain, where the catalytically active molybdenum is bound to an ene-dithiolate system of a pyranopterin, called molybdopterin. The basic structure of this molybdenum cofactor is conserved in all kingdoms and it is found in all molybdenum- (as well as tungstate-) containing enzymes, except nitrogenase. The catalytic cycle can be divided into three parts: a reductive half-reaction in which NADH reduces the FAD site, the electron transfer via the intermediate heme-domain to the molybdenum center, and an oxidative half-reaction in which the now reduced molybdenum center reacts with nitrate to generate nitrite. The enzyme is only active as a homodimer, which is mediated by the fourth, cofactor-free dimerization domain. The assembly and reaction mechanism of eukaryotic nitrate reductases is conserved among plants and fungi whereas they are structurally, functionally and mechanistically distinct from bacterial nitrate reductases, that contain a different type of molybdenum cofactor as well as iron-sulfur clusters as additional prosthetic groups [3]. Nitrate reductase belongs to the same class of dioxo-molybdenum-hydroxylases as animal and plant sulfite oxidases for which the crystal structures have been reported recently [1, 2].

We have determined the crystal structure of *Pichia angusta* nitrate reductase molybdenum domain at 1.7 Angstrom resolution, which is a representative member of eukaryotic nitrate reductases and presents the first structure for the catalytic domain of this protein family. The structure presented on the poster provides novel aspects for the understanding of the structure-function-relation between nitrate reduction and sulfite oxidation, two important and ubiquitous electron transfer reactions in nature.

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**Structural and functional characterization of bacterial YeiK nucleoside hydrolase from *E. coli*: catalytic mechanism and possible role in cancer gene therapy.**

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Enzymes with nucleoside hydrolase (NH) activity are crucial for the salvage of nucleic acid components in purine auxotrophic protozoan parasites. Several genes encoding for putative NH proteins have been annotated in genomes from both prokaryotes [1] and eukaryotes [2] [3] based on the presence of a consensus sequence formed by a N-terminal aspartate cluster [4]. Here we show the characterization of the YeiK (RihB) gene product from *Escherichia coli* as a calcium-dependent, pyrimidine-specific NH. We obtained single crystals of YeiK belonging to the triclinic crystal system in space group P1, with unit-cell parameters  $a=44.81$ ,  $b=85.71$ ,  $c=90.68$  Å,  $\alpha=112.95^\circ$ ,  $\beta=101.95^\circ$ ,  $\gamma=85.92^\circ$ . The structure of YeiK was solved using the molecular replacement technique for phase determination. The crystal structure of YeiK to 1.7 Å defines the basis for its substrate specificity, and identifies new residues involved in the catalytic mechanism. Large variations in the tetrameric quaternary structure compared to non-specific protozoan NHs are brought forth by minor differences in the interacting surfaces. The overall structure of the YeiK monomer resembles the NH fold [4], with an open ( $\alpha,\beta$ ) structure. To best characterize the catalytic mechanism of this pyrimidine specific enzyme, we performed kinetic studies on both native and site-specific mutants of the protein. The structure-based mutagenesis of YeiK supports a different mechanism for pyrimidine nucleoside hydrolysis compared to both non-specific and purine-specific NHs [5]. The first structural and functional characterization of a non-parasitic NH suggests a possible role for these enzymes in the metabolism of unusual tRNA nucleosides. The high catalytic efficiency of YeiK towards 5'-fluorouridine could be exploited for a suicide gene therapy approach in cancer treatment.

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