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Crystal structure of Tpa1 from *Saccharomyces cerevisiae.* Hye Jin Yoon[a], Hyoun Sook Kim[a], Hye Lee Kim[a], Kyoung Hoon Kim[a], Do Jin Kim[a], Sang Jae Lee[a], Ji Young Yoon[a], Se Won Suh[a,b]. [a]*Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea.* [b]*Department of Biophysics and Chemical Biology, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea.*
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Tpa1 (for *t*ermination and *p*oly*a*denylation) from *Saccharomyces cerevisiae* is a component of a messenger ribonucleoprotein complex at the 3' untranslated region of mRNAs. It comprises an N-terminal Fe(II)- and 2-oxoglutarate-dependent dioxygenase domain and a C-terminal domain. The N-terminal dioxygenase domain of a homologous Ofd1 protein from *Schizosaccharomyces pombe* was proposed to serve as an oxygen sensor that regulates the activity of the C-terminal degradation domain. Members of the Tpa1 family are also present in higher eukaryotes including humans. Here we report the crystal structure of *S. cerevisiae* Tpa1 as a representative member of the Tpa1 family. Structures have been determined as a binary complex with Fe(III) and as a ternary complex with Fe(III) and 2-oxoglutarate. The structures reveal that both domains of Tpa1 have the double-stranded β-helix fold and are similar to prolyl 4-hydroxylases. However, the binding of Fe(III) and 2-oxoglutarate is observed in the N-terminal domain only. We also show that Tpa1 binds to poly(rA), suggesting its direct interaction with mRNA in the messenger ribonucleoprotein complex. The structural and functional data reported in this study support a role of the Tpa1 family as a hydroxylase in the messenger ribonucleoprotein complex and as an oxygen sensor [1].

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Structure and function of a novel Dienelactone hydrolase Christian Roth[a], Michael Schlömann[b] Norbert Sträter[a]. [a]*Faculty for Chemistry and Mineralogy, Structural Analytics of Biopolymers, University Leipzig, Germany.* [b]*Interdisciplinary Ecological Center, Technical University Bergakademie Freiberg, Germany.*
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Fluoroaromatic compounds are widely used as pesticides or pharmaceuticals and well known environmental pollutants. The bacterium *Cupriavidus necator* is able to degrade such compounds via a modified variant of the β-ketoadipate pathway. One of the key enzymes is the *trans*-dienelactone hydrolase (t-DLH) which degrades (fluoro)dienelactone leading to maleylacetate. The *t*-DLH from C. necator is a monomeric enzyme with a molecular weight of approximately 38 kDa. The enzyme shows no significant similarity to other known dienelactone hydrolases. Furthermore C. necator t-DLH shows activity only in the presence of divalent cations like Mn^{2+} or Mg^{2+}.
The gene of *t*-DLH was cloned expressed in *E.coli*. The protein was purified to at least 90 % homogenity. The wildtype was subjected to crystallization trials using sparse matrix screens. Suitable crystallization conditions could not be identified for this variant. Mutants designed based on the principle of surface entropy reduction were created and purified. Crystals, suitable for diffraction experiments, could be obtained for a double mutant. The phase problem was solved using MIRAS and a model of the enzyme was built and the metal center was assigned. We currently try to get a complex structure to study the reaction mechanism of this new class of dienelactone hydrolases.

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