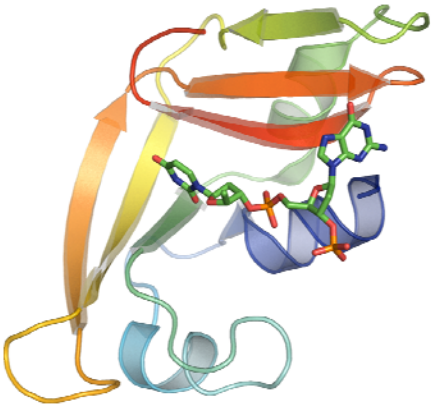
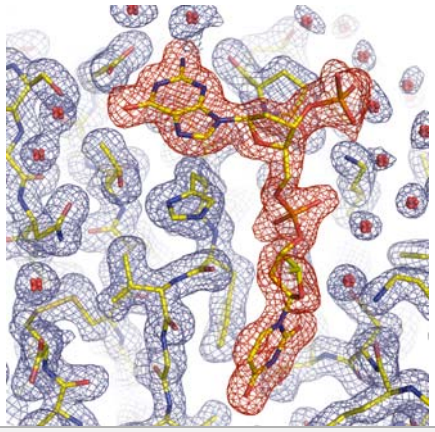
Center for Eukaryotic Structural Genomics

Protein Structure Initiative

Target ID	GO.80160	
Source Organism	*Rana pipiens*	
Target Name	Onconase-wt	
PDB Entry	2I5S	Deposition: 25-Aug-2006
Function	Onconase, P-30 protein, onconase with bound nucleic acid	
Produced From	*E. coli* BL21(DE3) pET22b(+)	
Structure by X-ray	Resolution: 1.90 Å	R-value (R-free): 18.1% (24.0%)
	No. of Residues/ASU: 104	Monomers/ASU: 1
Data Collected At	Rotating anode, Bruker AXS Microstar 02-May-2006	
Authors	E. Bae, G.E. Wesenberg, G.N. Phillips, Jr., E. Bitto, C.A. Bingman	





Structural Features

Onconase (ONC) is a homolog of bovine pancreatic ribonuclease (RNase A) from the frog *Rana pipiens*. ONC displays antitumoral activity and it is in advanced clinical trials for the treatment of cancer. ONC shares 30% of sequence identity with RNase A and their 3-D structures are very similar. Both proteins adopt a characteristic ribonuclease fold, which comprises of V-shaped β-sheet motif surrounded by three α-helices. The main differences between RNase A and onconase include the presence of disulfide bond between Cys104 and Cys87 and the unusual N- terminal pyroglutamate in the amphibian enzyme. The catalytic triad (His10, Lys31, and His97 in ONC) as well as several residues involved in the substrate binding are conserved in RNase A homologs. Despite this similarity, the ONC activity is 3-5 orders of magnitude lower in comparison with RNase A. ONC displays an unusually high thermal stability (T_m = 90°C), which has been linked to the renal toxicity of this enzyme. Extensive structure-function studies have been carried out in order to improve the properties of ONC for anticancer chemotherapy. CESG contributed to this effort by solving the first atomic structure of ONC with nucleic acid: a T89N/E91A ONC-5'-AMP complex and a wild type ONC-d(AUGA) complex (PDB entries 2GMK and 2I5S, respectively). In the ONC-d(AUGA) structure, one oligonucleotide is bound per one enzyme molecule. The flanking adenosines are disordered and have not been included in the final model. Site-directed mutagenesis along with structural data was used to reveal the basis of substrate recognition and turnover by ONC. Kinetic studies established that Glu91 and Thr89 are the primary determinants of guanine base preference of ONC in B2 position of the active site. The extensive network of hydrogen bonds and eletrostatic interactions is altered in T89N/E91A mutant leading to the reversal of substrate specificity. In addition to wild type ONC (PDB entry 1ONC), crystal structures of several ONC mutants are available in PDB (1YV4, 1YV6, and 1YV7). These structures help to explain molecular basis for low catalytic activity and unusually high thermal stability of the amphibian enzyme.

Reference: (1) Lee, J.E., Bae, E., Bingman, C.A., Phillips, G.N., Jr., Raines, R.T. (2008) Structural basis for catalysis by onconase. *J Mol Biol* 375(1):165-77.

Percent Identity with Nearest PDB Structure at Time Solved	100% (1ONC) novel ligand
Pfam Cluster	RnaseA
Sequence Cluster Size	172

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