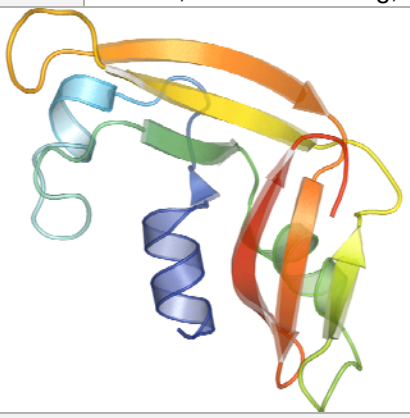
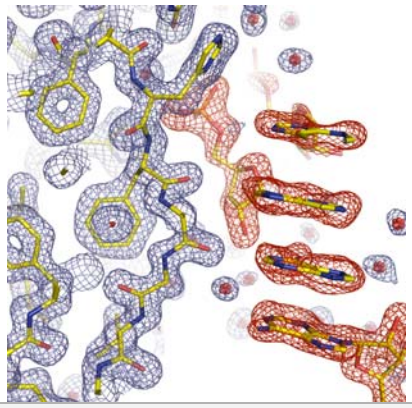
Center for Eukaryotic Structural Genomics

Protein Structure Initiative

Target ID	GO.80161	
Source Organism	*Rana pipiens*	
Target Name	Onconase-T89-E91A	
PDB Entry	2GMK	Deposition: 06-Apr-2006
Function	onconase double mutant with spontaneously-assembled (AMP) 4 stack	
Produced From	*E. coli* BL21(DE3) pET22b(+)	
Structure by X-ray	Resolution: 1.65 Å	R-value (R-free): 16.5% (21.7%)
	No. of Residues/ASU: 104	Monomers/ASU: 1
Data Collected At	Rotating anode, Bruker AXS Microstar 27-Jan-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 anti-tumor 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 among RNase A homologs. Despite this similarity, the ONC activity is 3-5 orders of magnitude lower in comparison with RNase A. The poor catalytic performance of ONC has been attributed to the low flexibility of ONC structure, which may prevent the induced fit of RNA substrate in the active site necessary for efficient catalysis (1). 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 T89N/E91A ONC-5'-AMP complex, four 5'-AMP molecules form a stack that makes contact with multiple enzyme molecules in the crystal lattice. One of the four 5'-AMP molecules binds its phosphate group in the active site, while the remaining nucleosides have only limited interaction with the enzyme. In addition to wild type ONC (PDB entry 1ONC), crystal structures of several mutant ONC 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 (1).

References: (1) Merlino, A., Mazzarella, L., Carannant, A., Di Fiore, A., Di Donato, A., Notomista, E., Sica, F. (2005) The importance of dynamic effects on the enzyme activity: X-ray structure and molecular dynamics of onconase mutants. *J Biol Chem* 280(18):17953-60.

Percent Identity with Nearest PDB Structure at Time Solved	98% (1ONC)
Pfam Cluster	RnaseA
Sequence Cluster Size	131

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