

Structure and Mechanism of Activity of the Cyclic Phosphodiesterase of Appr>p, the Product of tRNA Splicing Reaction

A. Hofmann^{1,2}, A. Zdanov¹, P. Genschik^{3,4}, S. Ruvinov⁵, W. Filipowicz³, and A. Wlodawer¹ (¹Protein Structure Section, Macromolecular Crystallography Laboratory, Program in Structural Biology, NCI-FCRDC, ³Friedrich Miescher-Institut, ⁵Laboratory of Biochemistry, Division of Basic Sciences, NCI, NIH)

Abstract No. Hofm4000

Beamline(s): **X9B**

The crystal structure of the cyclic phosphodiesterase (CPDase) from *Arabidopsis thaliana*, an enzyme involved in the tRNA splicing pathway, was determined at 2.5 Å resolution. CPDase hydrolyzes ADP-ribose 1'', 2''-cyclic phosphate (Appr>p), a product of the tRNA splicing reaction, to the monoester ADP-ribose 1''-phosphate (Appr-1''p). The 181 amino acid protein shows a novel, bilobal arrangement of two $\alpha\beta$ modules. Each lobe consists of two α -helices on the outer side of the molecule, framing a three- or four-stranded antiparallel β -sheet in the core of the protein. The active site is formed at the interface of the two β -sheets in a water-filled cavity involving residues from two H-X-T/S-X motifs. This previously noticed motif participates in coordination of a sulfate ion. A solvent-exposed surface loop (residues 100–115) is very likely to play a flap-like role, opening and closing the active site. Based on the crystal structure and on recent mutagenesis studies of a homologous CPDase from *Saccharomyces cerevisiae*, we propose an enzymatic mechanism, which employs the nucleophilic attack of a water molecule activated by one of the active site histidines.