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Structure and Mechanism of Activity of the Cyclic Phosphodiesterase of Appr>p, a Product of the tRNA Splicing Reaction
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ABSTRACT: 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 alphabeta modules. Each lobe consists of two alpha-helices on the outer side of the molecule, framing a three- or four-stranded antiparallel beta-sheet in the core of the protein. The active site is formed at the interface of the two beta-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 that employs the nucleophilic attack of a water molecule activated by one of the active site histidines.