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human NUDT16

Human NUDT16, a decapping enzyme belonging to the Nudix superfamily, plays a pivotal role in U8 snoRNA stability. Recombinant NUDT16 expressed in *Escherichia coli* was crystallized using the hanging-drop vapour-diffusion method. The crystals, which diffracted to 2.10 Å resolution, belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 44.47$, $b = 79.32$, $c = 97.20$ Å. The Matthews coefficient and the solvent content were calculated to be $1.92 \text{ Å}^3 \text{ Da}^{-1}$ and 35.84%, respectively, for two molecules per asymmetric unit.

1. Introduction

Members of the superfamily of Nudix hydrolases are widespread in bacteria, viruses, eukaryotes and archaea. They catalyze the hydrolysis of a nucleoside diphosphate linked to another moiety X . These enzymes are characterized by a highly conserved 23-amino-acid Nudix motif, $GX_5EX_7REUXEEXGU$, where U is an aliphatic or hydrophobic residue (Bessman *et al.*, 1996). This motif is involved in catalysis and the binding of Mg^{2+} , Mn^{2+} or other divalent cations (Mildvan *et al.*, 2005). The substrates of these enzymes are N_pN , (d)NTP and capped RNAs (Ito *et al.*, 2005; Fisher *et al.*, 2004; Hori *et al.*, 2005). NUDT16 is the human homologue of *Xenopus laevis* X29 protein, which binds and decaps U8 snoRNA and other nuclear RNAs *in vitro* (Ghosh *et al.*, 2004; Tomasevic & Peculis, 1999). The varying divalent metal can determine the RNA-substrate specificity and the efficiency of the decapping activity of NUDT16 and X29 (Peculis *et al.*, 2007). The results of Peculis and coworkers showed that U8 snoRNA is involved in the processing of 5.8S and 28S rRNAs and that the decapping of U8 snoRNA leads to its degradation. Thus, X29 may be involved in the maturation of the large ribosomal subunit through regulating the stability of U8 snoRNA (Ghosh *et al.*, 2004). NUDT16 shares 51% identity with X29, but its structure and function is unclear.

Several structures of Nudix hydrolases have been determined, *e.g.* those of *X. laevis* X29 (Scarsdale *et al.*, 2006), *Schizosaccharomyces pombe* Dcp2p (She *et al.*, 2006) and *Escherichia coli* ADPRase (Gabelli *et al.*, 2001). Structural similarity is only observed in the Nudix domain of these proteins, which is the catalytic centre responsible for catalysis and the binding of divalent cations. There are several important regions that determine the substrate specificity of Nudix hydrolases (Mildvan *et al.*, 2005). The nucleotide diphosphatase activity of X29 is metal-dependent and requires the presence of a significant part of the RNA substrate, but no known RNA-binding motif has been observed. However, from its crystal structure we can observe a plausible RNA-binding channel on the positive face of the protein created by a striking surface dipolarity and unique structural features (Scarsdale *et al.*, 2006). A high-resolution crystal structure would provide much valuable information on the catalytic mechanism of NUDT16 and other Nudix hydrolases.

In this study, we report the cloning and expression of NUDT16 in *E. coli*. Purified recombinant NUDT16 protein was crystallized using