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Structures of new crystal forms of *Mycobacterium tuberculosis* peptidyl-tRNA hydrolase and functionally important plasticity of the molecule

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PDB References: *Mt*Pth, form IV, 3tck; form V, 3td2; form V at 50% PEG, 3td6; new form II, 3tcn.

The X-ray structures of new crystal forms of peptidyl-tRNA hydrolase from *M. tuberculosis* reported here and the results of previous X-ray studies of the enzyme from different sources provide a picture of the functionally relevant plasticity of the protein molecule. The new X-ray results confirm the connection deduced previously between the closure of the lid at the peptide-binding site and the opening of the gate that separates the peptide-binding and tRNA-binding sites. The plasticity of the molecule indicated by X-ray structures is in general agreement with that deduced from the available solution NMR results. The correlation between the lid and the gate movements is not, however, observed in the NMR structure.

1. Introduction

A variety of causes result in the premature stalling of translation, leading to the dropping-off of peptidyl-tRNA from the ribosome. Accumulation of peptidyl-tRNA is toxic to the cell and also results in the non-availability of free tRNA for further protein synthesis (Atherly, 1978; Menninger, 1976). This is eventually prevented by the action of the enzyme peptidyl-tRNA hydrolase (Pth), which cleaves the ester bond between tRNA and the peptide, thus releasing tRNA for further use (Cuzin *et al.*, 1967; Kössel & RajBhandary, 1968; Das & Varshney, 2006). In eubacteria Pth is a monomeric enzyme that has been shown to be essential for cell viability and therefore qualifies as a potential drug target. Pth from *Mycobacterium tuberculosis* (*Mt*Pth) is a 191-amino-acid protein coded by the gene Rv1014c. As part of a long-range programme on the structural biology of mycobacterial proteins (Vijayan, 2005; Prabu *et al.*, 2006; Krishna *et al.*, 2007; Roy *et al.*, 2008; Kaushal, Talawar *et al.*, 2008; Chetnani *et al.*, 2010), we cloned, overexpressed, purified and crystallized *Mt*Pth in three crystal forms, which were analyzed by X-ray crystallography (Selvaraj *et al.*, 2006, 2007). In the crystal structure of the *Escherichia coli* enzyme (*Ec*Pth), which has previously been analyzed, the C-terminus of the molecule is bound to the peptide-binding site of the adjacent molecule, which is a cleft between the body of the molecule and a peptide stretch encompassing a loop and a short helix (Schmitt *et al.*, 1997). This stretch closes on the bound C-terminus with a movement similar to that observed in other proteins (Ruzheinikov *et al.*, 2004; Qin *et al.*, 1998; Oliveira *et al.*, 2001). The C-terminal stretch thus mimics the peptide component of the peptidyl-tRNA. In the structure of *Mt*Pth the C-terminal region of the enzyme is disordered and the loop in the peptide-binding region has an open conformation (Selvaraj *et al.*, 2007). Furthermore, the peptide-binding region and tRNA-binding region in *Mt*Pth are not contiguous as the gate separating them has a closed conformation. In the structure of peptide-bound *Ec*Pth, on the other hand, the loop in the peptide-binding region is closed over the bound peptide and the gate separating the two binding regions is open so that peptidyl-tRNA can bind to the enzyme. Thus, together the two structures appeared to describe the structural changes associated with the action of Pth.