

Site-directed mutagenesis and X-ray crystallographic analyses of wild-type and mutant fluoroacetyl-coenzyme A thioesterase FIK from *Streptomyces cattleya*

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FIK, a thioesterase catalysing hydrolysis of fluoroacetyl-coenzyme A (FAc-CoA) but not acetyl-coenzyme A (Ac-CoA) from the fluoroacetate (FAc)-producing *Streptomyces cattleya*, has been cloned and characterised. We have suggested that FIK acts as a resistance enzyme in the cell (1). Observations both *in vivo* and *in vitro* have demonstrated that FAc is accepted as a substrate by Ac-CoA synthase to form FAc-CoA. FAc-CoA in turn is a substrate for citrate synthase, and reacts with oxalacetate to form 2-fluorocitrate. Lauble *et al.* (2) have shown that the (-)-*erythro* diastereomer of 2-fluorocitrate can be further converted to 4-hydroxy-*trans*-aconitate, a lethal inhibitor of aconitase, thereby blocking the tricarboxylic acid (TCA) cycle. The activity of FIK provides an effective self-defence mechanism to prevent any FAc-CoA formed from entering the TCA cycle with concomitant regeneration of CoA. In order to understand the mechanism of FIK activity, especially the ability of FIK to distinguish between FAc-CoA and Ac-CoA, we have carried out site-directed mutagenesis on FIK and have obtained X-ray crystal structures of the wild-type and mutant FIK proteins, with or without bound fluoroacetate or acetate. Enzyme activity assays and analyses of the crystal structures indicate that the catalytic triad is composed of T42, H76 and a water molecule, and that interactions between the fluorine atom of FAc-CoA and residues G69, R120 and E50 may be involved in positioning the substrate in a correct orientation for the reaction to occur.

References

(1) Huang F, Haydock SF, Spiteller D, Mironenko T, Li TL, O'Hagan D, Leadlay PF, Spencer JB. The gene cluster for fluorometabolite biosynthesis in *Streptomyces cattleya*: a thioesterase confers resistance to fluoroacetyl-coenzyme A. *Chem Biol.* 2006 May;13(5):475-84.

(2) Lauble H, Kennedy MC, Emptage, Beinert H, Stout CD, The reaction of fluorocitrate with aconitase and the crystal structure of the enzyme-inhibitor complex, *Proc. Natl. Acad. Sci. USA* 1996; **93**:13699–13703.