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Tetartohedral twinning in IDI-2 from *Thermus thermophilus*: crystallization under anaerobic conditions

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Abstract

Type-2 isopentenyl diphosphate isomerase (IDI-2) is a key flavoprotein involved in the biosynthesis of isoprenoids. Since fully reduced flavin mononucleotide ($FMNH_2$) is needed for activity, it was decided to crystallize the enzyme under anaerobic conditions in order to understand how this reduced cofactor binds within the active site and interacts with the substrate isopentenyl diphosphate (IPP). In this study, the protein was expressed and purified under aerobic conditions and then reduced and crystallized under anaerobic conditions. Crystals grown by the sitting-drop vapour-diffusion method and then soaked with IPP diffracted to 2.1 Å resolution and belonged to the hexagonal space group $P6_322$, with unit-cell parameters $a = b = 133.3$, $c = 172.9$ Å.

Keywords

isopentenyl diphosphate isomerase; IDI-2; flavoprotein; anaerobic; isoprenoid; twinning

1. Introduction

Type-2 isopentenyl diphosphate:dimethylallyl diphosphate isomerase (IDI-2) is a key enzyme involved in the biosynthesis of isoprenoids, which are essential compounds for all organisms. IDI-2 is the common isoform in some pathogenic bacteria and is not found in humans. Thus, IDI-2 is a logical target for antibiotics. Discovered by Kaneda et al. (2001), IDI-2 requires a divalent cation and reduced flavin ($FMNH_2$) for activity. Crystal structures with the oxidized cofactor reveal that isopentenyl diphosphate substrate is parallel and adjacent to the flavin in the active site (Berthelot et al., 2012; de Ruyck et al., 2011). The structural information that we seek will facilitate the rational design of covalent and noncovalent inhibitors for the enzyme.

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2. Materials and methods

2.1. Cloning and expression

Steven C Rothman generously provided a plasmid encoding Histagged IDI-2 from *Thermus thermophilus* (*tt*-IDI-2). *Escherichia coli* M15 cells (Qiagen) were cultivated on MDG and (LB + 1% glucose) media containing ampicillin and kanamycin at 100 and 25 µg ml^{-1}, respectively. Overexpression and purification were performed as described previously (Rothman et al., 2007).

2.2. Crystallization

Recombinant *tt*-IDI-2 was concentrated to 17 mg ml^{-1} (Tris pH 8.0, 10% glycerol) as calculated by a BCA assay. The His tag was not removed prior to the crystallization trials. After setting up the anaerobic chamber (COY Laboratories) to ensure an atmosphere with <5 p.p.m. O_2, the crystallization solutions (100 m*M* HEPES pH 6.5–8.0, 25–50% PEG 400) were gas-exchanged for 1 h by vacuum pumping for 2 min, then filled for 30 s with argon before they were placed in the anaerobic chamber. The solution containing the enzyme was also gas-exchanged, placed in the chamber and reduced by the addition of 100 m*M* $Na_2S_2O_4$. Anaerobic crystallization was performed using the sitting-drop vapour-diffusion method in 24-well crystallization plates at 298 K. Drops were prepared by mixing 4 µl protein solution with 4 µl reservoir solution. After 1 month, colourless hexagonal crystals appeared in 100 m*M* HEPES pH 7.5, 40% PEG 400 (Fig. 1). A portion of the crystals were soaked in modified assay buffer (2 m*M* IPP, 10 m*M* $MgCl_2$, 100 m*M* HEPES pH 7.5, 100 m*M* $Na_2S_2O_4$).

2.3. Data collection and processing

One soaked and one native crystal were separately mounted in a loop and flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K on beamline BL7-1 at the Stanford Synchrotron Radiation Lightsource (SSRL) and data sets were processed with *XDS* (Kabsch, 2010) (Table 1).

3. Results and discussion

The novelty of this experiment was to crystallize reduced flavin-bound IDI-2 to investigate the IDI-2-IPP-$FMNH_2$ complex.

Crystallization conditions were adapted from those for the recently solved IDI-2 structure from *Sulfolobus shibatae* (Nagai et al., 2011; Nakatani et al., 2012). The initial crystals diffracted to ~4 Å resolution (Fig. 2a) in the absence of a ligand. When the crystal was soaked with IPP, the resolution increased to 2.1 Å (Fig. 2b).

We collected a complete data set. The crystals belonged to space group $P6_322$, according to *XDS*, with unit-cell parameters $a = b = 133.3$, $c = 172.9$ Å. However, the *L* and *H* twinning tests output by *phenix.xtriage* indicated that the crystal was perfectly twinned (twin fraction = 42.6%). We obtained a starting structure by molecular replacement (*AutoMR*) using the previously solved structure of oxidized *tt*-IDI-2 (PDB entry 3dh7; de Ruyck et al., 2008).

Unfortunately, refinement using *phenix.refine* was not successful ($R_{cryst} \simeq 50\%$), even applying the $-k, -h, -l$ twin law (Adams et al., 2010).

4. Conclusions

The reduced flavin structure should provide the location and orientation of the reduced cofactor and active-site amino acids of the catalytically active state of IDI-2. Of particular interest is the increase in resolution when IPP is bound owing to conformational changes in the enzyme. This is in agreement with evidence that the flexible N-terminal segment becomes a structured part of the active site upon binding IPP (Nakatani et al., 2012).

Acknowledgments

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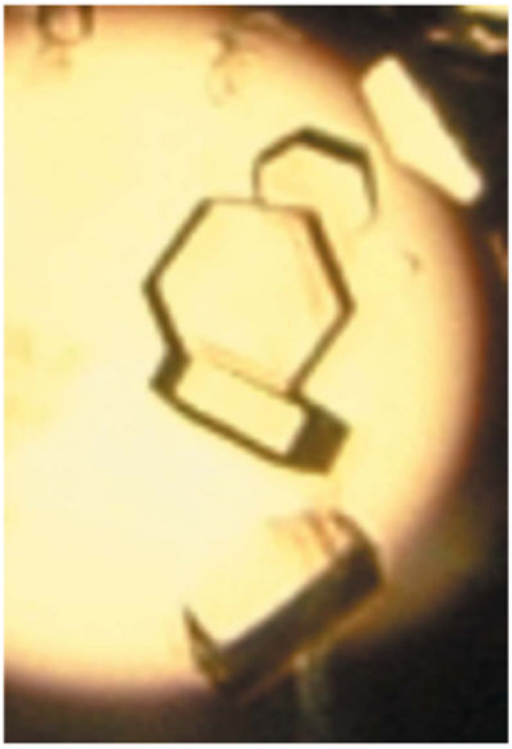

Figure 1.
Crystals of colourless *tt*-IDI-2.

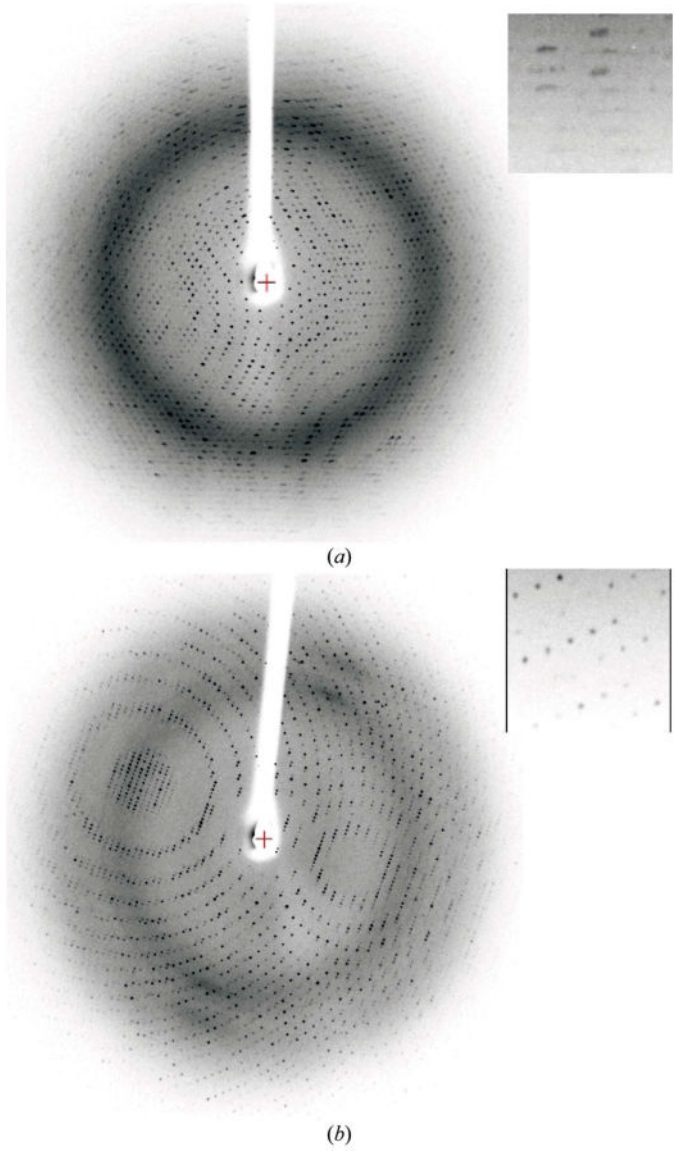

Figure 2.

Diffraction patterns of (*a*) native and (*b*) soaked crystals. These patterns were taken on an in-house diffractometer prior to synchrotron data collection.

Table 1

Data-collection and processing statistics for *T. thermophilus* IDI-2. Values in parentheses are for the outermost resolution shell.

	***tt*-IDI-2**	***tt*-IDI-2 + IPP**
Crystal data		
Space group	$P6_322$	$P6_322$
Unit-cell parameters (Å)	$a = b = 133.3$, $c = 172.9$	$a = b = 133.3$, $c = 172.9$
Data statistics		
Resolution range (Å)	33–3.8	33–2.1 (2.2-2.1)
Unique reflections		55077
Completeness (%)		99.3 (97.6)
R_{merge} (%)		6.2 (30.5)
$\langle I/\sigma(I)\rangle$		17.7 (4.5)