Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Monoclinic form of isopentenyl diphosphate isomerase: a case of polymorphism in biomolecular crystals

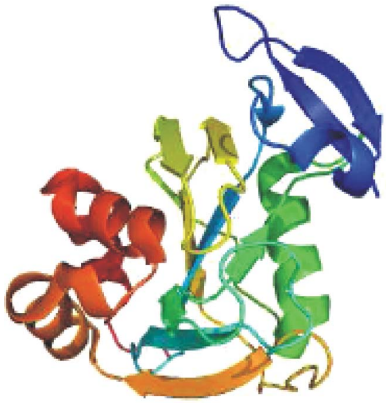
Jérôme de Ruyck,[a]* Yamina Oudjama[b] and Johan Wouters[a]

[a]Laboratoire de Chimie Biologique Structurale, FUNDP University of Namur, 61 Rue de Bruxelles, 5000 Namur, Belgium, and [b]Institut de Recherches Microbiologiques J. M. Wiame, 1 Avenue E. Gryzon, 1070 Bruxelles, Belgium

Correspondence e-mail: jerome.deruyck@fundp.ac.be

Received 1 November 2007
Accepted 28 February 2008

PDB References: IDI-1, 2vnq; IDI-1–NIPP complex, 2vnp.



Type 1 isopentenyl diphosphate isomerase (IDI-1) has been crystallized in a new crystal form. After data collection from small thin needle-shaped crystals, a new monoclinic form of the studied protein was identified. In this article, the three crystal forms of IDI-1 (orthorhombic, monoclinic and trigonal) are compared.

1. Introduction

The overall process of protein crystallization is mainly governed by the nature of the protein molecules (Vekilov *et al.*, 2002). The intermolecular contacts in the crystal lattice include groups of surface atoms bound by various types of intermolecular contacts, such as hydrogen bonds and ionic and van der Waals interactions, which frequently involve bound water molecules on the surface of the molecules. These intermolecular contacts occupy various areas on the molecular surface and have various strengths depending on the nature of the protein molecules. Those various intermolecular interactions can generate different crystal packing, leading to so-called packing polymorphism. For instance, the extensively studied lysozyme has entries in the PDB for triclinic (PDB code 2lzt), monoclinic (1lma), orthorhombic (1bgi) and tetragonal (193l) modifications (Berthou *et al.*, 1983; Hodsdon *et al.*, 1990; Madhusudan *et al.*, 1993; Vaney *et al.*, 1996). Other cases of biomolecular polymorphism have been reported, for example in apolipoproteins involved in Alzheimer's disease (Kodali & Wetzel, 2007), in proteins involved in signal transduction (Loughlin, 2005) and in prions (Baxa *et al.*, 2006).

Recently, we identified a new example of biomolecular packing polymorphism in crystals of type 1 isopentenyl diphosphate isomerase (IDI-1; EC 5.3.3.2). IDI-1 catalyzes the isomerization of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are the basic building blocks of isoprenoids, a diverse family of metabolites comprising over 23 000 natural compounds (Sacchettini & Poulter, 1997). This metalloprotein, which adopts a compact globular form and belongs to the class of α/β proteins (Carrigan & Poulter, 2003), is composed of about 200 amino acids. Two crystal forms of this protein have been reported to date. The metal-free protein (PDB code 1hzt) crystallizes in the trigonal space group $P3_221$, while the metal-bound protein (PDB code 1hx3) crystallizes in the orthorhombic space group $P2_12_12_1$ (Durbecq *et al.*, 2001).

We have obtained a third form of IDI-1 that crystallizes in the monoclinic space group $P2_1$. In this paper, we describe this form and discuss the crystal polymorphism of IDI-1.

2. Material and methods

Protocols for the cloning, overexpression and purification of *Escherichia coli* IDI-1 have been described previously (Oudjama *et al.*, 2001).

2.1. Crystallization

The purified enzyme samples were concentrated to about 5 mg ml^{-1} by ultrafiltration (YM10, Amicon) and the protein con-

centration was estimated by UV absorption. Crystallization trials were set up using the hanging-drop vapour-diffusion method at 293 K. Each drop was made up of a mixture of 4 µl protein solution and the same volume of reservoir solution (homemade screen) and was suspended over 0.5 ml reservoir solution. Crystals of IDI-1 were obtained using 10%(*w*/*v*) polyethylene glycol 2000 monomethyl ether (PEG 2000 MME), 100 m*M* Tris–maleate buffer pH 5.5 in the presence of 100 m*M* ammonium sulfate, 20 m*M* $MnCl_2$ and 20 m*M* $MgCl_2$.

A complex was obtained by soaking crystals of the wild-type (WT) protein with *N*,*N*-dimethyl-2-amino-1-ethyl diphosphate (NIPP; $K_i = 1.2 \times 10^{-10}$ *M*), a transition-state analogue. The inhibitor solution (25 m*M* in 100 m*M* Tris–maleate pH 5.5, 10% PEG 2000 MME, 100 m*M* ammonium sulfate, 10 m*M* $MnCl_2$ and 25% glycerol) was replaced every 8 h for at least 24 h.

2.2. Data collection and refinement

After flash-freezing, complete data sets were collected to 2.2 Å resolution using a MAR Research CCD on beamline BM30A (ESRF) for both the wild-type enzyme and the complex. Data were processed with *XDS* (Kabsch, 1993). IDI-1 crystallizes in space group $P2_1$. Model building was performed by molecular replacement using the metal-bound form of *E. coli* IDI-1 (PDB code 1hx3) as a starting model. Refinements were performed with the program *REFMAC*5 (Murshudov *et al.*, 1997). Electron-density maps were inspected with the graphics program *Coot* (Emsley & Cowtan, 2004) and the quality of the models was analyzed with the program *PROCHECK* (Laskowski *et al.*, 1993). The main crystallographic details are given in Table 1.

3. Results and discussion

The shapes of the IDI-1 crystals obtained in the different crystal forms studied present important differences. The orthorhombic protein (space group $P2_12_12_1$, two molecules in the asymmetric unit, unit-cell parameters $a = 69.3$, $b = 72.6$, $c = 92.5$ Å) crystallizes as large parallelepiped crystals, while the monoclinic form (space group $P2_1$, two molecules in the asymmetric unit, unit-cell parameters $a = 43.2$, $b = 69.1$, $c = 62.4$ Å, $\beta = 100.3^\circ$) crystallizes as small thin needles. The metal-free protein, which belongs to the trigonal space group $P3_221$ with unit-cell parameters $a = 71.4$, $b = 71.4$, $c = 61.8$ Å (one molecule in the asymmetric unit), crystallizes as small bipyramidal crystals (Fig. 1*a*).

The global fold of the orthorhombic form corresponds to an α/β-fold. The N-terminal residues fold into a small β-sheet consisting of two antiparallel strands. This folding leads to the formation of the

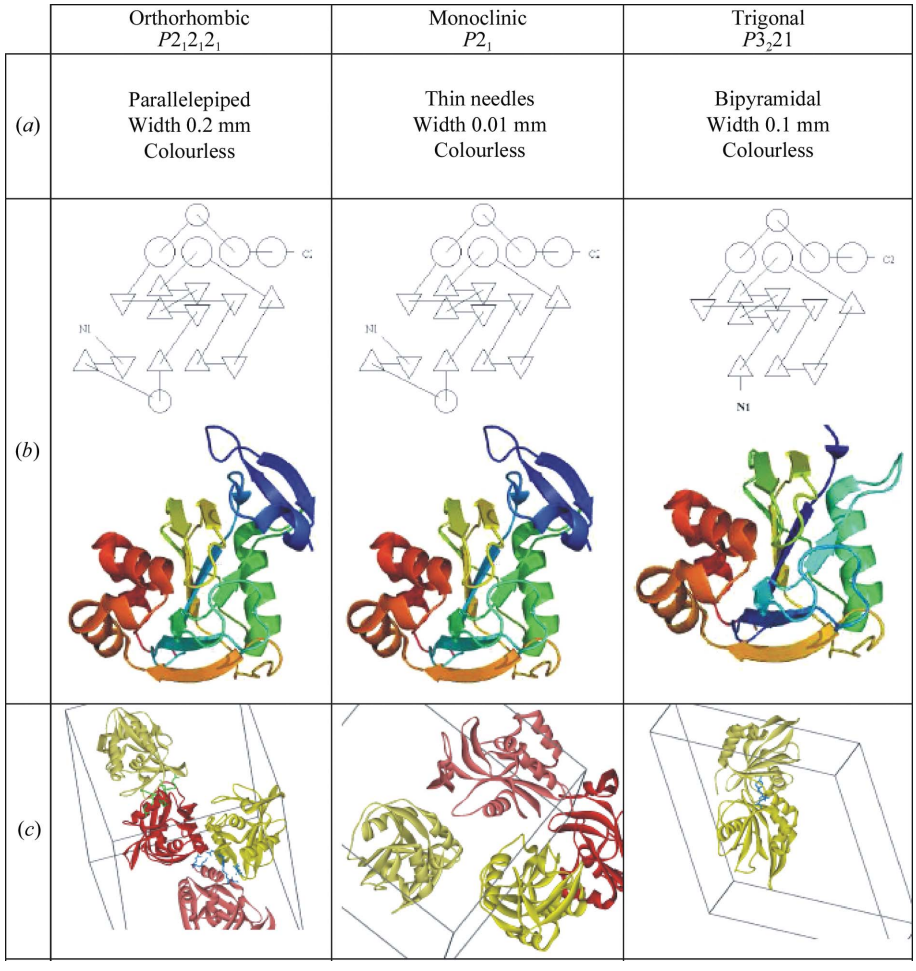


Figure 1
(*a*) The studied crystal forms are compared in terms of macroscopic properties (crystal shape, width and colour). (*b*) Sketches and pictures of the global tertiary structures. The two antiparallel N-terminal β-sheets are not observed for the metal-free trigonal crystal form. (*c*) Crystal packing of the various polymorphs studied. Monomer *A* is coloured yellow and a red colour is used for monomer *B*. Pale colours indicate monomers *A* or *B* of a neighbouring protein molecule.

Table 1
Data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell (2.4–2.2 Å).

	WT enzyme	WT–NIPP complex
Space group	$P2_1$	
Unit-cell parameters (Å, °)	a = 43.2, b = 69.1, c = 62.4, β = 100.3	
Wavelength (Å)	0.9797	
Highest resolution (Å)	2.2	
Observed reflections [$I > 4\sigma(I)$]	66888	68550
Unique reflections	17775	18119
Completeness (%)	96.0 (91.4)	97.8 (95.6)
R_{merge} (%)	6.7 (27.4)	6.0 (16.5)
$I/\sigma(I)$	17.8 (6.5)	17.5 (9.0)
No. of protein atoms	2824†	2821
No. of ligand atoms	22	32
No. of water molecules	69	61
R_{work}/R_{free}‡ (%)	19.7/26.0	19.7/25.5
Average B factors (Å^2)		
All protein atoms	21.3	23.9
All ligand atoms	30.9	22.4
All water molecules	24.1	25.3
Root-mean-square deviations§		
Bond lengths (Å)	0.022	0.022
Bond angles (°)	1.94	1.94
NCS restraints		
Medium positional (Å)	0.50	0.50
Ramachandran plot statistics¶ (%)		
Most favoured regions	90.1	87.9
Additional allowed regions	9.9	12.1
PDB code	2vnq	2vnp

† This value is higher than that for WT in complex with NIPP because of the two alternate conformations of Ala67. ‡ The free test subset represents 5% of the total unique reflections. § As defined by *REFMAC*5 (Murshudov *et al.*, 1997). ¶ As defined by *PROCHECK*; Gly and Pro residues are excluded (Laskowski *et al.*, 1993).

Table 2
Comparison of selected interactions in the crystal packing of the three polymorphs of IDI-1.

Asymmetric unit protein	Symmetry-related protein	Distance (Å)
Orthorhombic, $P2_12_12_1$, V_M = 0.75 Å^3 Da^{-1}		
Asp28*A* OD2	Arg82*B* NH2^{i}	3.2
Arg30*A* NH2	Gly89*B* N^{i}	3.0
Arg30*A* NE	Tyr86*B* O^{i}	3.2
Arg30*A* NH1	Gln130*B* O^{i}	2.9
Arg30*A* NH1	Arg85*B* O^{i}	3.0
Leu129*A* O	Ser97*B* N^{ii}	2.8
Gln130*A* OE1	Ser97*B* OG^{ii}	3.3
Asp138*A* OD1	Lys182*B* NZ^{ii}	3.0
Asp138*A* OD2	Gln180*B* OE1^{ii}	3.1
Tyr139*A* OH	Glu96*B* OE1^{ii}	2.6
Monoclinic, $P2_1$, V_M = 0.95 Å^3 Da^{-1}		
Leu129*A* O	Ser97*B* N^{iii}	2.8
Gln130*A* OE1	Ser97*B* OG^{iii}	2.8
Asp138*A* OD2	Gln180*B* OE1^{iii}	3.2
Tyr139*A* OH	Glu96*B* OE1^{iii}	2.6
Trigonal, $P3_221$, V_M = 0.96 Å^3 Da^{-1}		
Glu96*A* OE1	Tyr139*A* OH^{iv}	2.6
Ser97*A* N	Leu129*A* O^{iv}	2.8
Asp138*A* OD2	Gln180*A* OE1^{v}	3.0

Symmetry codes: (i) $x + 1/2, -y + 1/2, -z$, (ii) $-x, y + 1/2, -z + 1/2$, (iii) $-x, y + 1/2, -z$, (iv) $-y, x - y, z + 2/3$, (v) $-x + y, -x, z + 1/3$.

first metal (M^{2+}) binding site, which coordinates His25 and His32 and brings them close to His69, Glu114 and Glu116. A second metal site coordinates the carbonyl group of the highly conserved Cys67 residue, the side-chain O atom (OE2) of Glu87 and four water molecules to form an MO_6 octahedral coordination sphere. Compared with the active protein, which crystallizes as a dimer, the apoenzyme also folds as an α/β protein with the N-terminal residues (1–32) disordered. This unfolding of the N-terminal region results in the absence of the M^{2+}-binding site and the protein crystallizes as a monomer in a different space group. Nevertheless, the second binding site formed by Cys67, Glu87 and four water molecules is still present (Fig. 1*b*).

Our new polymorphic form of the active protein shows the same tertiary α/β-fold as the orthorhombic crystal form. The complex formed between IDI-1 and NIPP, a well known transition-state analogue of IPP, was then refined in order to visualize the conservation of the active site. This active site is the same as that observed for the protein crystallized in the orthorhombic form in complex with NIPP (PDB code 1nfs; Wouters *et al.*, 2003).

The crystal packing of the three different forms was also compared (Fig. 1*c*). Selected hydrogen bonds and salt bridges between the molecules in the crystal packing are presented in Table 2. Conservation of interactions involving aliphatic and polar residues (Leu129, Gln130, Asp138 and Tyr139) with polar residues (Ser97, Lys182, Gln180 and Glu96) of another neighbouring molecule is observed. This type of interaction mostly involves hydrogen bonding. In the case of the monoclinic and trigonal forms, the compactness of the crystal cell is such that the monomers stabilize each other by relatively weak interactions (*e.g.* atomic contacts and van der Waals interactions). In the case of the orthorhombic form, additional contacts are observed between Asp28 or Arg30 and Arg82, Arg85, Tyr86, Gly89 or Gln130 of another neighbouring molecule. Stronger interactions are needed to maintain the crystal packing because of the lower occupancy of the crystal cell in this polymorphic form (Table 2, Fig. 1*c*).

In this study, we demonstrated that two different types of crystals could be obtained using the same crystallogenesis conditions. After refinement of the new crystal form (monoclinic, space group $P2_1$), we observed that the fold and the active site are the same as in the orthorhombic $P2_12_12_1$ crystal form. IDI-1 thus presents packing polymorphism as described for lysozyme or other proteins involved, for example, in Alzheimer's disease.

JdR is grateful for financial support from the Belgian Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA). The authors thank the local team of beamline BM30A (ESRF, Grenoble) for assistance during data collection. Financial support from FNRS (IISN No. 4.4505.00) is acknowledged. Figures were produced using *PyMOL* (DeLano, 2002).

References

Baxa, U., Cassese, T., Kajava, A. V. & Steven, A. C. (2006). *Adv. Protein Chem.* **73**, 125–180.
Berthou, J., Lifchitz, A., Artymiuk, P. & Jollès, P. (1983). *Proc. R. Soc. Lond. B Biol. Sci.* **217**, 471–489.
Carrigan, C. N. & Poulter, C. D. (2003). *J. Am. Chem. Soc.* **125**, 9008–9009.
DeLano, W. L. (2002). *The PyMOL Molecular Graphics System.* http://www.pymol.org.
Durbecq, V., Sainz, G., Oudjama, Y., Clantin, B., Bompard-Gilles, C., Tricot, C., Caillet, J., Stalon, V., Droogmans, L. & Villeret, V. (2001). *EMBO J.* **20**, 1530–1537.
Emsley, P. & Cowtan, K. (2004). *Acta Cryst.* D**60**, 2126–2132.
Hodsdon, J. M., Brown, G. M., Sieker, L. C. & Jensen, L. H. (1990). *Acta Cryst.* B**46**, 54–62.
Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.
Kodali, R. & Wetzel, R. (2007). *Curr. Opin. Struct. Biol.* **17**, 48–57.
Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). *J. Appl. Cryst.* **26**, 283–291.
Loughlin, J. (2005). *Curr. Opin. Rheumatol.* **17**, 629–633.
Madhusudan, Kodandapani, R. & Vijayan, M. (1993). *Acta Cryst.* D**49**, 234–245.

Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst.* D**53**, 240–255.
Oudjama, Y., Durbecq, V., Sainz, G., Clantin, B., Tricot, C., Stalon, V., Villeret, V. & Droogmans, L. (2001). *Acta Cryst.* D**57**, 287–288.
Sacchettini, J. C. & Poulter, C. D. (1997). *Science*, **277**, 1788–1789.
Vaney, M. C., Maignan, S., Riès-Kautt, M. & Ducruix, A. (1996). *Acta Cryst.* D**52**, 505–517.
Vekilov, P. G., Feeling-Taylor, A., Yau, S.-T. & Petsev, D. (2002). *Acta Cryst.* D**58**, 1611–1616.
Wouters, J., Oudjama, Y., Barkley, S. J., Tricot, C., Stalon, V., Droogmans, L. & Poulter, C. D. (2003). *J. Biol. Chem.* **278**, 11903–11908.