Structures of *Escherichia coli* tryptophanase in holo and 'semi-holo' formsAnna Kogan,^{a,‡} Leah Raznov,^{a,‡} Garik Y. Gdalevsky,^a Rivka Cohen-Luria,^a Orna Almog,^b Abraham H. Parola^{a,c,d,*}§ and Yehuda Goldgur^{d,*}

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Keywords: tryptophanase; *Escherichia coli*; polymorphism.**PDB references:** tryptophanase, 'semi-holo' form, 4w1y; holo form, 4w4h**Supporting information:** this article has supporting information at journals.iucr.org/f^aDepartment of Chemistry, Ben Gurion University of the Negev, Beer Sheva 84105, Israel, ^bDepartment of Clinical Biochemistry and Pharmacology, Ben Gurion University of the Negev, Beer Sheva 84105, Israel, ^cNew York University Shanghai, Shanghai 200122, People's Republic of China, and ^dStructural Biology Program, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. *Correspondence e-mail: aparola@bgu.ac.il, goldgur@mskcc.org

Two crystal forms of *Escherichia coli* tryptophanase (tryptophan indole-lyase, Trpase) were obtained under the same crystallization conditions. Both forms belonged to the same space group $P4_32_12$ but had slightly different unit-cell parameters. The holo crystal form, with pyridoxal phosphate (PLP) bound to Lys270 of both polypeptide chains in the asymmetric unit, diffracted to 2.9 Å resolution. The second crystal form diffracted to 3.2 Å resolution. Of the two subunits in the asymmetric unit, one was found in the holo form, while the other appeared to be in the apo form in a wide-open conformation with two sulfate ions bound in the vicinity of the active site. The conformation of all holo subunits is the same in both crystal forms. The structures suggest that Trpase is flexible in the apo form. Its conformation partially closes upon binding of PLP. The closed conformation might correspond to the enzyme in its active state with both cofactor and substrate bound in a similar way as in tyrosine phenol-lyase.

1. Introduction

Tryptophanase (Trpase, tryptophan indole-lyase) is a bacterial PLP-dependent enzyme that catalyses the degradation of L-tryptophan to indole, pyruvate and ammonia. It is a homotetramer with a molecular mass of about 210 kDa. In the active form, each subunit binds one molecule of the cofactor pyridoxal 5'-phosphate (PLP; holo form). The three-dimensional structure of Trpase has been the subject of several studies. The first crystals of the *Escherichia coli* enzyme were obtained by Kawata *et al.* (1991) and improved by Dementieva *et al.* (1994). The crystal structure of Trpase from *Proteus vulgaris* in a holo form, which shares 51% identity and 69% similarity with the *E. coli* enzyme, was reported by Isupov *et al.* (1998). Two crystal structures of *E. coli* Trpase in the apo form were determined by Ku *et al.* (2006) (referred to below as the apo I structure) and by Tsesin *et al.* (2007) (referred to below as the apo II structure).

Trpase is closely related to another PLP-dependent lyase, tyrosine phenol-lyase (TPL). TPL is a tetrameric enzyme that catalyzes the reversible cleavage of L-tyrosine to phenol, ammonium ion and pyruvate. Both TPL and Trpase are classified as β -eliminating lyases and their reaction mechanism is similar except that TPL is specific for the phenol side chain rather than indole. A number of crystal structures of TPL from *Citrobacter freundii* and *Erwinia herbicola* have been published (Antson *et al.*, 1993; Sundararaju *et al.*, 1997; Pletnev *et al.*, 1996; Milić *et al.*, 2006). The sequence of TPL from *C. freundii* is 40% identical to those of the Trpases (Kamath & Yanofsky, 1992; Phillips *et al.*, 2003). Trpase and TPL share the

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same fold consisting of two domains: the large domain and the small domain. Both domains are built around β -sheets with flanking α -helices. In both enzymes, the large and small domains can undergo significant movement with respect to each other. In various crystal structures, TPL can be found in closed (Milić *et al.*, 2006) and open (Sundararaju *et al.*, 1997) conformations. For Trpase, three different conformations were identified: closed (Ku *et al.*, 2006), open (Isupov *et al.*, 1998) and wide open (Tsesin *et al.*, 2007). A similar domain shift has been observed in aspartate amino-transferase (McPhalen *et al.*, 1992). It was postulated that the transition between conformations is important for the mechanism of catalysis. Here, we report crystal structures of *E. coli* Trpase in the holo open conformation similar to that found in *P. vulgaris* Trpase and in a 'semi-holo' form with one subunit found in the holo form and the other in the apo form. This is the first example of two different catalytic states of Trpase coexisting in the same crystal.

2. Materials and methods

Trpase was overexpressed in *E. coli* SVS 370 cells and isolated as described previously (Kogan *et al.*, 2004). Crystallization was carried out by the hanging-drop vapour-diffusion method. The protein at a concentration of 15–25 mg ml⁻¹ in 50 mM sodium phosphate buffer pH 6.0, 5 mM β -mercaptoethanol and 0.5–2 mM PLP were mixed in a ratio of 3:1 with a precipitant solution consisting of 10–15% (w/v) ammonium sulfate in 25 mM potassium acetate pH 5.4 and 3–10 mM β -mercaptoethanol. Crystals appeared after 48 h and grew to a maximum size of 0.4 mm in about a week. The crystals were cryoprotected by stepwise transfer to a solution containing 25% glycerol in the mother liquor. Data were collected on a Rigaku RU-300 rotating-anode generator equipped with a MAR Research image-plate detector at 100 K. The crystals belonged to space group $P4_32_12$. Despite the fact that the crystals grew readily under a wide range of conditions, the diffraction quality varied greatly even for crystals that were harvested from the same drop. Out of several dozen crystals that we screened, only about ten diffracted beyond 3.5 Å resolution. These could be grouped into two slightly different crystal forms, both with two subunits in the asymmetric unit, with the tetramer being generated by a crystallographic twofold axis. In form I one subunit is in the apo conformation and the other in the holo conformation, while in form II both are in the holo conformation. The structure was solved by molecular replacement using *AMoRe* (Navaza, 1993) from the *CCP4* suite (Winn *et al.*, 2011). The coordinates of apo Trpase (PDB entry 2oqx; Tsesin *et al.*, 2007) were used as the search model. The structures were refined using *REFMAC5* (Murshudov *et al.*, 2011) and manual model rebuilding was performed with *O* (Jones *et al.*, 1991). Both crystal forms contained two subunits in the asymmetric unit. Data-collection and refinement statistics are presented in Table 1. The clashscore, the percentage of nonrotamer side chains and the Ramachandran statistics are worse than expected for the resolution limit. We believe that the reason is that the degree of disorder is high,

Table 1

Summary of data-collection and refinement statistics.

Crystal	Form I (PDB entry 4w1y; 'semi-holo')	Form II (PDB entry 4w4h; holo)
Wavelength (Å)	1.5418	1.5418
Resolution range (Å)	15–3.2 (3.30–3.20)	15–2.9 (2.95–2.90)
Space group	$P4_32_12$	$P4_32_12$
Unit-cell parameters (Å)	$a = b = 110.0$, $c = 238.4$	$a = b = 112.4$, $c = 232.7$
Average multiplicity	5.9 (6.2)	9.4 (9.5)
Completeness (%)	94.8 (97.2)	98.7 (99.2)
$R_{\text{merge}}^\dagger$	0.124 (0.52)	0.167 (0.75)
$\langle I \rangle / \langle \sigma(I) \rangle$	19.2 (3.1)	19.0 (2.9)
Wilson B factor (Å ²)	87	64
Subunits in asymmetric unit	2	2
No. of reflections		
Total	22389 (1737)	31660 (2319)
Working set	21182 (1655)	29973 (2199)
R_{free} set	1207 (82)	1687 (120)
$R_{\text{work}}/R_{\text{free}}$	0.213/0.289 (0.318/0.400)	0.219/0.269 (0.377/0.440)
No. of atoms		
Total	6957	6906
Protein	6840	6865
Water	107	42
Ions	10	—
Mean B value (Å ²)	85	58
R.m.s.d. from ideal values (Engh & Huber, 1991)		
Bond lengths (Å)	0.024	0.019
Bond angles (°)	2.4	1.8
Overall clashscore‡	67	13
Percentage of nonrotameric side chains	19	8
Ramachandran analysis§		
Favoured	80.9	91.7
Allowed	17.5	7.4
Disallowed	1.6	0.8

[†] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where I is intensity, h , k and l are Miller indices and i runs over all observations of each reflection. Angle brackets indicate averaging over i . Vertical bars denote the absolute value. [‡] Clashscore is defined as the number of clashes calculated for the structure per 1000 atoms (including H atoms) of the structure. [§] Ramachandran analysis was performed using *RAMPAGE* (Lovell *et al.*, 2003).

probably owing to the simultaneous presence of different conformations of the protein in both crystal forms. As a result, poor electron density in many areas makes it impossible to determine the actual local conformation. Representative electron density for both structures is shown in Fig. 1. The structures were deposited in the Protein Data Bank (PDB entries 4w1y for the 'semi-holo' form and 4w4h for the holo form).

3. Results and discussion

Together with those previously published, there are three structures of Trpase in the holo form and three in the apo form. Comparison of these structures allows an analysis of the flexibility and the conformational changes that take place upon binding of the cofactor.

3.1. The structure of holo *E. coli* Trpase

The crystals of holo *E. coli* Trpase contain two subunits in the asymmetric unit. The two other subunits of the tetramer are generated by twofold crystallographic symmetry. The final

model contains 871 residues of the two polypeptide chains. The following regions are disordered in the structure: the N-termini (residues 1–4) in both subunits and residues 402–412 in both subunits, as well as the C-terminal stretches (A448–A471 and B453–B471). Lys270 is covalently bound to PLP in both subunits, forming an aldimine bond. The overall conformations of Trpase from *E. coli* and *P. vulgaris* (Isupov *et al.*, 1998) are very similar, with an r.m.s.d. of 0.6 Å for 373 superposed C α atom pairs. The similarities in the active site are even closer. The pattern of interactions of PLP with the active site residues is the same. All residues contacting PLP are conserved in both enzymes: Phe132 from *P. vulgaris* Trpase corresponds to Phe136 from *E. coli* Trpase, Arg101 to Arg103, Gln99 to Gln101, Arg226 to Arg230, Tyr72 to Tyr74 and Tyr301 to Tyr307, respectively. In addition, both subunits in the asymmetric unit of *E. coli* Trpase crystal superimpose with

Table 2

The width of the catalytic cleft in various structures of Trpase.

Trpase structure	Distance between C α atoms of Lys127 and Ala373 (or equivalent) (Å)
Holo <i>E. coli</i> (PDB entry 4w4h): open cleft	34
Holo <i>P. vulgaris</i> (PDB entry 1ax4): open cleft	34
Holo subunit of the 'semi-holo' (PDB entry 4w1y chain A): open cleft	35
Apo I (Ku <i>et al.</i> , 2006; PDB entry 2c44): closed cleft	29
Apo II (Tsesin <i>et al.</i> , 2007; PDB entry 2oqx): wide-open cleft	38
Apo subunit of the 'semi-holo' (PDB entry 4w1y chain B): wide-open cleft	37

an r.m.s.d. of 0.2 Å for 429 pairs of C α atoms. Fig. 2(a) shows the superposition of the three structures of holo Trpase.

3.2. The structure of apo Trpase

In contrast to the high degree of similarity between the structures of holo Trpase, the apo form exhibits a significant degree of flexibility resulting from the domain shift (Fig. 2b).

The movement of the small domain affects the width of the catalytic cleft. To quantitate the conformational change that occurs in Trpase, we compare the distance between the C α atoms of Lys127 and Ala373 (numbering according to the sequence of *E. coli* Trpase). The pair was chosen for the following reasons: (i) these residues are located on opposite sides of the catalytic cleft, (ii) they are ordered in all structures of Trpase known to date and (iii) they belong to secondary-structure elements rather than to flexible loops. The structural equivalent of this pair in *P. vulgaris* Trpase are Asn123 and Gly368. The 'catalytic cleft widths' as defined above are listed in Table 2 for various Trpases.

As seen from Table 2, the apo subunit of the 'semi-holo' crystal form is found in a wide-open conformation. Therefore, the closure of the apo Trpase conformation observed in Ku *et al.* (2006) cannot be attributed to the binding of sulfates, as they are found in both structures. Rather, the conformational change results from the crystal packing. Given that the crystallization conditions of the apo II and 'semi-holo' crystals are dissimilar, we can hypothesize that in solution apo Trpase adopts the wide-open conformation, which partially closes upon binding of PLP. The closed conformation might be also relevant, representing the state in which both substrate and cofactor are bound.

The new structures of *E. coli* Trpase resolve the mystery of the poor reproducibility and the limited order of *E. coli* holo Trpase crystals. They diffract to anywhere between 8 and 3 Å resolution, with large variations even between crystals from the same drop. Both holo and semi-holo form crystallize under the same conditions in the same space group and with similar unit-cell parameters, representing an example of concomitant crystallization of polymorphs, as reviewed by Bernstein *et al.* (1999). At high concentrations of ammonium sulfate Trpase readily loses PLP. Therefore, both the apo and the holo forms are present simultaneously in solution, especially at high

Figure 1

Representative electron density for the two structures. $2mF_o - DF_c$ OMIT maps are shown. Sulfate ions and Lys270/PLP were excluded from phase calculation. (a) The area of Lys270 bound to PLP in the holo structure. The map is contoured at 0.13 e Å⁻³ (1σ). (b) Lys270 and the sulfate ions in the apo subunit of the 'semi-holo' structure. The map is contoured at 0.12 e Å⁻³ (1σ).

Figure 2

Superposition of the Trpase subunits aligned using the large domains (residues 60–325). (a) In all structures of holo Trpase known to date the orientation of the two domains and hence the width of the catalytic cleft are similar. Trpase from *P. vulgaris* is shown in red, *E. coli* holo Trpase (PDB entry 4w4h) is in yellow and the holo subunit of the ‘semi-holo’ structure is in cyan. The PLP moieties covalently bound to the side chains of lysines are depicted as sticks in the corresponding colours and are found in the same position. (b) Apo I is shown in magenta (closed conformation) and the apo subunit of the ‘semi-holo’ structure is in green (wide open). The shift of the small domain is clearly seen. For a comparison, the structure of holo Trpase is shown in cyan (open conformation). Sulfate ions in apo structures are depicted as spheres of the corresponding colours. One of the sulfate ions is bound in place of the phosphate moiety of PLP. The second presumably occupies the position of the carboxylic group of Trp substrate.

concentrations of SO_4^{2-} , which binds in place of the phosphate group of PLP. Most probably, each crystal of holo/semi-holo Trpase contains crystal domains of two forms. This explains both the poor diffraction and the poor reproducibility. The crystals that diffracted best contained predominantly one crystal form.

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