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Preliminary X-ray diffraction studies of rabbit muscle triose phosphate isomerase (TIM)

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Triose phosphate isomerase (TIM) is responsible for the interconversion between GAP and DHAP in the glycolytic pathway. Two crystal forms belonging to space group $P2_12_12_1$ were obtained by the hanging-drop method and were designated *A* and *B*. Synchrotron X-ray diffraction data were collected for both forms. Form *A* had unit-cell parameters $a = 65.14$, $b = 72.45$, $c = 93.24$ Å and diffracted to 2.25 Å at 85 K, whereas form *B* had unit-cell parameters $a = 73.02$, $b = 79.80$, $c = 172.85$ Å and diffracted to 2.85 Å at room temperature. Molecular replacement was employed to solve the structures, using human TIM as a search model. Further refinement of both structures is under way and is expected to shed light on the recently reported conformational studies for rabbit TIM.

1. Introduction

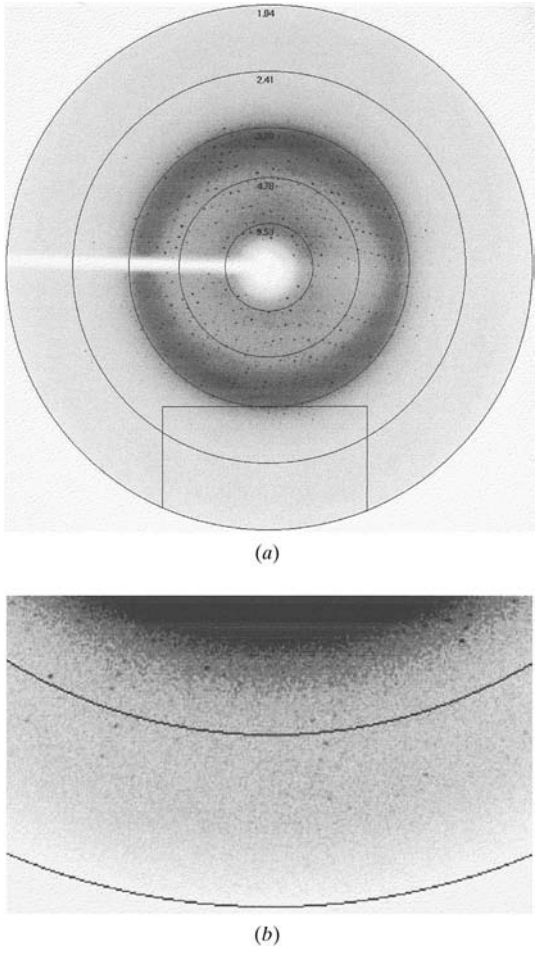
Glycolysis is the biochemical pathway by which glucose is converted to pyruvate with the generation of two moles of ATP per mole of glucose, providing part of the energy utilized by most cells. Triose phosphate isomerase (TIM, D-glyceraldehyde-3-phosphate ketol-isomerase; E.C. 5.3.1.1) catalyzes the fifth reaction of the glycolytic pathway, namely the interconversion, probably *via* an enediol (or enodiolate) intermediate, of glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), two ketose–aldose isomers (Yüksel & Gracy, 1991). Rabbit muscle TIM is a dimer of identical subunits, each containing 248 residues and with a molecular weight of 26 627 Da. Although the dimer is the physiologically active species, a number of monomeric TIM mutants possessing catalytic activity have been described (Borchert *et al.*, 1993; Mainfroid *et al.*, 1996; Schliebs *et al.*, 1996, 1997). At *in vivo* concentrations of substrate, the rate of reaction catalyzed by TIM (Knowles, 1991) is essentially limited by diffusion. The α/β-barrel structural motif, a supersecondary structure in which eight β-strands alternate in sequence with eight α-helices forming a parallel β-sheet closed into a cylindrical topology, was first recognized in the structure of chicken muscle TIM (Banner *et al.*, 1975). Similar structures are now known to occur in many other proteins and this folding motif is frequently named the TIM barrel (Branden & Tooze, 1991). The active site of almost all known α/β-barrel enzymes is located at the carboxyl end of the barrel. The fact that most of the members of α/β-barrel proteins are enzymes serves as an argument for their divergent evolution from a common ancestor (Farber, 1993). In *Schistosoma mansoni*, TIM is being considered as a possible target in attempts to obtain a vaccine against schistosomiasis (Doenhoff, 1998). In addition, TIM from *Trypanosoma brucei brucei* (the aetiologic agent of sleeping sickness) has been actively investigated from a structural point of view (Wierenga *et al.*, 1991, 1992), with the goal of developing drugs that might inhibit its function and be of therapeutic value against infection. Furthermore, TIM deficiency in humans results in a genetic disease of autosomal dominant inheritance (locus 12p13), causing developmental retardation, myopathy, cardiac failure and anaemia (Daar *et al.*, 1986; Watanabe *et al.*, 1996). However, despite being one of the first glycolytic enzymes to be purified (Krietsch *et al.*, 1970; Scopes, 1977), the structure of rabbit muscle TIM has not yet been reported. The amino-acid sequence of this protein is known (Corran & Ley, 1975) and its kinetic properties have been extensively studied (Garza-Ramos *et al.*, 1992; Fernandez-Velasco *et al.*, 1995) and compared with those of *T. brucei brucei* and yeast (*Saccharomyces cerevisiae*) TIMs (Lambeir *et al.*, 1987). Biophysical studies of the enzyme have been carried out by infrared spectroscopy (Castresana *et al.*, 1988) and electron microscopy (Tanaka *et al.*, 1994). Recently, in order to relate conformational stability to the chemical stability and function of TIM, unfolding and refolding studies of rabbit muscle TIM using hydrostatic pressure and chaotropic agents as perturbing agents were monitored by fluorescence resonance energy-transfer (FRET) measurements (Rietveld & Ferreira, 1996, 1998). An interesting result from the latter

Table 1
Data-collection statistics for the *A* and *B* crystal forms.

Values in parentheses refer to the last resolution shell.

Parameter	*A* form	*B* form
No. of images	75	50
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)		
a	65.14	73.02
b	72.45	79.80
c	93.24	172.75
Resolution range (Å)	13.5–2.25	14.00–2.85
Last shell (Å)	2.30–2.25	2.92–2.85
No. of measured reflections	49411 (2645)	34316 (1344)
No. of unique reflections	19851 (1270)	19037 (1012)
Multiplicity	2.49 (2.08)	1.80 (1.33)
R_{sym} (%)	10.7 (36.6)	12.4 (26.4)
Completeness (%)	93.1 (91.8)	78.8 (63.8)
$\langle I/\sigma(I)\rangle$	8.39 (2.14)	5.51 (1.99)

studies was the finding that rabbit muscle TIM exhibits persistent conformational/energetic heterogeneity in solution. This led to the proposal of the existence of a heterogeneous ensemble of TIM dimers, probably arising from long-lived heterogeneity of subunit interactions (Rietveld & Ferreira, 1996). This surprising observation has been subsequently investigated from an energetic point of view (Rietveld & Ferreira, 1998), but a detailed investigation of the structural basis of the heterogeneity of TIM dimers is still lacking. Determination of the crystallographic structure of rabbit muscle TIM would contribute to the above-mentioned functional and physicochemical studies, as well as adding to knowledge of the structures of triose phosphate isomerases from various organisms. Here, we report the crystallization, data collection and molecular-replacement solutions obtained for two crystalline forms of TIM, one determined at 85 K and the other at 298 K. Subsequent comparison of the refined structures in the two crystal forms will provide information on the influence of crystal contacts on the protein structure. Furthermore, analysis of the temperature factors of the models determined at room temperature and at 85 K might contribute to our understanding of the nature of the disorder (*i.e.* static or dynamic) of the flexible parts of the molecule, with possible implications regarding the above-described conformational heterogeneity of TIM dimers.



(*a*)

(*b*)

Figure 1
(*a*) A representative diffraction pattern from the cryo-collected data set (crystal type *A*); (*b*) enhanced contrast close-up of the marked region.

2. Crystallization and data collection

Rabbit muscle TIM (type X) was purchased from Sigma Chemical Co. The sample was >98% pure as judged by SDS–PAGE analysis. Initial crystallization conditions were screened at room temperature using the sparse-matrix method (Jancarik & Kim, 1991) and the hanging-drop method. Some conditions produced small crystals. These crystallization conditions were used as a guide for further trials. Two crystalline forms have been obtained by growing TIM crystals for 1–2 weeks in closely related conditions. A single data set was collected for each crystal form. Data were collected on a 345 mm MAR Research imaging-plate detector at the LNLS Protein Crystallography beamline (Polikarpov, Oliva *et al.*, 1998; Polikarpov, Perles *et al.*, 1998) by the oscillation method and were processed using the *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) packages. We describe below the growth conditions and details of the data collection and reduction for both crystal forms, which will henceforth be referred to as types *A* and *B*.

2.1. Crystal type *A*

This crystal form grew when reservoir solution containing 24% PEG 4000, 0.2 *M* $MgCl_2$, 0.1 *M* Tris–HCl pH 8.5 was mixed with protein solution (10 mg ml^{-1} in water) in equal amounts and equilibrated against the reservoir solution. The crystals were needles measuring 0.6 × 0.2 × 0.2 mm. To prevent radiation damage, the crystal was briefly soaked in a cryoprotectant solution containing 10%(*v*/*v*) ethylene glycol and rapidly frozen. A data set was collected from a single flash-cooled crystal at 85 K. The X-ray wavelength employed was 1.31 Å with a crystal-to-detector distance of 210 mm, giving an outer-edge resolution of 1.94 Å. The oscillation range was 1° and the exposure time per image (Fig. 1) was about 3 min. Data processing and reduction led to the statistics summarized in Table 1.

2.2. Crystal type *B*

This crystal form grew under similar conditions as type *A*, except that the concentrations of PEG 4000 and $MgCl_2$ in the reservoir solution were 23% and 0.33 *M*, respectively. These crystals formed clusters of plates and grew to dimensions of 1.0 × 0.5 × 0.2 mm. Unfortunately, a suitable cryoprotectant solution for these crystals was not easy to obtain, as the crystals dissolved almost instantaneously when placed in the cryosolution described above. Therefore, we collected X-ray data for this crystal form at room temperature (298 K). Data were collected from a single crystal at a wavelength of 1.37 Å and a crystal-to-detector distance of 205 mm. The oscillation range was 1° and the exposure time was about 3 min per frame (Fig. 2). The results of data processing are shown in Table 1.

3. Molecular-replacement solutions

Phasing was carried out by the molecular-replacement method, as implemented in the program *AMoRe* (Navaza, 1994). The primary sequence search and sequence alignments were made using *ENTREZ* and *BLAST* (Altschul *et al.*, 1997). Human (Maquat *et al.*, 1985) and rabbit TIM

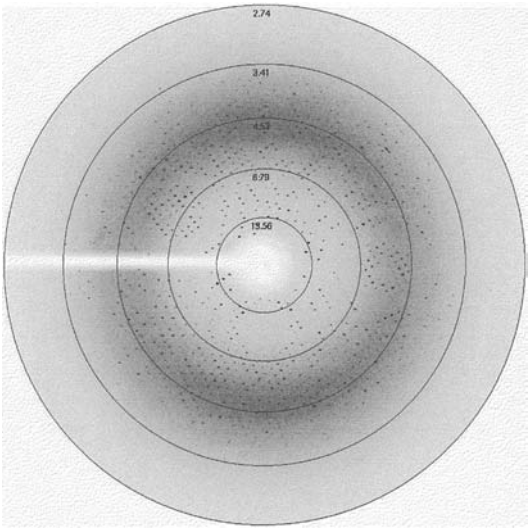


Figure 2
A diffraction pattern from the data set collected for crystal type *B*.

(Corran & Ley, 1975) show 98% sequence identity and 99% similarity. Therefore, the human TIM X-ray dimer structure (PDB entry 1hti; Mande *et al.*, 1994) was used as a search model. For the cryo-data set (crystal type *A*), the Matthews coefficient (Matthews, 1968) for a dimer in the asymmetric unit is V_M = 2.07 Å Da^{-1}, with a solvent content of 41%. For the type *B* crystal data set, V_M = 2.37 Å Da^{-1} indicated two dimers in the asymmetric unit and a solvent content of 48%. In the first case, calculations using 10–3.5 Å resolution limits and default parameters led to a single clear solution with a correlation coefficient of 73.5% and an *R* factor of 30.8% after fitting. The molecular-replacement procedure for the second (room-temperature) data set included a search for two dimers in the asymmetric unit cell and resulted in a clear solution with a correlation coefficient of 72.1% and an *R* factor of 33.0%. Further refinement of both structures is under way.

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