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Minireview

Crystallographic binding studies with triosephosphate isomerases: conformational changes induced by substrate and substrate-analogues

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TIM catalyses the interconversion of a triosephosphate aldehyde into a triosephosphate ketone. This is a simple chemical reaction in which only protons are transferred. The crystallographic studies of TIM from chicken, yeast and trypanosome complexed with substrate and substrate analogues are discussed. The substrate binds in a deep pocket. On substrate binding, large conformational changes are induced in three loops. As a result of these conformational changes in the liganded structure, the active site pocket is sealed off from bulk solvent and the sidechain of the catalytic glutamate becomes optimally positioned for catalysis.

Triosephosphate isomerase; Conformational change; Dimer; Catalysis; Crystallographic binding study

1. INTRODUCTION

TIM is a dimeric glycolytic enzyme. It catalyzes the interconversion of DHAP into GAP (Fig. 1) via a cis-enediolate and/or cisenediol intermediate. TIM is a very efficient enzyme and in-vivo the reaction is diffusion rate limited. The reaction mechanism is being studied by a wide range of techniques, including site-directed mutagenesis, NMR and crystallography. These mechanistic studies have been well reviewed by Knowles [1]. Crystal structures have been reported of TIM from chicken [2,3], yeast [4] and trypanosome [5], and recently the crystal structure of *E. coli* TIM has been determined at 2.6 Å resolution (Noble et al., unpublished observations). TIM is an α_2-homodimer of 27-kDa subunits. Each subunit has a regular fold, consisting of 8 ($\beta\alpha$)-units. The 8 β-strands form a parallel β-barrel in the core of the protein. This core is surrounded by 8 α-helices (Fig. 2). The active site is at the C-terminal ends of the β-strands. The loops at the C-terminal ends of these β-strands are therefore major determinants of the active site architecture. These loops will be referred to as loop-1 to loop-8. This TIM-barrel topology is observed in many other enzymes, which catalyse a wide range of reactions. The possibly divergent evolution of these enzymes has been reviewed by Farber and Petsko [6] and Brändén [7].

Here we will focus on the mode of binding of substrate analogues, as well as on the conformational changes induced by these compounds. In Table I the available crystallographic data are summarised. Some structures are obtained via cocrystallysation procedures and some via soaking experiments. Our reference model will be the structure of trypanosomal TIM-glycerol-3-phosphate [8]. The numbering scheme of glycerol-3-phosphate is given in Fig. 3. The protein residue numbering refers to the sequence of trypanosomal TIM. Subunit-1 consists of residues 2–250, and the subunit-2 residues are numbered from 302 to 550; the catalytic residues are therefore Asn-11, Lys-13, His-95 and Glu-167 in subunit-1 and Asn-311, Lys-313, His-395 and Glu-467 in subunit-2.

At present the best refined TIM-structures are from yeast (1.9 Å) and trypanosome (1.83 Å). Structures of liganded and unliganded TIM have been characterised. There is a major conformational switch between unliganded TIM (the 'open' conformation) and liganded TIM (the 'closed' conformation). This was first observed at low resolution for chicken TIM in crystal soaking experiments in the presence of DHAP [3]. The liganded and unliganded structures of chicken TIM, yeast TIM and trypanosomal TIM have been compared [9]. The conformational flexibility, as illustrated schematically in Fig. 2, is observed in all three TIMs, despite different crystal packings.

Abbreviations: G3P, glycerol-3-phosphate; PGH, phosphoglycolohydroxamate; TIM, triosephosphate isomerase (E.C. 5.3.1.1.); 2PG, 2-phosphoglycollate; DHAP, dihydroxyacetone phosphate; GAP, D-glyceraldehyde-3-phosphate; RMS, root mean square.

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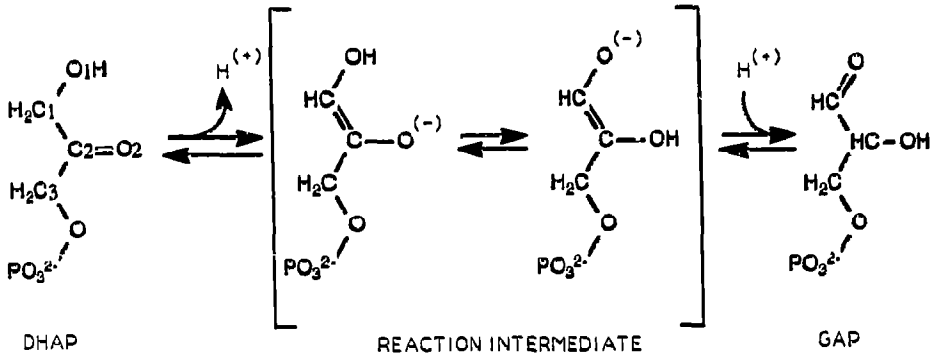


Fig. 1. The reaction catalysed by TIM. In step-1 a proton is abstracted from C1 by the sidechain of Glu-167. Subsequently a negative charge develops on O2, stabilised by the interactions of O2 with NZ (Lys-13) and NE2 (His-95). In step-2 proton transfer, assisted by the sidechain of His-95, from O1 to O2 takes place. In step-3 a proton is added back from Glu-167 to C2.

2. LIGAND BINDING AND ACTIVE SITE GEOMETRY

The active site is at the bottom of a deep pocket. In the open unliganded structure this pocket is accessible to bulk solvent. This pocket is rather polar except for the sidechains of Leu-232 (at the end of β-strand-8, forming the bottom of the active site pocket) and Ile-172 (from loop-6, closing off the active site from solvent in the closed structure). The comparison of unliganded yeast TIM and trypanosomal TIM shows that in both structures a well defined water molecule is hydrogen bonded to the sidechains of Asn-11 and His-95 at the bottom of the active site (WAT600 in trypanosomal TIM is at 3.2 Å from ND2 (Asn-11) and at 3.1 Å from NE2 (His-95)). On superposition of the protein CA-atoms these active site waters superimpose within 0.4 Å. Substrate analogues bind with the phosphate moiety close to the surface and with the aldehyde/ketone part at the bottom of the active site pocket. Upon binding of such a ligand WAT600 is displaced and the CA of Ile-172 moves 3.1 Å into the pocket, due to the conformational switch of loop-6. No buried water is left after the binding of the ligand, and the ligand oxygen atoms at the bottom of the active site are not hydrogen bonded to waters. The ligand is not completely dehydrated, since the phosphate moiety is hydrogen bonded to waters. The phosphate moiety is also hydrogen bonded to main chain nitrogen atoms of residues Gly-173 (loop-6), Ser-213 (loop-7) and Gly-234, Gly-235 (loop-8). These hydrogen bonds stabilise the position of the phosphate moiety of the ligand [10]. The hydrogen bonds with loop-6 and loop-7 are only possible because of conformational changes of these loops. In the unliganded form NH of Gly-212 (loop-7) points into the protein core, towards the aromatic ring of Tyr-210 [5]. In the closed structure it points outwards and interacts via a water with the phosphate moiety. As a result of the conformational switch the ϕ/ψ values of Ser-213 (loop-7) change from (−80,120) in the open state to (50,50) in the closed state. This conformational switch could have a high energy barrier. Model calculations [11] suggest that for a simple alanine dipeptide this barrier could be as high as 10 kcal, but within the context of the protein structure the height of this energy barrier will be modulated by the presence of the neighbouring atoms.

The structure of loop-5 also changes somewhat as a result of the closure of loop-6. The conformational change of loop-5 is probably related to the different hydrogen bonding interactions of the Glu-129 (loop-5) side chain with loop-6 residues. In the unliganded structure there is a direct hydrogen bond between OH(Tyr-166) and NE1(Trp-170) of loop-6, whereas in the closed structure the tryptophan sidechain has moved away,

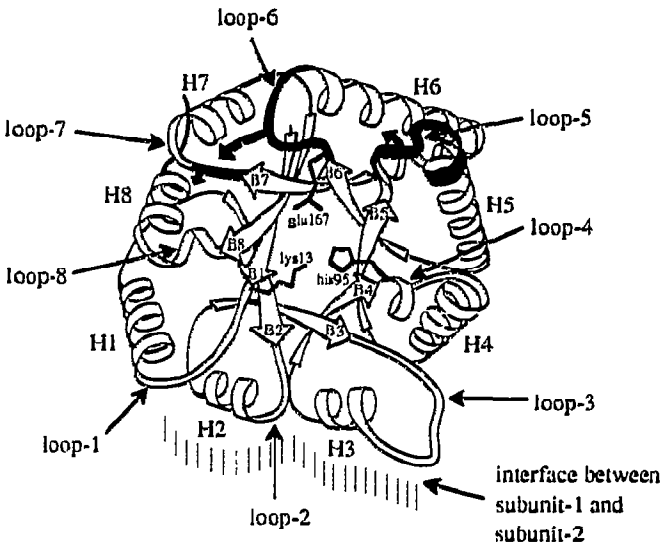
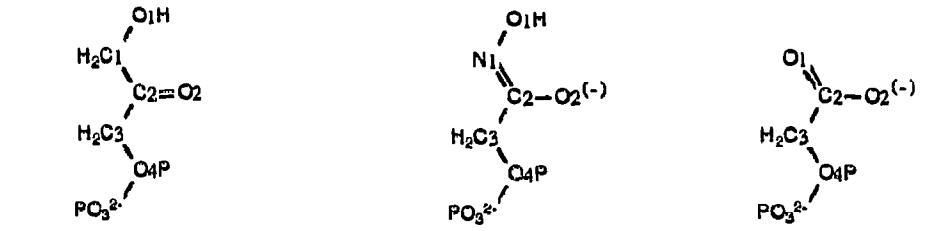


Fig. 2. Schematic drawing of the TIM-monomer. View into the active site of subunit-1. The approximate positions of the catalytic residues Lys-13, His-95 and Glu-167 are shown. The polypeptide segments that undergo rearrangements are in black. The arrows indicate qualitatively the movement from open to closed. Subunit-2 is below subunit-1; most of the interactions between subunit-1 and subunit-2 are mediated via loop-1, loop-2 and loop-3.



G3P (glycerol-3-phosphate) PGH (phosphoglycolohydroxamate) 2-PG (2-phosphoglycollate)

Fig. 3. The numbering scheme of G3P, PGH and 2PG.

and the interaction between OH(Tyr-166) and NE1(Trp-170) is now mediated via the carboxylate oxygens of Glu-129 (Fig. 4).

Loop-6 is a long loop consisting of 14 residues. The conformational switch involves residues 167 up to 180. The tip of this loop, residues Ile-Gly-Thr[174]-Gly, moves as a rigid body (12,13), due to small changes of the main chain angles of the flanking regions. The CA atom of residue Thr-174, moves over a distance of 6.3 Å. The first residue of loop-6 which changes its conformation is the catalytic glutamate, residue 167. In some complexes the side chain of Glu-167 is in a 'swung-out' position (away from the ligand), whereas in other complexes it is in a 'swung-in' position, with direct interaction with ligand atoms. The side chain of the catalytic glutamate is only competent for catalysis in the swung-in position. So far the swung-in position is only observed when loop-6 is in a closed conformation. Therefore it seems that loop closure is also needed to facilitate the switch to the swung-in conformation [5].

3. LIGAND BINDING AND CATALYSIS

TIM catalyzes the conversion of DHAP into GAP with a rate which is 10^{10} times faster than the non-enzymatic base catalysed conversion. At the same time an undesired phosphate elimination reaction is strongly disfavoured [14]. For the reaction to occur, protons have to be transferred from C1 to C2, and from O1 to O2 (Fig. 1) [15]. At an intermediate stage (either as a transition state or as a reaction intermediate) there ex-

Table 1

Crystallographic data of various TIM-ligand complexes

	Ligand	Concentration of ligand	Experimental conditions	PDB-entry	Reference	Comments
Chicken	Sulphate	2.4 M	cocrystallisation		[3]	
TIM	Dihydroxyacetone phosphate	200 mM	soaking		[3]	
Yeast	–*	–	cocrystallisation	1YPI	[3]	
TIM	Phosphoglycolohydroxamate	10 mM	cocrystallisation		[16]	
	2-Phosphoglycollate	2.5 mM	cocrystallisation	2YPI	[10]	
	Phosphoglycolohydroxamate	1.5 mM	cocrystallisation	3YPI	[28]	mutant $His^{95}Gln$
Trypanosomal	–**	–	–	5TIM	[5]	subunit-1
TIM	Sulphate**	2.4 M	cocrystallisation	5TIM	[5]	subunit-2
	Glycerol-3-phosphate	6 mM	soaking	6TIM	[8]	
	3-Phosphonopropionate	100 mM	soaking		[8]	
	3-Phosphoglycerate	7 mM	soaking		[8]	
	2-Phosphoglycerate	30 mM	soaking	4TIM	[29]	
	2-(*N*-formyl-*N*-hydroxy-amino)-ethylphosphonic acid	2.5 mM	soaking		Noble et al., unpublished observations	
	Phosphoglycolohydroxamate	30 μM	soaking		Noble et al., unpublished observations	
	Phosphate	40 mM	soaking		[30]	
	N-Hydroxy-4-phosphonobutamide	3.3 mM	soaking		Verlinde et al., unpublished observations	

*The crystals were grown in the presence of 200 mM ammomium sulphate, but data was collected from crystals after transfer to an ammonium sulphate-free solution [3].

**Crystals were grown in the presence of 2.4 M ammonium sulphate. The conformation of subunit-1 and subunit-2 is affected by crystal contacts: subunit-1 is in the unliganded, open conformation, whereas subunit-2 is in the liganded (sulphate), almost-closed conformation.

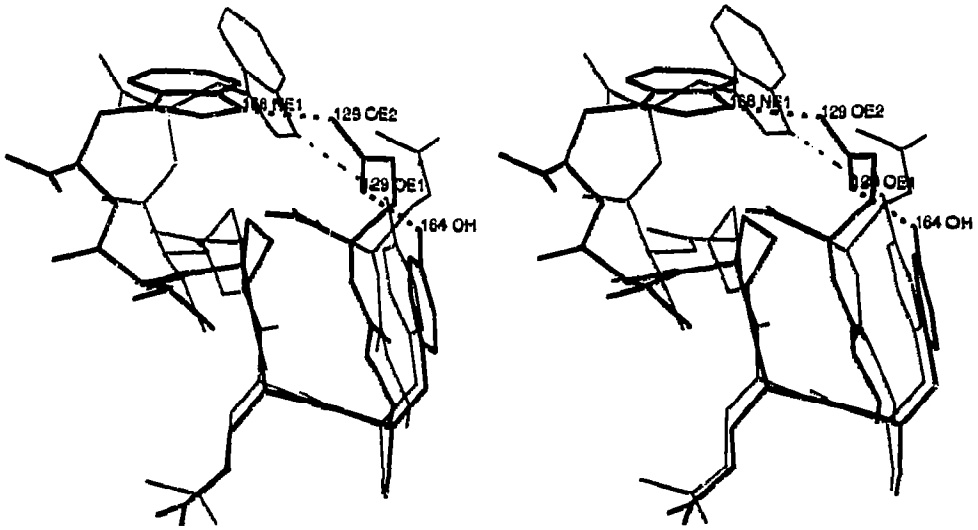


Fig. 4. The Tyr–Trp interaction of loop-6 in the open and closed form. Thick lines represent the closed form (yeast TIM-2PG complex). Thin lines represent the open form (yeast TIM, unliganded). The dotted lines are hydrogen bonds. Some sidechain atoms of the tyrosine, glutamate and tryptophan residues of the closed structure are labelled (yeast TIM numbering).

ists a negatively charged enediolate molecule. The Glu-167 sidechain is responsible for proton transfer between the two carbon atoms, whereas His-95 is important for proton transfer between the oxygen atoms. The structure of chicken TIM complexed with DHAP has been studied at 6 Å resolution [3], but high resolution structures of TIM-DHAP or TIM-GAP have not been determined. Therefore, the catalytic importance of the various sidechain arrangements is based on the analogy between DHAP, GAP and the substrate analogues used in the crystallographic studies summarised in Table I. The conclusions are in agreement with solution experiments, such as the labelling studies of the catalytic glutamate [27]. Structures of yeast TIM complexed with 2PG [10] and PGH [16] have been described in great detail. 2PG and PGH are reaction intermediate analogues [17] with inhibition constants of 8 μM and 4 μM, respectively, for rabbit muscle TIM [18]. NMR studies have shown that 2PG binds as a trianion [19]. PGH (Fig. 3) mimics the enediolate structure; the hydroxamate moiety is planar and the pK of this group is 9.5 [18]. As indicated in Table I, the TIM-PGH structure is known for trypanosomal TIM (by soaking) as well as for yeast TIM (by cocrystallisation). The observed mode of binding of PGH is identical (RMS difference in position of the 10 PGH atoms is 0.35 Å). Fig. 5 shows for trypanosomal TIM the positions of the catalytic residues Asn-11, Lys-13, His-95 and Glu-167 in the unliganded, the G3P-bound and the PGH-bound structures. In the unliganded structure Glu-167 is in the swung-out position, whereas in the complexes it interacts with the ligand. The position of the conserved water molecule, WAT600, is in the G3P structure occupied by a carbon atom (C1 of G3P). In this position this carbon atom is in a special environment. C1(G3P) is at 3.3 Å from NE2(His-95), at 3.1 Å from ND2(Asn-11) and at 3.3 Å from OE2(Glu-167). The equivalent N-atom of PGH is at 4.4 Å from ND2(Asn-11), 4.1 Å from

Table II

Geometric data of ligands bound in the active site of trypanosomal TIM and yeast TIM

		distances (Å)					dihedral(°)**
		WAT600*	ND2 (Asn-11)	NE2 (His-95)	OE1 (Glu-167)	OE2 (Glu-167)	
Trypanosomal TIM	C1 (glycerol-3-phosphate)	0.4	3.1	3.3	4.0	3.3	16.5
	N1 (phosphoglycolohydroxamate)	0.9	4.4	4.1	3.4	2.4	−28.4
Yeast TIM	O1 (2-phosphoglycollate)	0.9	3.6	3.1	3.3	2.8	−15.1
	N1 (phosphoglycolohydroxamate)	0.7	4.0	3.8	3.4	2.7	−11.4

*This is the distance between WAT600 (unliganded structure) and the listed atom after superposition of the 105 CA-atoms of the $\beta\alpha$-units of the liganded structure on the unliganded structure.

**This is the dihedral of the atoms corresponding to O4P, C3, C2, O2 in G3P.

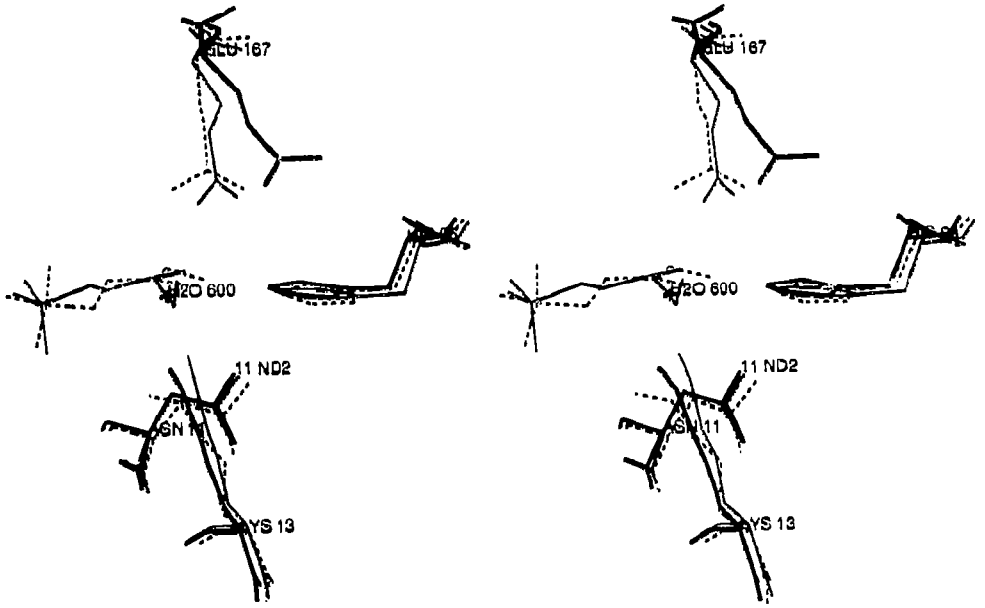


Fig. 5. The catalytic residues Asn-11, Lys-13, His-95 and Glu-167 of trypanosomal TIM in the unliganded structure (thick lines), the G3P complex (thin lines) and the PGH complex (dotted lines). WAT600 of the unliganded structure is hydrogen bonded to ND2 (Asn-11) and NE2 (His-95), see text. Also shown are the positions of G3P (thin lines) and PGH (dotted lines) as observed in the respective complexes. Note that only the sidechain conformation of the catalytic glutamate differs significantly when comparing the structures of the liganded enzyme (swung-in conformation) and unliganded enzyme (swung-out conformation).

NE2(His-95) and 2.4 Å from OE2(Glu-167) (Table II). By analogy this polar environment will also effect the properties of the equivalent C-atom of the substrate, from which proton transfer occurs. As is seen in Fig. 5, the electrophilic atoms ND2(Asn-11) and NZ(Lys-13) are below the ligand whereas the carboxylate oxygens of Glu-167 are above it. This geometric arrangement facilitates proton transfer by the glutamate sidechain.

The torsion angle of O4P-C3-C2-O2 of G3P and other ligands is close to zero (Table II). This strained conformation disfavours the phosphate elimination reaction [10]. A common feature in the mode of binding of substrate analogues (as listed in Table I) is also the position of the O2-atom, always hydrogen-bonded to NZ(Lys-13) and NE2(His-95). These interactions will stabilise a negative charge on this oxygen atom, which is believed to be transiently present during the reation. The ND1 atom of His-95 is hydrogen bonded to a mainchain NH, which means that His-95 is neutral in the ground state, with a proton bound to NE2. Ab initio

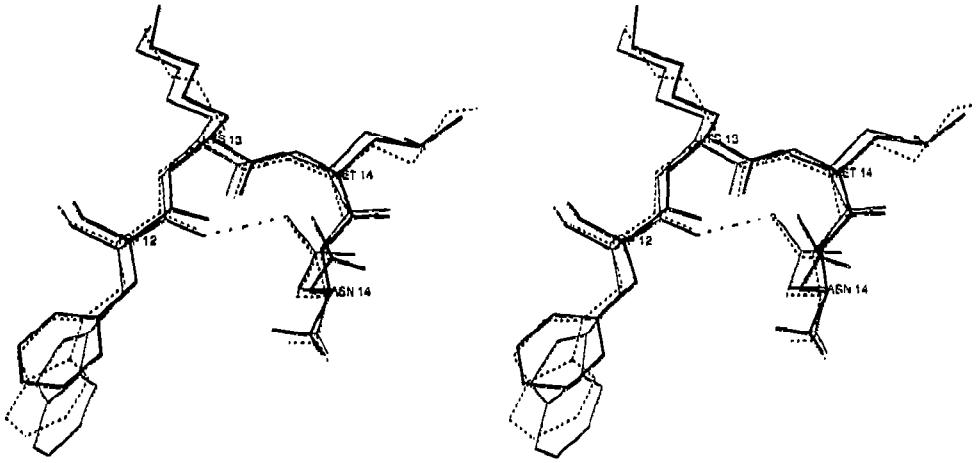


Fig. 6. The loop-1 conformation of chicken TIM (residues Trp-Lys-Met-Asn, thin dotted lines), yeast TIM (residues Phe-Lys-Leu-Asn, thick lines) and trypanosomal TIM (Trp-Lys-Cys-Asn, thin lines). The hydrogen bond between O (Trp-12) and ND2 (Asn-14) in trypanosomal TIM is indicated by a dotted line.

and molecular dynamics calculations have suggested that this histidine might become transiently negatively charged during the proton transfer between the two oxygen atoms [20]. NMR-studies [21] also suggest the intermediate formation of an enediol-imidazolate pair.

4. TRIOSEPHOSPHATE ISOMERASE IS ONLY ACTIVE AS A DIMER

All the interactions between a substrate analogue and the protein are with atoms of one subunit. The OG1 atom of Thr-375, which is at the tip of loop-3, the interface loop, of the other subunit (Fig. 2), comes closest to the substrate analogue, at a distance of 5.5 Å. Nevertheless TIM is only active as a dimer [22,23], although the active sites function independently [24]. The structure gives several reasons for this: (i) the Thr-375 sidechain hydrogen bonds to Glu-97 which forms a salt bridge with NZ of the catalytic lysine, Lys-13. OG1(375 is also hydrogen bonded to ND2(Asn-11). NZ(Lys-13) and ND2(Asn-11) interact with a ligand atom (see Table II); (ii) the main chain dihedrals of Lys-13 are unusual, causing strain in this region of the molecule. This strained geometry could be stabilised by two mainchain–mainchain hydrogen bonds between Cys-14 and Gly-372 of the other subunit (Cys-14 and Gly-372 from a one-residue long antiparallel β-sheet). These two hydrogen bonds are the only mainchain–mainchain hydrogen bonds across the dimer interface; (iii) the sidechain of Lys-13 is stabilised by van der Waals contacts with atoms of residues 372, 373, 375 of the other subunit. At present we are investigating, by site directed mutagenesis, the importance of the dimer interface interactions of trypanosomal TIM. Such mutagenesis experiments have also been described for yeast TIM, in which Asn-14 and Asn-78 have been mutated into other residues [25,26]. Asn-14 is a completely conserved residue and could be important for the stability of loop-1 (Fig. 6). The studies with N78D seem to indicate that monomeric TIM can exist as a folded molecule [25].

5. CONCLUDING REMARKS

The analysis of the currently available structures, summarised above, shows that TIM has acquired during its evolution a complicated catalytic machinery. Residues 20 Å away from the active site change conformation during the catalytic cycle [9]. This might explain why so many residues (56 out of 250) are completely conserved in 13 different sequences [9]. Most of these residues are part of the β-strands or of the subsequent loops. It is surprising to see that such a complicated protein machinery is required for optimal catalysis of such a simple chemical reaction, stressing that the design of efficient enzymes is not at all trivial.

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