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Reflections on the catalytic power of a TIM-barrel

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ABSTRACT

The TIM-barrel fold is described and its propagation throughout the enzyme universe noted. The functions of the individual front loops of the eponymous TIM-barrel of triosephosphate isomerase are presented in a discussion of: (a) electrophilic catalysis, by amino acid side chains from loops 1 and 4, of abstraction of an α-carbonyl hydrogen from substrate dihydroxyacetone phosphate (DHAP) or D-glyceraldehyde 3-phosphate (DGAP). (b) The engineering of loop 3 to give the monomeric variant monoTIM and the structure and catalytic properties of this monomer. (c) The interaction between loops 6, 7 and 8 and the phosphodianion of DHAP or DGAP. (d) The mechanism by which a ligand-gated conformational change, dominated by motion of loops 6 and 7, activates TIM for catalysis of deprotonation of DHAP or DGAP. (e) The conformational plasticity of TIM, and the utilization of substrate binding energy to "mold" the distorted active site loops of TIM mutants into catalytically active enzymes. The features of the TIM-barrel fold that favor effective protein catalysis are discussed.

1. Introduction

Protein structure determines function in obtaining large enzymatic rate enhancements [1–6]. An interesting and difficult problem is to define the mechanism by which specific features of folded α-helices, β-strands and flexible loops contribute to these rate enhancements. The classical TIM-barrel fold $(\beta\alpha)_8$ consists of eight repeating (βα) units arranged so that eight parallel β-strands form a central protein core that is covered by α-helices on the outside. The TIM-barrel is reportedly the most common protein fold in the Protein Data Bank, and at one time accounted for about 10% of all proteins with known three-dimensional structures [7–9]. The properties of this protein fold [8,10], and the divergent evolution of the progenitor TIM-barrel into proteins that display a vast array of enzymatic activities, have been discussed in many excellent reviews [8–14]. This essay will consider the specific features of the TIM-barrel, which favor efficient catalysis by the eponymous enzyme triosephosphate isomerase (TIM); and, the general features of this protein fold, which favored propagation of the TIM-barrel throughout the enzyme universe.

The secondary structural elements of the TIM-barrel are numbered sequentially from the N-terminus as β1–β8 and α1–α8. These strands and helices are connected by a total of sixteen loops, which are referred to as βα loops (front loops), such that the βα loop 1 follows after strand β1; and, αβ loops (back loops), such that αβ loop 1 follows after helix α1. The active sites of TIM-barrel enzymes lie at the C-terminal ends of the β-strands, which are localized at the "catalytic" end of the barrel. They are often constructed by the folding of the eight βα front loops into a structured *cage* for the substrate, whose formation is driven by the development of stabilizing interactions between the substrate and the catalytic front loops [15] The eight back loops lie at the end of the barrel distant from the front loops, and play an important role in stabilizing this protein fold [8,11]. The TIM-barrel provides a robust framework to support an incredible variety of front loop structures, which are used in construction of the active sites for enzymes found in 21 homologous superfamilies and 76 different sequence families [10].

2. Triosephosphate isomerase

[1]The TIM-barrel fold was first characterized for the crystal structure for triosephosphate isomerase (TIM), which catalyzes isomerization of D-glyceraldehyde 3-phosphate (GAP) to dihydroxyacetone phosphate (DHAP, Scheme 1), through a pair of enediolate reaction intermediates [20]. Enzyme-catalyzed isomerization proceeds with proton transfer between carbons 1 and 2, mediated by the carboxylate side chain of Glu165 [16,17], and between oxygen 1 and 2, mediated by the imidazole side chain of His95 [18,19].

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[1] We note the following small differences in the numbering of the amino acid residues at TIM from the different sources: (*c*TIM or *y*TIM and *Tbb*TIM): Lys 12 and 13, Glu165 and 167, Pro166 and 168, Ile170 and 172, Leu230 and 232.

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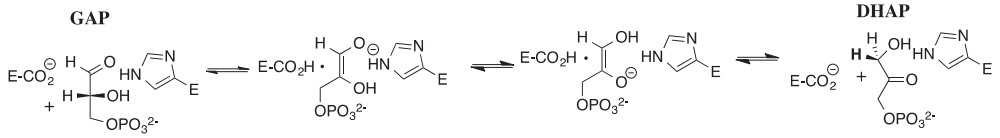

Scheme 1. An abbreviated mechanism for TIM-catalyzed isomerization of D-glyceraldehyde 3-phosphate (GAP) to dihydroxyacetone phosphate (DHAP). Proton transfer reactions at carbon and oxygen are carried out, respectively, by the carboxylate side chain of Glu165 [16,17] and the imidazole side chain of His95 [18,19].

The side chains of Asn10 and Lys12 are positioned to provide stabilization of the developing negative charge at either O-1 or O-2 of enediolate phosphate reaction intermediates [21,22]. The mechanism of action of TIM has been investigated by kinetic and isotope labeling studies [23–27], the specific role of the active site side chains has been probed by site-directed mutagenesis [20,28–31], and the role of an extended ligand-gated conformational change has been examined in solid state [32,33] and solution NMR studies [34,35] and by temperature jump relaxation fluorescence spectroscopy [36].

This essay will focus on the function of the front loops of the TIM-barrel in catalysis of isomerization of DGAP, and will conclude by drawing generalizations from these studies about the advantages of the TIM-barrel that favor its evolution into proteins that catalyze a broad range of reactions. Fig. 1A and B shows representations of chicken muscle TIM (*c*TIM) from the top and the side of the TIM-barrel, respectively, which highlight the β1–β8 front loops from a single enzyme subunit, and the β1–β8 loop 3′ from the neighboring subunit. The loops are grouped by colors and the codes are given in the figure legend.

2.1. Loops 1 and 4

Efficient catalysis by TIM requires that the enzyme provide a similar lowering of the activation barriers to proton transfer at carbon-1 of DHAP and carbon-2 of GAP, in order to avoid one of these steps become strongly rate-determining for turnover. Consequently, the basic side chain of Glu165 shows a similar high reactivity toward deprotonation of C-1 and C-2 of DHAP and DGAP, respectively. It is not widely appreciated that the reactivity of this side chain depends upon both its intrinsic basicity and upon the assistance to carbon deprotonation from electrophilic catalysis at the carbonyl oxygen of DHAP or DGAP. This sets TIM an additional challenge of providing strong stabilization of transition state negative charge that develops at O-1, when the substrate is DGAP, and at O-2 when the substrate is DHAP; while, at the same time promoting fast intramolecular transfer of hydrogen between O-1 and O-2 in the overall isomerization reaction (Scheme 1).

Three acidic side chains are positioned to stabilize negative charge at the two enediolate intermediates (Fig. 2A): the amide side chain from Asn10, the alkylammonium side chain from Lys12 (loop one, residues 12–17 for *c*TIM) [22,41,42], and the imidazole side chain of His95 (loop 4, residues 94–105 for *c*TIM) [18,19,43]. The imidazole side chain lies equidistant from O-1 and O-2 at the complexes to the intermediate analog PGH (Fig. 2B) for TIM from *L. mexicana* [38]. This implies a role for the side chain in mediating proton transfer between O-1 and O-2 (Scheme 1). Elimination of this side chain at the H95Q mutant results in a dramatic change in the products of wild type TIM-catalyzed isomerization of GAP and of DHAP in tritium-labeled water [19]. It was proposed that this change was due to substitution of the remaining carboxylate side chain of Glu165 for the deleted imidazole of His95 in mediating proton transfer reactions between the enediolate O-1 and O-2, so that this carboxylate effects proton transfer at both carbon and oxygen for the H95Q mutant enzyme-catalyzed reaction. The requirement that the imidazole side chain interact with the O-1 and O-2 oxyanions at the two reaction intermediates (Scheme 1) will limit the strength of the interaction, in order to avoid that interconversion of these intermediates become strongly rate-determining. This provides a simple explanation for the utilization of the weakly basic neutral imidazole side chain [43], instead of the more strongly acidic imidazolium cation.

The cationic side chain of Lys12 runs across the surface of TIM, forms a solvent separated ion pair with the ligand phosphodianion,

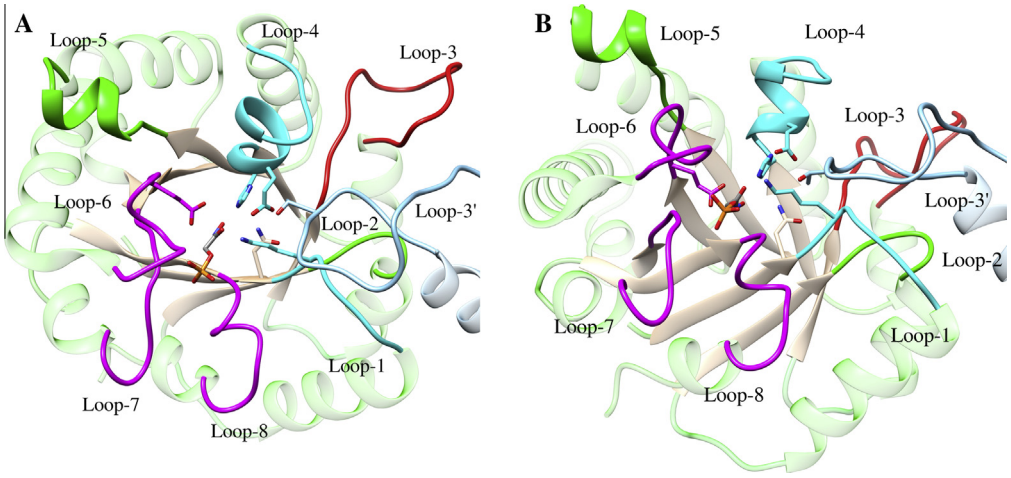

Fig. 1. Cartoons (PDB entry ITPH) drawn to show perspectives of a single subunit of the complex between *c*TIM and the competitive inhibitor phosphoglycolohydroxamate (PGH). The eight βα front loops are labeled, along with loop 3′ provided by the neighboring protein subunit. (A) A view from the "top" of the barrel, which shows the convergence of the catalytic side chains at the active site. The basic side chain from Glu165 (loop 6) abstracts the acidic carbon proton, the acidic side chain from His95 (loop 4) shuttles a proton between the enediolate oxygens, and the acidic side chains of Asn10 and Lys12 (loop 1) stabilize negative charge at these oxygen atoms. (B) A view from the "side" of the barrel, which illustrates the placement of front loops across the "top" of the barrel. The loops are color-coded: loops 1 and 4 are shown in cyan; loop 3 is shown in red; loops 6, 7 and 8 are shown in magenta; loops 2 and 5 are shown in green; and, loop 3′ from the second subunit is shown in light blue.