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An alternative allosteric pathway in thermophilic methylglyoxal synthase

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ABSTRACT

Methylglyoxal synthase (MGS) is a homohexameric enzyme responsible for converting dihydroxyacetone phosphate (DHAP) to methylglyoxal and phosphate in the methylglyoxal bypass of glycolysis. Phosphate acts as an allosteric inhibitor and strong regulator for this enzyme. Previous studies on MGS from *Thermus* sp. GH5 (TMGS) had indicated a pathway for transmitting the signal through Pro82, Arg97 and Val101 to the active site. The necessity of these residues for heterotropic negative cooperativity between subunits of TMGS were also proposed. In this study, it has been shown that a path via a salt bridge between Arg80 and Asp100 in the narrow dimer interface provides an alternative pathway for transmission of the allosteric inhibitory signal through subunit interfaces.

1. Introduction

Allostery by definition involves the propagation of signals between functionally important regulatory and distant allosteric sites [1]. Any event such as binding of molecules, covalent modifications, or changes in environmental factors that perturb a protein at an allosteric site generates an allosteric signal and results in changes in function, structure, and flexibility of the protein at regulatory site [2,3]. The significance of allostery in control mechanisms has been long known and used in practical applications like drug design [4]. However, the exact allosteric signal transmission is strongly debated. In classical models for allostery, the MWC (or symmetric) and KNF (or sequential) models, allostery described as a conformational change between two well-defined alternative structures [5,6]. These models which result from experimental technologies such as X-ray crystallography are based on structural end states static images and are deficient in explaining how allosteric communication occurs by these structural changes [7]. Developments of the techniques which provide dynamic and thermodynamics information of proteins, propose that changes in protein dynamics are associated with allosteric communications [7–9]. These points of view that are called new view of allostery [10–12], have revealed that allosteric event does not create a new structural conformation but leads to a population shifts among existing conformational states. As a consequence, all non-fibrous proteins can be treated as ensembles of conformational states that their interconversion rates are altered by allosteric perturbations [13,14]. In this view allostery is "an intrinsic property of all dynamic proteins" [15]. As a result, many allosteric enzymes that were previously thought to induce cooperativity according to classical models have been proven to use these new approaches [15]. Examples of these enzymes can be found in the seven-helix receptor family [16], Notch receptors [17] and epidermal growth factors [18]. However with the comprehensive look, many recent studies are positing the idea that a network of physically interconnected and/or thermodynamically linked residues create allosteric pathways for transmission of informations [19–21]. For instance *Thermus* sp. GH5 methylglyoxal synthase (TMGS) also uses allostery to mediate its function.

Methylglyoxal synthase (MGS, EC 4.2.3.3) is a homohexameric enzyme that catalyzes an elimination reaction of dihydroxyacetone phosphate (DHAP) and produces phosphate and enol pyruvaldehyde. This enol then tautomerizes to methylglyoxal in solution [22]. MGS catalyzes the first reaction in the methylglyoxal bypass of the

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glycolysis pathway and has been explored in different microorganisms [23]. However, MGS from *E.coli* (EMGS) is the most extensively studied enzyme. In the absence of phosphate, MGS acts non-allosterically and there is a hyperbolical dependence of its kinetic data on the substrate concentration, while phosphate acts as a strong allosteric inhibitor of the Pazhang et al. [25]. The crystal structure of thermophilic [*26*] and mesophilic methylglyoxal synthases [27] in complex with transition state analogues showed that different types of monomer-monomer interactions within the hexamer that include three wide dimer interfaces (Fig. 1a: between C and E, B and F, D and A), three narrow dimer interfaces (Fig. 1a: between A and C, B and E, F and D) and two trimer interfaces (Fig. 1a: between A, E, and F and D, B, and C). Structural and mutagenesis studies suggested that these interfaces promoted second shell interactions between residues of neighboring monomers and participated in signal transmission between the active-site residues of one monomer with residues from the neighboring monomer [24,27–29]. The structure of the EMGS enzyme in complex with phosphate suggests two possible pathways for transmitting allosteric changes throughout the hexamer. In the first pathway, interactions of Pro92, Arg107 and Val111 in the trimer interface, and in the second pathway, formation of a salt bridge between Asp20 and Arg150 of the neighboring subunit, conveys the allosteric signal [27]. Amino acid sequence alignment of TMGS and EMGS shows 66% similarity and indicates the conservation of the first pathway's amino acids while the second allosteric pathway is absent in TMGS (Fig. 2) [25].

Crystallographic data of phosphate bound to TMGS introduces a new unique salt bridge between Arg80 and Asp100 in the narrow dimer interface that is not conserved in phosphate bound EMGS (Fig. 1b). Formation of this salt bridge was evaluated by ESBRI web server [30]. Incidence of this specific salt bridge upon phosphate binding may provide a pathway for transmission of the allosteric inhibitory signal through the narrow dimer interfaces. Therefore, this interaction can act as an alternative route to compensate for the absence of Asp20 and Arg150 salt bridge in TMGS. In order to examine this hypothesis, in the current study, this new salt bridge was disrupted by site-specific mutagenesis. Arg80 was mutated to lysine, glutamine, isoleucine and glutamate, and Asp100 was substituted with asparagine, valine and lysine. In the case of lysine substitution the positive charge remained at the location and is considered as a conservative mutation. This mutation partially maintained the salt bridge. To survey the impact of opposite charges in the location, Arg80Glu and Asp100Lys mutations were designed. To study the impact of hydrophobicity at the location of salt bridge, Arg80 and Asp100 were substituted with polar and hydrophobic amino acids. Arg80Gln and Asp100Asn mutations were polar substitutions while Arg80Ilu and Asp100Val were hydrophobic substitutions. Moreover, to investigate the exact role of the salt bridge, we constructed a variant with substitutions at both Arg80 and Asp100 (R80D/D100R). Kinetic parameters and structural properties of these variants were measured. Information obtained from the kinetic data provided new aspects of TMGS allosteric behavior.

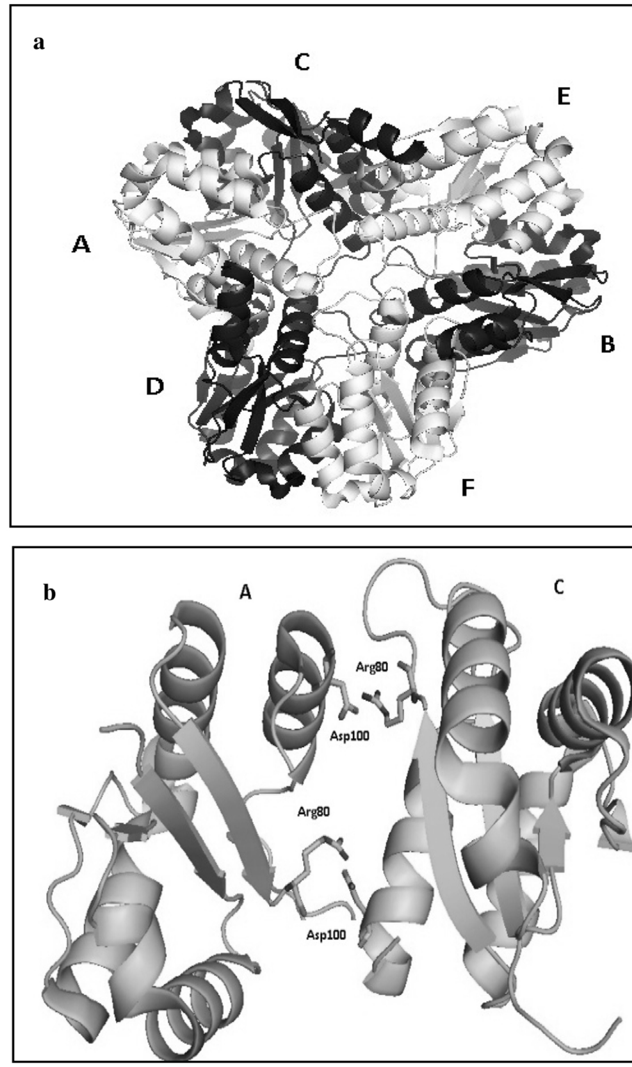

Fig. 1. (a) Cartoon diagram showing the homohexamer of TMGS and its three different types of monomer-monomer interactions prepared from the coordinates of Shahsavar et al. [14] (PDB ID: 2XW6) rendered in PyMol. Wide dimer interfaces occurs between subunits A and D, B and F and C and E. Narrow dimer interfaces occurs between subunits A and C, B and E and F and D. Trimer interfaces occurs between subunits A, E and F and between D, B and C. (b) Narrow dimer interfaces between A and C subunits in the phosphate bound TMGS. Crystallographic data introduce a new salt bridge formation in narrow interface between Arg80 and Asp100.

2. Materials and methods

2.1. Chemicals, bacterial strain and plasmids

Dihydroxyacetone phosphate (DHAP), isopropyl-β-*D*-thiogalactopyranosid (IPTG) and bovine serum albumin (BSA) were purchased from Sigma (USA). High-fidelity DNA polymerase, PCR reagents and restriction enzyme were supplied by Fermentase Life Sciences (Germany). All components of growth media were

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                                                                                                               ↓
E. coli MGS   MELTTRTLPA RKHIALVAHD HCKQMLMSWV ERHQPLLEQH VLYATGTTGN LISRATGMNV NAMLSGPMGG DQQVGALISE GKIDVLIFFW DPLNAVPHDP  100
TMGS          ---------- MRALALIAHD AKKEEMVAFC QRHREVLARF PLVATGTTGR RIEEATGLTV EKLLSGPLGG DQQMGARVAE GRILAVIFFR DPLTAQPHEP   90

                         ↓
              DVKALLRLAT VWNIPVATNV ATADFIIQSP HFNDAVDILI PDYQRYLADR LK   152
              DVQALLRVCD VHGVPLATNP MAAEALIP-- WLQSLVGYQT PQGQ------ --   132
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Fig. 2. Amino acid sequence alignment of MGS *E.coli* enzyme and TMGS. Mutagenesis sites marked by arrows. The alignment was performed by ClustalW version 2.0.