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Comparative analysis of malate synthase G from *Mycobacterium tuberculosis* and *E. coli*: Role of ionic interaction in modulation of structural and functional properties

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Dedicated to Vinod Bhakuni who left for the heavenly abode on 15th July 2011 when the manuscript was under review.

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ABSTRACT

Metabolic plasticity of Mycobacterium renders high degree of adaptive advantages in the persistence through the upregulation of glyoxylate shunt. The malate synthase (MS), an important enzyme of the shunt belongs to the G isoform and expressed predominantly as monomer. Here we did a comparative unfolding studies of two homologous MS from *Mycobacterium tuberculosis* (MtbMS) and *Escherichia coli* (ecMS) using various biophysical techniques. Despite having high sequence identities, they show different structural, stability and functional properties. The study suggests that the differences in the stability and unfolding of the two enzymes are by virtue of differential electrostatic modulation unique to their respective molecular assembly.

1. Introduction

Tuberculosis is the second leading infectious cause of mortality across the globe claiming approximately 2.5 million deaths annually [1]. The problem has become more severe with the emergence of multidrug resistance strains and stresses the need of new and effective drugs. The enzymes of glyoxylate shunt have received global focus of contemporary drug research because of its proven role as virulence factor [2–4]. This pathway is present in most prokaryotes, lower eukaryotes and plants, but has not been observed in vertebrates. As humans do not have functional glyoxylate pathway the enzymes of the pathway are promising drug target [5–8]. The glyoxylate cycle has two key enzymes; isocitrate lyase (ICL; EC4.1.3.1) and malate synthase (MS; EC 2.3.3.9). In the first step, ICL catalyzes the cleavage of D-isocitrate (six carbons) to glyoxylate (two carbons) and succinate (four carbons). In the second step, glyoxylate formed by ICL reaction is condensed with acetyl-CoA to produce L-malate (four carbons) by malate synthase [9].

Malate synthase (MS) is a multifunctional protein which, besides its traditional enzymatic role, has evolved to promote the adherence of the bacterium to host cells by its ability to bind laminin thus acting as virulence factor [3–10]. All malate synthases described till date fall broadly in to two major families, isoforms A and G. The 80 kDa monomeric malate synthase isoform G (MSG) has been found exclusively in bacteria whereas, the oligomeric malate synthase isoform A (MSA, 65 kDa per subunit) occurs in plants and several other organisms including prokaryotes. MSG is a magnesium-dependent enzyme [11]. The members of this family of enzymes are structurally based on TIM barrel fold. They also contain an insertion forming a separate α/β domain and an additional C-terminal helical plug. The cleft between the TIM barrel and C-terminal plug forms the active site [12]. Members of the MSG family share about 50% amino acid sequence identity [9]. The *Escherichia coli* and Mycobacterium MSGs have about 56% sequence identity with identical backbone conformation [13] and active site.

The present study was carried out to delineate the structure, activity, and stability properties of recombinant MtbMS

Abbreviations: MtbMS, *Mycobacterium tuberculosis* malate synthase G; ecMS, *E. Coli* malate synthase G; SEC, size exclusion chromatography; GdnHCl, guanidine hydrochloride; CD, circular dichroism; GRASP, graphical representation and analysis of structural properties.

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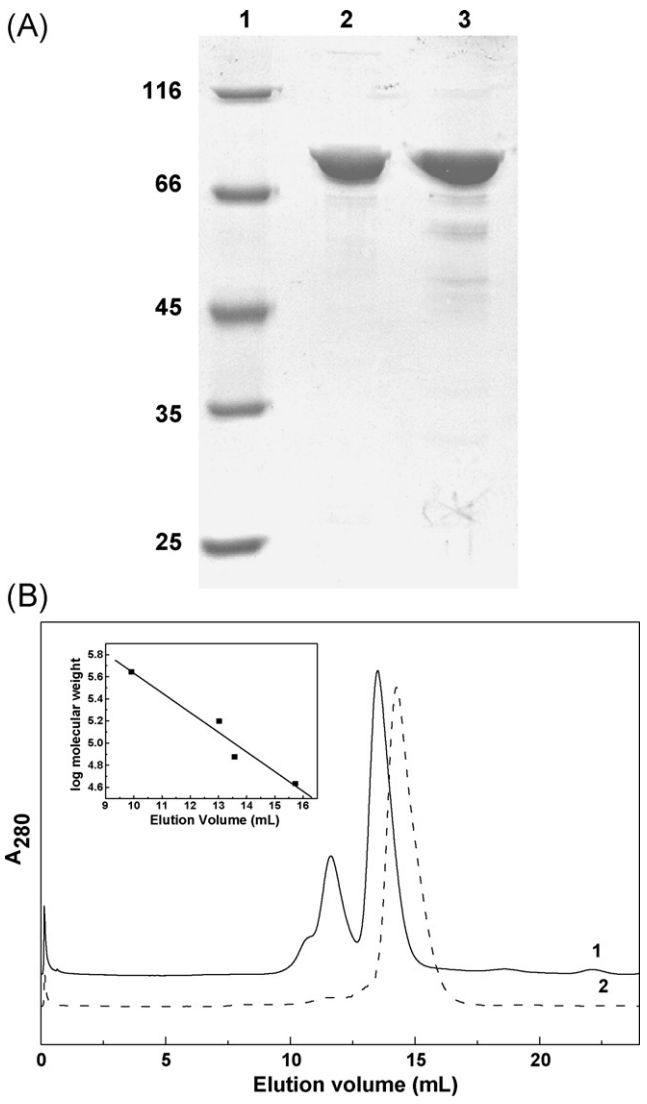

Fig. 1. Purification of MtbMS and ecMS. (A) SDS-PAGE showing purified recombinant MtbMS and ecMS protein. Lanes 1–3 represent molecular weight markers and, purified MtbMS and ecMS, respectively. (B) SEC profile of MtbMS (1) and ecMS (2) on Superdex 200HR column at pH 8.0 and 25 °C. The inset shows the curve of elution volume plotted against log of molecular mass of standard protein markers. The proteins are (1) 440 kDa (ferritin), (2) 158 kDa (aldolase), (3) 75 kDa (conalbumin) and (4) 43 kDa (ovalbumin).

and *E. coli* MSG (ecMS). As significant differences in the structural properties of the two enzymes were observed so attempts were made to unveil the differential role of ionic interactions in modulation of structure, stability and function of these enzymes using biophysical and biochemical techniques.

2. Results and discussion

2.1. MtbMS and ecMS are stabilized predominantly as a monomer

The *Mycobacterium tuberculosis* (MtbMS) and *E. coli* (ecMS) were over-expressed and purified. The SDS PAGE profile of both the enzymes shows the expected molecular mass of about 80 kDa and a purity of about 95% (Fig. 1A). The size exclusion chromatography on Superdex^{TM} 200 column calibrated with various molecular weight standards showed two peaks with high abundance of one compared to other in case of MtbMS whereas ecMS showed a single prominent peak. For MtbMS, the prominent peak was observed at a retention

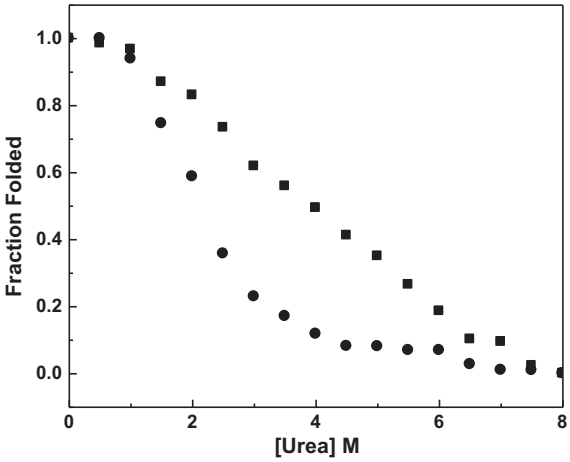

Fig. 2. Urea induced perturbations in secondary structure MtbMS and ecMS. Changes in CD ellipticity at 222 nm for MtbMS (circle) and ecMS (square) on incubation with increasing concentrations of urea at pH 8.0 and 25° C. Represented as fraction folded where one represent the native and zero represent the unfolded form in the two state model.

volume of 13.9 ml and a smaller peak at 12.3 ml, corresponding to the molecular mass of about 80 kDa (monomer) and 160 kDa (dimer) (Fig. 1B and inset). For ecMS, the single peak with a retention volume of about 14.36 ml was observed which corresponds to the molecular mass of 70 kDa (monomer) (Fig. 1B).

The long term incubation of MtbMS does not change the equilibrium and hence suggests that the enzymes predominantly present as a monomers. The other peak was found to be active dimer has been characterized and reported independently [14]. An interesting observation was difference in the retention volume for MtbMS (13.9 ml) and ecMS (14.36 ml), despite having the similar molecular mass suggesting that ecMS may be stabilized as compact conformation compared to MtbMS.

2.2. Effect of chaotropic agents on the structure and stability of MtbMS and ecMS

The spectral changes associated with the unfolding of MtbMS and ecMS were studied by CD spectroscopy at 222 nm by chaotropic denaturation. The equilibrium unfolding studies was done by measuring the overnight incubated samples for each denaturant concentration.

2.3. Urea-induced unfolding

The comparative equilibrium unfolding and stability characteristics of the MtbMS and ecMS were studied by monitoring the urea-induced denaturation (Fig. 2). For both the enzymes, the loss in secondary structure appeared to be sigmoidal. However, the denaturation curve observed for MtbMS was significantly steeper as compared to that observed for ecMS suggesting that MtbMS was more susceptible to urea denaturation than ecMS. This is evident from the Cm value associated with the urea denaturation of the two enzymes MtbMS (2 M) and ecMS (4.0 M), respectively.

2.4. GdnHCl-induced unfolding

For MtbMS, a sigmoidal loss of the CD signal at 222 nm was observed between 1.0 and 3.0 M GdnHCl, with Cm of about 1.0 M (Fig. 3A). This demonstrates that MtbMS undergoes GdnHCl-induced two-state unfolding. However, for ecMS, the loss of secondary structure monitored by CD appeared to be biphasic. The first phase of unfolding of ecMS was a sharp decrease in CD sig-