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Structural and thermodynamic properties of kappa class glutathione transferase from *Camelus dromedarius*

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ABSTRACT

The Arabian camel, *Camelus dromedarius* is naturally adapted to extreme desert climate and has evolved protective mechanisms to limit oxidative stress. The mitochondrial kappa class glutathione transferase enzyme is a member of GST supergene family that represents an important enzyme group in cellular Phase II detoxification machinery and is involved in the protection against oxidative stress and xenobiotics. In the present study, *C. dromedarius* kappa class glutathione transferase (CdGSTK1-1) was cloned, expressed in *E. coli* BL21, purified and its structural, thermodynamic and unfolding pathway was investigated. The results showed that CdGSTK1-1 has unique trimeric structure, exhibits low thermostability and a complex equilibrium unfolding profile. It unfolds through three folding states with formation of thinly populated intermediate species. The melting points (Tm) of the first unfolding transition was 40.3 ± 0.2 °C and Tm of the second unfolding transition was 49.1 ± 0.1 °C. The van't Hoff enthalpy of the first and second transition were 298.7 ± 13.2 and 616.5 ± 2.4 kJ/mol, respectively. Moreover, intrinsic fluorescence and near-UV CD studies indicates that substrate binding does not leads to major conformational changes in CdGSTK1-1.

1. Introduction

The one-humped camel (*C. dromedarius*) lives most of its life under high environmental stress due to the intense heat and dryness in the Arabian Desert. It is exposed to many intrinsic and extrinsic factors that are known to cause damage of important biomolecules such as DNA, proteins and enzymes. Generally, living organisms have evolved many strategies to protect themselves against xenobiotics and reactive oxygen species (ROS). One of the most powerful mechanism is achieved by the contribution of phase II detoxification enzyme system. This internal system works with many types of enzyme classes such as glutathione S-transferases (GSTs; EC 2.5.1.18). GSTs belong to a widely distributed, highly conserved multigene family complex that found in all living organisms. It is classified into over increasing classes based on their subcellular localization, amino acid sequence, 3D structure, substrate specificity and immunological identity. In mammals, nine classes were identified: alpha, mu, Pi, omega, theta, sigma, zeta, kappa and microsomal GST [1,2].

The biochemical and physiological importance of GSTs relies on their catalytic ability to conjugate the tripeptide glutathione (γGlu-Cys-Gly; GSH) to a wide variety of deleterious compounds, including products of oxidative stress, chemical carcinogens and other electrophile compounds [3,4] rendering them water-soluble

Abbreviations: Amp, ampicillin; *Cd*GSTK1-1, kappa class glutathione transferase; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FPLC, fast protein liquid hromatography; IPTG, Isopropyl β-D-1-thiogalactopyranoside; LB, Luria-Bertani; Ni-NTA, nickel-nitrilotriacetic acid; OD_{600}, optical density at 600 nm; PMSF, phenylmethylsulfonyl fluoride; rpm, rotation per minute.

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and less toxic that easily excluded from the cell through membrane-based glutathione conjugate pumps [5,6].

The kappa GST is an ancient mitochondrial enzyme with orthologues in bacteria and eukaryotes. It was isolated for the first time from the rat liver mitochondria by Harris et al., [7]. Beside the mitochondria as a main site for kappa GST, it has recently been identified in the peroxisomes [8–10] and in the endoplasmic reticulum of adipose tissue [11,12].

Kappa GSTs exhibit structural, functional and catalytic differences compared with other soluble GSTs and it originates from a separate evolutionary pathway [13,14]. These enzymes show high transferase activity as well as peroxidase activity and are able to detoxify peroxides and ROS generated from lipid metabolism and from the respiratory chain in the mitochondria [9,15]. Recently, new physiological roles have been assigned to kappa GSTs. For example, the human enzyme serves as a chaperone to facilitate the assembly and folding of adiponectin [11,16,17]. In addition, the polymorphism in the human GSTK1 promoter is correlated with obesity, insulin secretion and fat deposition [18].

The aim of the present work is to purify *C. dromedarius* GSTK1-1 expressed in *E. coli* and elucidate its structural, thermodynamic and unfolding pathway. We have used analytical gel filtration for quaternary structure determination and spectroscopic techniques (intrinsic fluorescence and near-UV CD measurements) for investigating conformational changes upon substrate binding. We have used dynamic multimode spectroscopic method, an information rich technique which elucidates spectroscopic as well as thermodynamic properties of polypeptides. It can identify the presence of intermediate species along the protein unfolding pathway [19–21].

2. Materials and methods

2.1. Chemicals and instruments

The ORF of CdGSTK1-1 was cloned on pET30a vector [22] and *E. coli* BL21 (DE3)pLysS was the expression host. Superdex 75, low molecular weight protein markers and prepacked columns were from Amersham Biosciences. Kanamycin and IPTG was purchased from Biobasic. Chicken egg lysozyme was obtained from USB Corporation, Benzonase was from Sigma. Ni–NTA agarose was from Qiagen. All other chemicals used in this study were of reagent grade. Ultrospec 2100 pro Spectrophotometer, AKTA purification system, SDS-PAGE assembly was from Amersham Biosciences. Thermomixer and benchtop cooling centrifuge was from Eppendorf. Shaking incubator from Jeio Tech, South Korea, gel scanner from Epson and pH meter was from Sentron. Chirascan-Plus spectropolarimeter from Applied photophysics, UK.

2.2. Expression of cdGSTK1-1 *in* E. coli *BL21(DE3)pLysS*

E. coli BL21 (DE3)pLysS was used for expression of CdGSTK1-1 pET30a-cGSTkappa plasmid was used to transform *E. coli* BL21(DE3)pLysS competent cells [23,24]. Throughout this study, 200 μg/mL kanamycin was used in both solid and liquid medium to maintain plasmid. Single isolated colony of *E. coli* BL21 (DE3)pLysS strain harboring pET30a-cGSTkappa plasmid was inoculated into 20 ml LBkan and grown overnight in shaking incubator at 37 °C. Pre-inoculum 1% (v/v) was inoculated into 1L LBkan and the culture was grown at 37 °C until OD600 was reached 0.8. The expression of CdGSTK1-1 was induced with 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG) as described [25]. After 3 h of induction at 37 °C, culture was harvested at 5000 rpm for 30 min and wet biomass was stored at −80 °C.

2.3. Soluble protein extraction and cdGSTK1-1 purification

E. coli BL21(DE3)pLysS biomass (3 g) was resuspended in 30 mL of lysis buffer (25 mM Tris, pH 8.0 containing 100 mM NaCl, 1 mM PMSF, 5 mM MgCl2, 1 μL benzonase, 5 mM DTT, 20% v/v glycerol and 0.5 mg/mL lysozyme) and incubated on ice for 20 min. The slurry was sonicated three times for 10 s at 5 μm amplitude on ice [Soniprep 150, MSE (UK) Ltd]. The crude lysate was then centrifuged at 13,000 rpm for 30 min at 4 °C. Supernatant was further passed through 0.45 μm filter. In the filtered soluble protein extract, 500 mM sodium chloride and 10 mM imidazole was added. The soluble protein extract was passed (1 mL/min) through 5 mL Ni-NTA agarose pre-equilibrated with 50 mM Tris-HCl, pH 8.0, containing 500 mM NaCl, 5 mM DTT, 20% glycerol and 10 mM imidazole. The column was connected to AKTA purification system and run at 4 °C. Column washing was done extensively with cold equilibration buffer until absorbance at 280 nm reached basal level. Wash fractions also collected. The bound protein was eluted with 0–50% (w/v) imidazole gradient (elution buffer: equilibration buffer containing 500 mM imidazole). Wash, flow-through and eluted fractions were collected on ice. The fractions with high GST activity were pooled and analyzed by 4–20% (w/v) gradient SDS-PAGE (genscript). The Ni-NTA pooled fractions containing CdGSTK1-1 was further purified on Superdex 75 size-exclusion column. Ni-NTA pooled fractions was loaded onto Superdex 75 column 26/600 using superloop, connected with AKTA FPLC. The column was pre-equilibrated with cold 20 mM phosphate buffer, pH 7.0 containing 100 mM NaCl, 1 mM DTT and 20% v/v glycerol and run isocratically using the same buffer. All the purification steps were achieved under cold condition. The purity of eluted fractions was analyzed on 4–20% gradient SDS-PAGE. Pure fractions were pooled and buffer exchange was done with Centricon centrifugal filter

2.4. Quaternary structure characterization

Analytical gel filtration was performed using HiLoad 16/600 Superdex 200 prep grade prepacked XK columns which was pre-equilibrated with cold 20 mM phosphate buffer, pH 7.0 containing 100 mM NaCl, 1 mM DTT and 20% v/v glycerol. The column was calibrated with five proteins of different molecular weight (ferritin, 440 kDa; aldolase, 158 kDa; conalbumin, 75 kDa, ovalbumin 43 kDa and ribonuclease, 13.7 kDa). The Superdex 75 purified CdGSTK1-1 was loaded on Superdex 200 using superloop, connected with AKTA FPLC and run isocratically. The molecular weight of CdGSTK1-1 was calculated using standard curve of elution profile of standard proteins

2.5. Protein quantification

Gel filtration fractions containing pure CdGSTK1-1 were pooled and total protein was estimated by Bradford method [26].

2.6. Fluorescence spectroscopy

Fluorescence spectra of CdGSTK1-1 were recorded with a Cary Eclipse Fluorescence Spectrophotometer in a 10 mm path length quartz cell. 0.35 mg/mL protein was used for the studies. The CdGSTK1-1 samples in the presence and absence of 1 mM GSH were excited at 280 nm and the excitation and emission slits were kept 5 nm. The spectra was recorded at 25 °C in the wavelength range of 300–400 nm.

2.7. Circular dichroism

Chirascan-Plus CD spectrophotometer (Applied Photophysics, Leatherhead, UK) was used for near- and far-UV Circular