Available online at www.sciencedirect.com
ScienceDirect
Protein Expression and Purification 59 (2008) 153–160
Protein Expression & Purification
www.elsevier.com/locate/yprep

Cloning, expression, purification and characterization of recombinant glutathione-*S*-transferase from *Xylella fastidiosa*

Regiane F. Travensolo [a,1], Wanius Garcia [b,1], João R.C. Muniz [b], Célia S. Caruso [a], Eliana G.M. Lemos [c], Emanuel Carrilho [a], Ana P.U. Araújo [b,*]

[a] *Laboratório de Bioanalítica, Microfabricação e Separações, Instituto de Química de São Carlos (USP), São Carlos, Brazil*
[b] *Laboratório de Biofísica Molecular "Sérgio Mascarenhas", Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, 13560-970 São Carlos, SP, Brazil*
[c] *Laboratório de Bioquímica de Microrganismos e Plantas, Universidade Estadual Paulista (UNESP), Jaboticabal, Brazil*

Received 8 October 2007, and in revised form 18 January 2008
Available online 5 February 2008

Abstract

Xylella fastidiosa is an important pathogen bacterium transmitted by xylem-feedings leafhoppers that colonizes the xylem of plants and causes diseases on several important crops including citrus variegated chlorosis (CVC) in orange and lime trees. Glutathione-*S*-transferases (GST) form a group of multifunctional isoenzymes that catalyzes both glutathione (GSH)-dependent conjugation and reduction reactions involved in the cellular detoxification of xenobiotic and endobiotic compounds. GSTs are the major detoxification enzymes found in the intracellular space and mainly in the cytosol from prokaryotes to mammals, and may be involved in the regulation of stress-activated signals by suppressing apoptosis signal-regulating kinase 1. In this study, we describe the cloning of the glutathione-*S*-transferase from *X. fastidiosa* into pET-28a(+) vector, its expression in *Escherichia coli*, purification and initial structural characterization. The purification of recombinant *xf*GST (r*xf*GST) to near homogeneity was achieved using affinity chromatography and size-exclusion chromatography (SEC). SEC demonstrated that r*xf*GST is a homodimer in solution. The secondary and tertiary structures of recombinant protein were analyzed by circular dichroism and fluorescence spectroscopy, respectively. The enzyme was assayed for activity and the results taken together indicated that r*xf*GST is a stable molecule, correctly folded, and highly active. Several members of the GST family have been extensively studied. However, *xf*GST is part of a less-studied subfamily which yet has not been structurally and biochemically characterized. In addition, these studies should provide a useful basis for future studies and biotechnological approaches of r*xf*GST.

Keywords: *Xylella fastidiosa*; Glutathione-*S*-transferase (GST); Detoxification enzymes; Circular dichroism spectroscopy (CD); Fluorescence spectroscopy

Xylella fastidiosa (*X. fastidiosa*) [1] belongs to the gram-negative group, restricted to the xylem vessels of its host plants and has proven to be the causal agent of many plant disease including alfalfa, almond, blackberry, citrus, coffee, grape, peach, pear, plum and some ornamental plants [2]. Recently, a consortium of researchers in Brazil published the sequence of the *X. fastidiosa* isolated 9a5c genome with 2905 genes, from which half were similar to proteins with unknown proteins functions [3]. The understanding of complete genome sequences together with functional studies such as transcriptomics and proteomics were a great advancement towards the comprehension of the metabolic and replicative pathways. Several papers have explored the information generated by the genomic sequencing, highlighting a series of hypothesis related to the functioning of the energetic metabolism, nutrient transport, adherence, aggregation, toxicity, pathogenicity factors, secretion, intercellular interactions, iron homeostasis, and antioxi-

* Corresponding author.
E-mail address: anapaula@if.sc.usp.br (A.P.U. Araújo).
[1] These authors contributed equally to this work.

1046-5928/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2008.01.017

dant responses [3–9]. Additionally, the technology of microarrays provides a way to monitor the expression of several genes simultaneously. A nearly complete collection of *X. fastidiosa* open reading frames (ORFs)[2] was produced to analyze the expression ratio of the bacterium when present in disease plants. In the host, the bacteria presented some upregulated genes related to energy, protein, amino acids, and RNA metabolism, transport, detoxication or antioxidants, and hypothetical proteins, among many others.

From genes belonging to the group of antioxidants found and expressed in plants by *X. fastidiosa*, the XF1210 ORF that codes for the glutathione-*S*-transferase enzyme (GST: EC 2.5.1.18) with 205 amino acids (22.7 kDa) was chosen for this study due to its specific role in biodegradative metabolism. Glutathione-*S*-transferases form a group of multifunctional isoenzymes that catalyzes both glutathione (GSH)-dependent conjugation and reduction reactions involved in the cellular detoxification of xenobiotic and endobiotic compounds such as hormones, heme, bilirubin, drugs, and pesticides which often impairs the catalytic activity of enzymes [10–12]. GSTs are the major detoxification enzymes found in the intracellular space and mainly in the cytosol from prokaryotes to mammals. Other studies have demonstrated that certain GSTs may be involved in the regulation of stress-activated signals by suppressing apoptosis signal-regulating kinase 1 [13].

GSTs are normally dimeric enzymes (molecular mass approximately 50 kDa) that show eight independent classes to mammalian GSTs: Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta on the basis of N-terminal amino acid sequence similarity, substrate specificity and immunological cross reactivity [14–20]. To date, novel classes of non-mammalian GSTs such as Beta, Delta, Epsilon, U, Lambda, Phi, and Tau have been isolated and characterized from bacteria, insects, and plants [21].

Over the last years GST research has focused on resistance phenomenon in insect strains [11] and multidrug-resistant tumor cells [10]. GSTs have emerged as promising therapeutic targets due to their isoenzyme-specific overexpression in different tumors and may play a role in the etiology of other diseases [22].

GSTs have been studied in bacteria such as *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas*, and *Streptomyces griseus* [23–26]. However, the majority of prokaryotic GSTs enzymes have been shown to possess peroxidase activity, to act as epoxide thiolase and to catalyse reactions in the metabolism of exogenous compounds (gentisate, dichloromethane, lignin, and tetrachlorohydroquinone) [27]. The *X. fastidiosa* GST sequence revealed highest sequence similarity with *Haemophilus influenzae* GST (gene *bph*H) [3] but very little is know about the relationship of bacterial GST with their host.

In this study, we describe the cloning procedure and present an expression and purification system capable of providing a large-scale quantity of recombinant functional GST from *X. fastidiosa* (r*xf*GST). The cDNA encoding the *xf*GST was amplified and cloned into the expression vector pET-28a(+). The recombinant protein was expressed at high levels in BL21(DE3) and subsequently purified using affinity chromatography. To confirm its native conformation the activity assay was performed together with circular dichroism and fluorescence spectroscopy.

Materials and methods

Materials

The reagents for PCR and oligonucleotide primers were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). The bacterial expression pET-28a(+) was purchased from Novagen (Madison, WI, USA). Restriction endonucleases, isopropyl thio-β-D-galactopyranoside (IPTG), and T4 DNA ligase were obtained from Invitrogen. Proteins standard used as SDS–PAGE markers were from Sigma Chemical (St. Louis, MO, USA). For the enzyme assay, the activity of the GST was detected using GST detection module kit from GE Healthcare (Piscataway, NJ, USA). All chemicals used were of analytical grade.

Cloning and expression vector construction

Recombinant DNA techniques were performed using conventional protocols. The *gst* gene of *X. fastidiosa* (ORF 1210) was amplified using: forward 5′-CGCATATGAA GTTGTACATCATGCCAGGCGCTTGCTC-3′ and reverse primer 5′-GGAATTCTTATCAGATCAGCCCTTCCGC CTGTAATGC-3′. The amplified DNA encoding the GST produced a fragment of around 620 pb. Transformants *E. coli* DH5α were confirmed by restriction analysis and the plasmid pGEM-T Easy (Promega, Madison, WI, USA) was sequenced by dideoxy chain termination method [28] using an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequenced data were analyzed using BLAST—Basic local alignment search tools program [29] through the network service of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

The DNA encoding the GST was then subcloned into vector pET-28a(+) in NdeI and EcoRI sites which was used to transform *E. coli* DH5α. The new vector construct was named pET–GST. Sequencing of the cloned vectors revealed the open reading frame (ORF 1210) of the *xf*GST plus the expected 17 additional amino acid residues derived from pET-28a(+) vector at its amino terminus (MGSSH HHHHHSSGLVPRGSH), including the cluster of six histidine residues for protein purification by metal affinity

[2] *Abbreviations used:* ORFs, open reading frames; GST, glutathione-*S*-transferase enzyme; IPTG, isopropyl thio-β-D-galactopyranoside; SEC, size-exclusion chromatography; CD, circular dichroism; CDNB, 1-chloro-2,4-dinitrobenzene; r*xf*GST, recombinant *xf*GST.