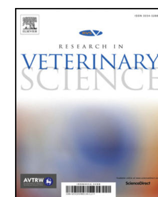
Research in Veterinary Science 111 (2017) 81–84

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc



Identification and characterization of a gamma-interferon-inducible lysosomal thiol reductase homolog from guinea pig (*Cavia porcellus*) that exhibits thiol reductase activity *in vitro*

Lei Ma [a,b], Fang Cao [a], Runze Tang [a], Jiaxin Zhang [c,*], Shuangquan Zhang [a,*]

[a] Jiangsu Province Key Laboratory for Molecular and Medical Biotechnology, Life Sciences College, Nanjing Normal University, Nanjing 210046, China
[b] Qilu Institute of Pharmaceutical Research, Qilu Pharmaceutical Co. Ltd, Jinan 250100, China
[c] Co-Innovation Center for Sustainable Forestry in Southern China, Key Laboratory of Forest Genetics & Biotechnology, Ministry of Education, Nanjing Forestry University, Nanjing 210037, China

ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 21 October 2016
Accepted 25 December 2016
Available online xxxx

Keywords:
Guinea pig
Gamma-interferon-inducible lysosomal thiol reductase
Immune response
Thiol reductase activity

ABSTRACT

Gamma-interferon-inducible lysosomal thiol reductase (GILT) is a key enzyme in the antigen processing and presentation pathway whereby it reduces disulfide bonds at an acidic pH. In this study, a homolog of GILT from guinea pigs (designated gpGILT) was identified and characterized using bioinformatic methods and bioactivity assays. The open reading frame of gpGILT is 705 bp in length and encodes 234 amino acids, with a putative molecular weight of about 25.85 kDa. The structure of gpGILT is similar to those of humans and zebrafish, containing six introns and seven exons. The deduced primary structure of the gpGILT protein includes all of the typical features of other known GILT proteins, including an active-site motif, CXXC, a GILT signature sequence, $CQHGX_2ECX_2NX_4C$, three potential Asn-linked glycosylation sites, and six other conserved cysteines. The predicted tertiary structures of gpGILT, human GILT, and mouse GILT are quite similar in shape and positional arrangement of the key motifs modeled on the same template. Amino acid sequence-based alignment and phylogenetic analysis showed that gpGILT is most closely related to that from the rat, with an identity of 68.40%. Additionally, the constitutive expression and immune response to lipopolysaccharide (LPS) challenge of gpGILT were tested using real-time quantitative polymerase chain reaction. A tissue-specific expression pattern in selected tissues and remarkable up-regulation of gpGILT mRNA in spleen and blood within 12 h of LPS stimulation were observed, suggesting that GILT functions as an immunological surveillance-related factor in both innate and adaptive immunity. Soluble recombinant gpGILT produced in *E. coli* could reduce the interchain disulfide bonds of IgG in an acidic reaction system *in vitro*, suggesting thiol reductase activity in antigen processing. The results of this study provide a better understanding of the molecular characteristics of gpGILT and are a useful reference for further investigation of its involvement in antigen processing and immunological surveillance using the laboratory guinea pig.

© 2016 Elsevier Ltd. All rights reserved.

Antigen processing and presentation by major histocompatibility complex (MHC) class I and II molecules are essential for immune responses in mammals (Kaufman, 2013). In this process, exogenous antigens are denatured, unfolded, and degraded, generating MHC-II-binding molecules in the endosomal/lysosomal system (Liu et al., 2007). The disulfide bonds in such antigens can be reduced to facilitate subsequent proteolysis by a critical molecule, gamma-interferon-inducible lysosomal thiol reductase (GILT), which is involved in the early phagosomal proteolytic events and steps in antigen processing (Balce et al., 2014; Phan et al., 2000). Evidence has shown that mice lacking GILT are deficient in generating MHC-II-restricted $CD4^+$ T-cell responses to protein antigens that contain disulfide bonds (Maric et al., 2001). Moreover, GILT plays a fundamental role in fibroblast proliferation *via* its influence on mitochondrial manganese superoxide dismutase protein activity and expression, and affects protein stabilization (Bogunovic et al., 2008). Recent studies have shown that GILT is directly involved in maintaining the activity of cathepsin S within the phagosome of macrophages under particular redox conditions (Balce et al., 2014). Guinea pigs, an important experimental animal, are widely used in the study of human diseases and comparative biology (Guo et al., 2012). In this study, gpGILT was identified and characterized using bioinformatics and a thiol reductase activity assay. The results presented herein will be useful for further structural biology research on GILT proteins.

Guinea pig peripheral blood mononuclear cells (PBMCs) and splenocytes were obtained by density centrifugation on Ficoll-Paque under sterile conditions. Cells cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) and 100 U/ml penicillin/streptomycin

* Corresponding authors.
E-mail addresses: zhangjiaxin60633@163.com (J. Zhang), zhangshuangquan@njnu.edu.cn (S. Zhang).

http://dx.doi.org/10.1016/j.rvsc.2016.12.006
0034-5288/© 2016 Elsevier Ltd. All rights reserved.

were exposed to 10 μg/ml LPS (*Escherichia coli* type 0111: B4; Sigma, St. Louis, MO, USA) for 0, 2, 4, 8, and 12 h for further assays. Total RNA from freshly collected heart, liver, spleen, lung, kidney, intestine, and the cells mentioned above was isolated, and first-strand cDNA was synthesized from 1 μg of RNA. To obtain the coding sequence, specific primers, gpGILT-F (ATGGCCCTGCCTTCCCGTGT) and gpGILT-R (TCATTTGAAGC AGACTTTCCTGGGGGTG), were used. The polymerase chain reaction (PCR) cycling conditions were as follows: one cycle at 94 °C for 5 min; 30 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; and then a final elongation step at 72 °C for 7 min. Purified PCR products were cloned into the pMD19-T vector and sequenced. We ultimately obtained a 705-bp ORF (accession number KF731758) encoding a protein of 234 amino acids with a calculated molecular mass of approximately 25.85 kDa and a theoretical pI of 5.33, namely, guinea pig GILT. This protein is highly homologous to other mammalian GILTs that contain the putative signal peptide, the CXXC active-site, the GILT signature sequence, potential Asn-linked glycosylation sites, and conserved cysteine residues, which were determined as described in previous reports (Ai et al., 2011) (Fig. S1). Genomic structure analysis showed that six introns separated the gpGILT gene into seven exons, exhibiting similar exon-intron organization to that in other mammals and zebrafish (Fig. S1). Despite some variation at the primary structure level, the active-site and signature sequence in the predicted gpGILT, human GILT (hGILT), and mouse GILT (mGILT) can be recognized at equivalent locations based on the fold recognition method using the same template (PBD: 3GHA) (Fig. 1A). Collectively, these results demonstrate the similar presumed mechanisms of action of the various GILTs, suggesting a parallel biological function of GILT *in vivo*. However, the crystal structure and biophysical properties of GILT have not been revealed. There is an urgent need to study the structural biology of this enzyme to reveal the molecular mechanisms involved and the optimal parameters to effectively express gpGILT, as shown in our work. Phylogenetic analysis among different species showed that all of the sequences selected grouped into two well-defined principal clusters of vertebrates and invertebrates, and could be further divided into three clusters: the cluster belonging to the phylum Chordata, the cluster belonging to the phylum Mollusca, and the rest belonging to the phylum Arthropoda (Fig. 1B), indicating significant divergence of GILT during the course of evolution. The species in the phylum Chordata could then be subdivided into four subclusters: mammal, fish, and chicken forming an independent cluster with green sea turtle, and African clawed frog forming a single independent cluster. gpGILT was located in the mammal cluster within the phylum Chordata and was most closely related to that of the rat, suggesting that gpGILT is derived from the same ancestor as other vertebrate GILTs. Moreover, the gpGILT amino acid sequence has the highest identity (69.57%) to black flying fox and shares 23.60–68.40% identity with other GILTs. The positions of these species in taxonomy decline along with the diminution of amino acid identity, which suggests that the amino acid identity may indicate the level of evolution. When all of the GILT amino acid sequences derived from different species listed in Table S1 were analyzed, we found that the cysteine residues in the CXXC motif were not completely conserved. The CXXS or CXXT active-site sequence motif is recognized and exists mostly in lower animals or some plants.

The different expressions of immune-related genes may reflect the functions of their protein products in the immune system. To reveal the expression level of gpGILT mRNA in selected tissues, we employed quantitative real-time PCR (qRT-PCR). Primer pairs gpGILT-QF (ACCTGGCTAATGGTGATGG) and pGILT-QR (AGGAAGGCGGCGTTTGG) were designed to amplify a PCR product of 176 bp. Primer pairs gpGAPDH-F (GTCGGTTGTGGATCTGACCT) and gpGAPDH-R (TGCT GTAGCCGAACTCATTG) were used to obtain a 245-bp fragment of cDNA from the guinea pig. GAPDH served as an internal control. Diethylpyrocarbonate-treated water instead of the cDNA template was used as a negative control. qRT-PCR using SYBR Premix Ex Taq was performed as described in detail elsewhere (Ai et al., 2011). The results showed that gpGILT is expressed in a tissue-specific manner. Prominent expression of gpGILT mRNA could be observed in the spleen (7.83-fold) and blood (6.97-fold), with lower expression levels in the lung (2.94-fold), intestine (1.82-fold), liver (1.35-fold), and kidney (1.07-fold), and the lowest in the heart. The high-level constitutive expression of gpGILT mRNA in immune organs suggested that gpGILT may be involved in innate immunity (Fig. 2A). Consequently, we further analyzed gpGILT in the spleen and blood for its involvement in immune responses at different time points within 12 h based on the fact that LPS from Gram-negative bacteria is a mitogen that induces GILT gene expression in mammals (Arunachalam et al., 2000; Luster et al., 1988; Srinivasan and Maric, 2011). Our findings revealed a trend of

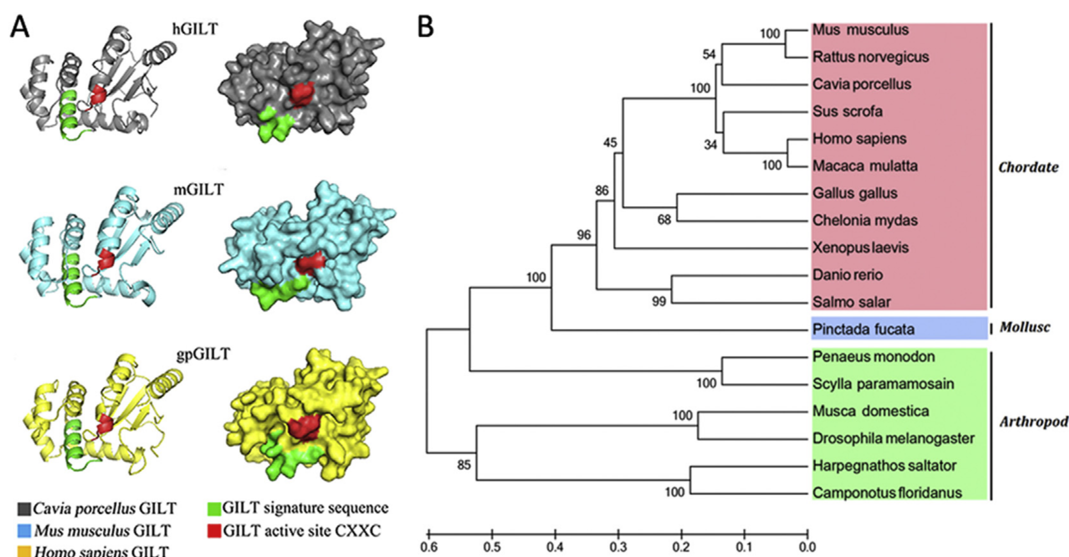


Fig. 1. (A) Fold recognition-based molecular modeling of hGILT, mGILT, and gpGILT monomers based on the crystal structures of disulfide oxidoreductase BdbD (PDB: 3GHA). Molecular structures of hGILT, mGILT, and gpGILT monomers are presented in gray, blue, and yellow, respectively. The GILT signature sequence (green) and the active-site motif CXXC (red) are highlighted in both the cartoon form (left panel) and the surface representation (right panel). (B) Phylogenetic analysis of GILT from 18 representative species. A neighbor-joining tree was constructed using MEGA6 software. The numbers above the branches represent the confidence level from 1000 bootstrap replications. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Download English Version:

https://daneshyari.com/en/article/5544021

Download Persian Version:

https://daneshyari.com/article/5544021

Daneshyari.com