DOI:10.13350/j.cjpb.230610

·论著·

粉尘螨谷氧还蛋白-1样蛋白基因原核表达质粒的构建及结构分析*

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【摘要】 **目的** 获得粉尘螨谷氧还蛋白-1样蛋白(Glutaredoxin-1,Grx1)的编码基因并构建表达质粒,对该基因及其编码蛋白质进行结构预测。 **方法** 以粉尘螨总RNA为模板,RT-PCR扩增获得编码基因后插入原核表达载体pET28a(+),构建的重组质粒转化入*E. coil* BL21(DE3)感受态细胞中,用异丙基-b-D-硫代半乳糖苷(isopropyl-beta-D-thiogalactoside,IPTG)诱导表达,SDS-PAGE分析表达产物。采用生物信息学软件分析该基因及其编码蛋白的结构等生物学特征。 **结果** 获得的粉尘螨Grx1编码基因全长315 bp,构建的原核表达质粒pET28a(+)-Grx1转化入大肠埃希菌感受态细胞后经IPTG诱导,表达相对分子质量为25.9×10^3的重组蛋白。生物信息学分析该基因编码的蛋白质由104个氨基酸组成,主要分布于细胞核(占87.0%)。该蛋白含有11个潜在磷酸化位点(4个苏氨酸,6个丝氨酸和1个酪氨酸),二级结构为α-螺旋(45.19%)、无规则卷曲(31.73%)、延伸链(14.42%)和β-转角(8.65%)。将该基因推导出的编码氨基酸序列进行Blast获得同源基因与屋尘螨谷胱甘肽样C8样蛋白(*Dermatophagoides pteronyssinus* glutaredoxin-C8-like)同源性最高(83.52%)。 **结论** 获得了粉尘螨Grx1的编码基因及其原核表达质粒,生物信息学分析该蛋白含有潜在的磷酸化位点且主要分布于细胞核,可为该基因的生理功能研究及粉尘螨的防制提供参考。

【关键词】 粉尘螨;谷氧还蛋白-1样蛋白;生物信息学;基因克隆
【中图分类号】 R384.4 【文献标识码】 A 【文章编号】 1673-5234(2023)06-0672-05

[*Journal of Pathogen Biology*. 2023 Jun;18(6):672-676, 682.]

Construction plasmid and Structural analysis of the glutaredoxin-1-like protein gene of *Dermatophagoides farinae*

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【Abstract】 **Objective** To obtain the cDNA coding for the glutaredoxin-1-like protein (Grx1) of *Dermatophagoides farinae* and to characterize that protein. **Method** By using the primers designed according to the sequence for Glrx1 inferred from annotation of *D. farinae* transcriptome data, the cDNA was amplified by RT-PCR from total RNA of *D. farinae* and inserted into pET28a(+), transformed into *E. coil* BL21 (DE3), expressed with the induction of isopropyl-beta-D-thiogalactoside (IPTG) and identified by SDS-PAGE. The structure analyses were conducted by using software and tools online. **Results** The product of amplification with RT-PCR showed a clear band on agarose gel electrophoresis, and nucleotide sequencing of the pET28a(+)-Grx1 plasmid yielded a coding gene 315 bp. Once the plasmid was transformed into *E. coil* and its expression was induced with IPTG, a specific band was produced on SDS-PAGE with a relative molecular mass of 25.9×10^3. Bioinformatic analysis of the protein encoded by this gene consists of 104 amino acids, subcellularly localized to the nucleus (87.0%). The protein contains 11 potential phosphorylation sites, including four Threonine, six Serine and one Tyrosine. And it's advanced structure consisted of random coil (31.73%), α-helix (45.19%), extended chain (14.42%), and β-turn (8.65%). Blast of the deduced amino acid sequence of this gene yielded homologous genes with the highest homology to *D. pteronyssinus* glutaredoxin-C8-like up to 83.52%. **Conclusion** We obtained the coding gene and prokaryotic expression plasmid for Grx1 of *D. farinae*. Bioinformatics analysis showed that this protein contains potential phosphorylation sites that distributed in the nucleus, providing

* 【基金项目】 国家自然科学基金项目(No. 81971511,31572319,31272369);无锡市太湖人才计划高端人才项目(No. 2020THRC-GD-7);无锡市科技局"太湖之光"科技攻关项目(No. Y20212006);无锡市卫生计生科研重大项目(No. Z201701)。

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references for the physiological function research about Grx1 and control measures for *D. farinae*.

【Key words】 *Dermatophagoides farina*; glutaredoxin-1-like protein; bioinformatics; gene clone.

尘螨与过敏性疾病的发生发展具有密切联系，如引起过敏性鼻炎、过敏性哮喘、特应性皮炎等[1-3]。在中度和重度持续性哮喘患者中，粉尘螨(*Dermatophagoides farinae*)的致敏率高达58.5%和77.2%[4]。对特应性皮炎儿童进行皮肤点刺试验，75.4%的受试者屋尘螨(*D. pteronyssinus*)、粉尘螨过敏原阳性或二者均阳性[5]。在我国中部地区儿童和成年人中引起过敏性鼻炎的粉尘螨过敏原致敏率高达91.8%和87.3%[6]。表明粉尘螨是引起过敏性疾病的重要螨种之一。

硫氧还蛋白(Thioredoxin，Trx)系统和谷氧还蛋白(Glutaredoxin，Grx)系统是生物体内两大主要的抗氧化系统，维持着细胞内氧化还原的平衡[7]。1967年Lillig等[8]报道在缺乏硫氧化蛋白的大肠埃希菌变异株中发现Grx。Grx是一种热稳定的二硫化物氧化还原酶，在真核和原核生物中广泛存在，参与多种细胞过程，如代谢、信号转导和免疫防御等[9]，但目前鲜有关于粉尘螨Grx1编码基因信息的报道。作者前期对粉尘螨转录组测序数据进行了基因注释，获得了Grx1样蛋白的编码基因。本研究通过RT-PCR扩增获得该基因，构建原核表达质粒，采用在线分析软件预测分析其生物学特征，为进一步探讨Grx1的生理功能及粉尘螨的防制提供参考。

材料与方法

1 粉尘螨总RNA提取

粉尘螨为本实验室培养。手工挑取粉尘螨，用75%酒精清洗后制备匀浆，使用RNAiso Plus试剂提取总RNA，按说明书步骤操作。

2 目的基因获取

以粉尘螨的总RNA为模板，逆转录获得cDNA(使用3′-Full RACE Core Set with PrimeScriptTM RTase)，同时设立M-MLV(-)对照。随后进行PCR扩增(使用TaKaRa LA Taq® with GC Buffer)。正向引物 F：5′-AATGGGTCGC GGATCC ATGTCTG AACAAA TTAAACA-3′(下划线为BamH I酶切位点)；反向引物 R：5′-ATCTCAGTGGTGGTGGTG-GTGGTGCTCGAGTTATGAAGAAAGTAATTT-GG-3′(下划线为Xho I酶切位点)。

3 表达质粒构建

将目的基因PCR产物和载体连接(使用In-Fusion® HD Cloning Kit)，连接产物热转化至*E. coli* Competent Cells JM109(Code No. 9052)中，涂布平板，37 ℃过夜培养。使用T7 /T7 terminator引物挑选阳性菌落，提取阳性克隆质粒，命名为pET28a(+)-Glrx1。

4 目的蛋白的表达及鉴定

将含有目的基因的质粒pET28a(+)-Grx1转入感受态细胞中，对其进行诱导表达。转化：取2 μL含有目的基因的质粒pET28a(+)-Grx1质粒转入100 μL Competent cell BL21(DE3)T1R感受态细胞中；取35 μL转化液涂布含有Amp的LB(100 μg/mL)平板，37 ℃培养过夜，同时设空载体pET-28a(+)对照。挑取单菌落至2 mL含Amp的LB培养基中，37 ℃培养至菌液浑浊。吸去1 mL种培养后的菌液放入到5 mL LB含有Amp(100 μg/mL)的新培养基中。37 ℃培养至A_{600}值为0.6～0.8，加入100 mmol/L IPTG 50 μL于37 ℃诱导培养4 h，采用SDS-PAGE检测表达的目的蛋白。

5 目的蛋白的生物信息学分析

利用表1中的生物学软件对粉尘螨Grx1蛋白进行氨基酸序列分析并分析和预测蛋白的理化性质及结构。

表1 生物信息学分析软件及用途
Table 1 Bioinformatics websites and tools

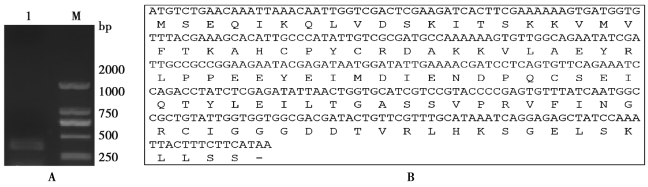
软件名称 Software	网址 Website	用途 Application
ORF Finder	https://www.ncbi.nlm.nih.gov/orffinder/	开放阅读框分析
BLAST	https://blast.ncbi.nlm.nih.gov/Blast.cgi	同源性比对分析
ProtParam	https://web.expasy.org/protparam/	蛋白质理化性质分析
ProtScale	https://web.expasy.org/protscale/	蛋白质亲疏水性分析
TMHMM	http://www.cbs.dtu.dk/services/TMHMM/	蛋白质跨膜结构预测
NetOGlyc1.0	https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0	N-糖基化位点预测
NetOGlyc4.0	https://services.healthtech.dtu.dk/service.php?NetOGlyc-4.0	O-糖基化位点预测
NetPhos3.1	https://services.healthtech.dtu.dk/service.php?NetPhos-3.1	磷酸化位点预测
SWISS-MODEL	https://swissmodel.expasy.org/interactive	蛋白质三级结构预测
SingalP4.1	http://www.cbs.dtu.dk/services/SignalP-4.1/	蛋白质信号肽分析

结 果

1 目的基因克隆

以粉尘螨总RNA为模板进行RT-PCR，经1%琼脂糖凝胶电泳分析，扩增的粉尘螨Grx1编码基因片段约为315 bp，与理论值相符(图1A)。使用NCBI的ORF Finder服务器对该序列进行开放阅读框分析，从起始密码子ATG到终止密码子TAA，编码104个氨基酸(图1B)。利用Bio Edit软件分析序列的碱基组

分，A、C、T 和 G 含量分别为 30.8%、19.7%、27.3% 和 22.2%。



A 粉尘螨 Grx1 编码基因 PCR 产物 1%琼脂糖凝胶电泳分析（M 为 DNA 标志物，DL 2000） 1 粉尘螨 Grx1 编码基因 PCR 产物） B 粉尘螨 Grx1 编码基因核苷酸序列分析

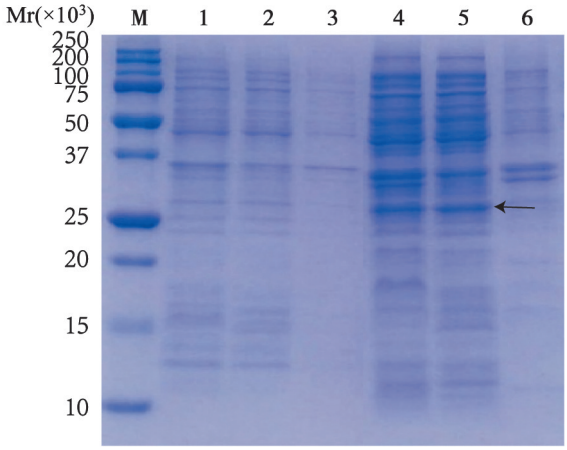
图 1 粉尘螨 Grx1 编码基因 PCR 产物 1%琼脂糖凝胶电泳检测及核苷酸序列分析

A Agarose gel elsctrophores analysis of PCR amplification product of Grx1(M:DNA marker(DL 2000) 1 PCR product) B Nucleotide sequencing of Grxl

Fig. 1 Agarose gel elsctrophores analysis of RT-PCR amplification product of Grx1 B for the total of *D. farinae* and Nucleotide sequencing of Grxl

2 目的蛋白的表达及检测

将含有目的基因的质粒 pET-28a（+）-Grx1 转入 BL21(DE3)感受态细胞中，用 IPTG 对阳性克隆进行诱导表达，SDS-PAGE 分析表达的重组粉尘螨 Grx1 蛋白相对分子质量为 25.9×10^3（图 2），与预期一致。



M 蛋白分子质量标准 1 pET-28(+)转化菌全细胞 2 pET-28(+)转化菌超声破碎上清 3 pET-28(+)转化菌超声破碎沉淀 4 pET-28a(+)-Grx1 全细胞 5 pET-28a(+)-Grx1 转化菌超声破碎上清 6 pET-28a(+)-Grx1 转化菌超声破碎沉淀

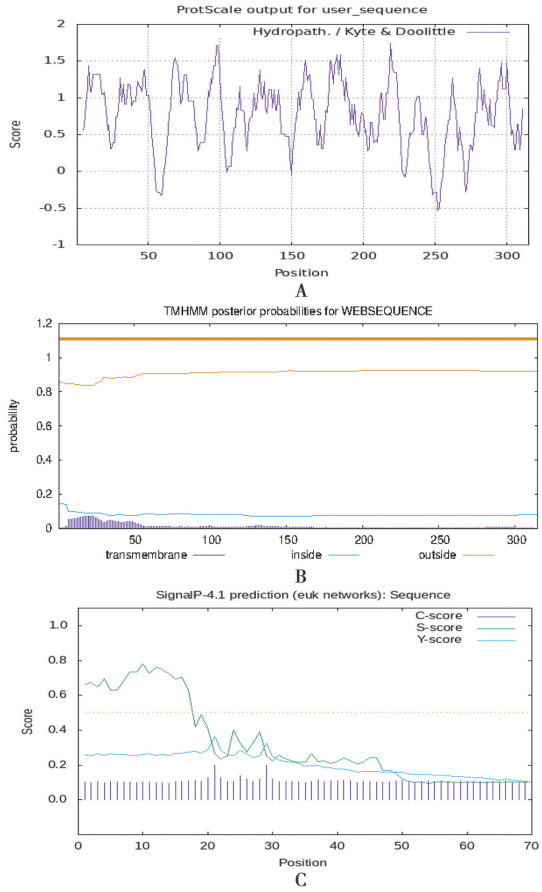
图 2 pET28a(+)-Grx1 转化 DE3 表达产物 SDS-PAGE 分析

M Protein MW marker(Broad) 1 pET-28(+)whole cell 2 pET-28(+)supernatant 3 pET-28(+)precipitation 4 pET-28a(+)-Grx1whole cell 5 pET-28a(+)-Grx1supernatant 6 pET-28a(+)-Grx1 precipitation

Fig. 2 Identification of pET28a(+)-Grx1expression in *E. coil* by SDS-PAGE

3 粉尘螨 Grx1 蛋白的生物信息学分析

3.1 理化性质 ProtParam 软件分析粉尘螨 Grx1 蛋白质的分子式为 $C_{961}H_{1609}N_{315}O_{402}S_{62}$，分子质量为 $25.995\ 94\times10^3$，等电点(pI)为 5.30。其氨基酸组成包括丙氨酸(30.8%)，半胱氨酸(19.7%)，甘氨酸(22.2%)和苏氨酸(27.3%)。脂肪系数(AI)30.79，不确定指数(II)27.09，体外半衰期 4.4 h，总平均亲水指数(GRAVY)0.766，属于疏水性蛋白(图 3A)。信号肽序列预测显示，该蛋白 C-max 为 0.200，Y-max 为 0.363，S-max 为 0.779，不存在信号肽区域，为分泌蛋白(图 3B)。对其跨膜区结构进行分析，该蛋白为膜外蛋白，无跨膜结构，不属于跨膜蛋白质(图 3C)。



A 疏水性分析 B 跨膜结构预测 C 蛋白信号肽预测

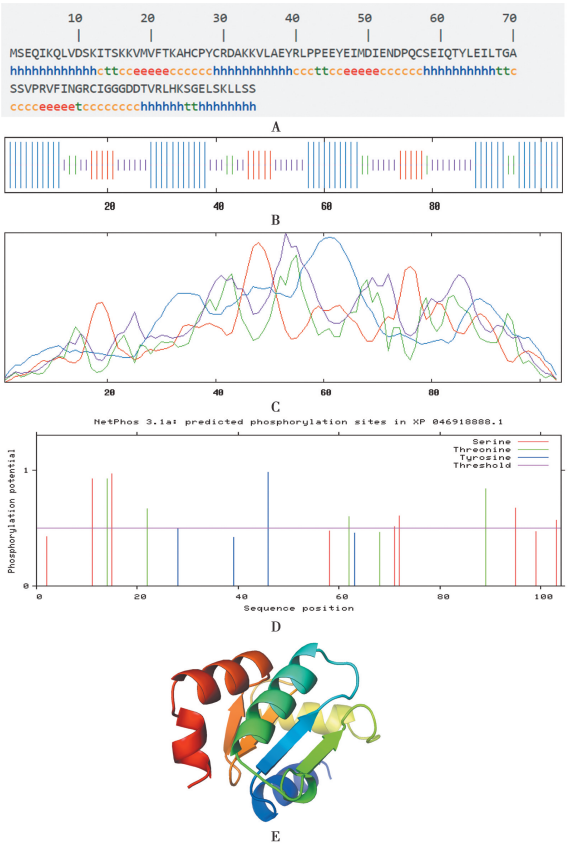
图 3 粉尘螨 Grx1 基因编码蛋白的理化性质分析

A Hydrophobic analysis B Transmembrane domain analysis C SignaIP analysis

Fig. 3 Physicochemical characterization of the protein encoded by the *D. farinae* glutaredoxin-1-like

3.2 蛋白质结构 粉尘螨 Grx1 蛋白质的二级结构由 α-螺旋(45.19%)、无规则卷曲(31.73%)、氨基酸残基连接成的延伸链(14.42%)和 β-转角(8.65%)组成，故该蛋白为混合型蛋白，且主要以 α-螺旋为主(图 4A、B、C)。亚细胞定位预测显示，该蛋白主要集中在细胞核(87.0%)、线粒体(8.7%)和过氧化物酶体(4.3%)。磷酸化位点预测显示，粉尘螨 Grx1 蛋白存在 11 个潜在磷酸化位点，包括 4 个苏氨酸，6 个丝氨酸和 1 个酪氨酸(图 4D)。以粉尘螨 Grx1 蛋白序列为模板，利用 SWISS-MODEL 同源建模预测三级结构，模型质量评估较好，GMQE 值为 0.75(GMQE 为 0-1，数值越接近 1 表明质量越好)；PyMol 可视化显示 5 个

α 螺旋包围 4 个 β 折叠(图 4 E)。NetOGlyc4.0 和 NetOGlyc1.0 预测该蛋白无糖基化位点。



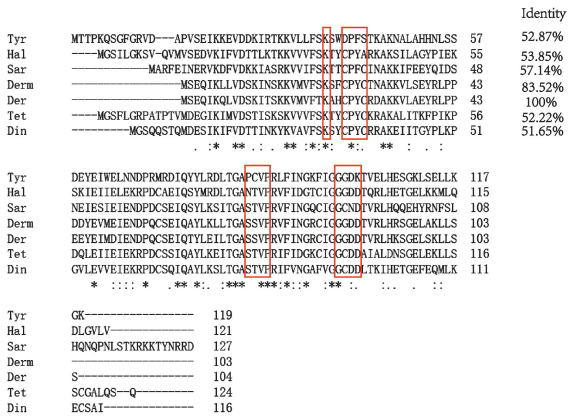
A SOMPA 预测的蛋白二级结构位点图(c:无规则卷曲;t:β-转角;e:延伸链) B SOMPA 分析蛋白质二级结构竖条纹图(蓝色:α-螺旋;紫色:无规则卷曲;绿色:β-转角;红色:延伸链) C SOMPA 分析蛋白质二级结构峰图 D NetPhos3.1 预测蛋白潜在的磷酸化位点 E SWISS-MODEL 预测的蛋白三级结构

图 4 粉尘螨 Grx1 蛋白结构分析

A Protein secondary structure site map (c:random coil;t:β-turn;e:Extended chain) B Protein secondary structure vertical stripe map (blue:α-helix;purple:random coil;green:β-turn;red:Extended chain) C Protein secondary structure peak map D Potential phosphorylation site analysis; E Tertiary structure analysis

Fig. 4 Protein structure analysis of Grx1 from *D. farinae*

3.3 同源性分析及其结构域对比 根据粉尘螨 Grx1 的氨基酸序列进行同源性分析,选取的螨种包括屋尘螨(*D. pteronyssinus*)、疥螨(*Sarcoptes scabiei*)、红足土螨(*Halotydeus destructor*)、二斑叶螨(*Tetranychus urticae*)、染色大绒螨(*Dinothrombium tinctorium*)和腐食酪螨(*Tyrophagus putrescentiae*)。其中与屋尘螨谷胱甘肽样 C8 样蛋白(*D. pteronyssinus* glutaredoxin-C8-like)同源性最高(83.52%)。通过 Cluatal Omega 在线比对与粉尘螨 Grx1 序列的相似性,显示存在 25 个保守氨基酸和 22 个半保守氨基酸(图 5)。CD-Search 分析粉尘螨 Grx1 基因编码蛋白,结果显示粉尘螨 Grx1 氨基酸序列的结构域为 GRX-GRXh-1-2-like,并且具有其典型的保守催化位点(CXXC)和与 GSH 结合的活性位点(SVP,GGD,Gly-Gly)。通过 PyMol 对粉尘螨 Grx1 基因编码蛋白和其他同源序列的催化位点进行可视化,结果如图 6。

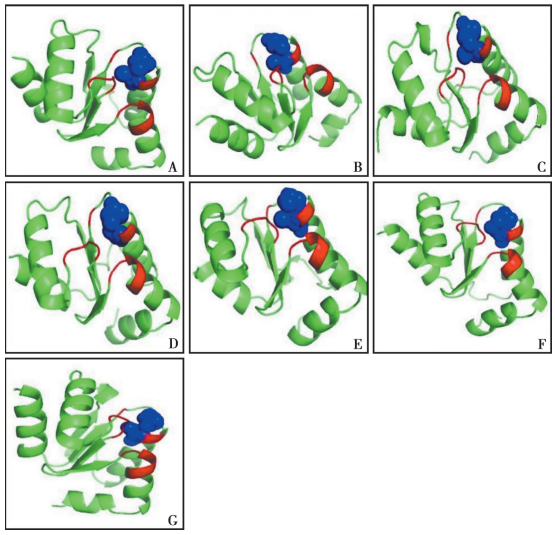


注:Tyr、Hal、Sar、Derm、Der、Tet 和 Din 依次表示腐食酪螨、红足土螨、疥螨、粉尘螨、屋尘螨、二斑叶螨和染色大绒螨(红色框表示与 GSH 的结合位点)。

图 5 粉尘螨 Grx1 与其他螨类同源氨基酸序列的 Clustal Omega 在线比对分析

Notes: Tyr, Hal, Sar, Derm, Der, Tet and Din indicate *Tyrophagus putrescentiae*, *Halotydeus destructor*, *Sarcoptes scabiei*, *D. farinae*, *D. pteronyssinus*, *Tetranychus urticae and D. tinctorium*.

Fig. 5 Comparison of the homologous amino acid sequences of the *Dermatophagoides farinae* Grx1 with other dust mite



A 粉尘螨 B 屋尘螨 C 疥螨 D 红足土螨 E 二斑叶螨 F 染色大绒螨 G 腐食酪螨。其中红色表示 GSH 结合位点,蓝色表示催化残基。

图 6 粉尘螨 Grx1 与其他螨类同源氨基酸序列催化位点的 PyMol 可视化

A *D. farinae* B *D. pteronyssinus* C *Sarcoptes scabiei* D *Halotydeus destructor* E *Tetranychus urticae* F *Dinothrombium tinctorium* G *Tyrophagus putrescentiae*. Red indicate GSH binding site; Blue indicate catalytic residues.

Fig. 6 Visualization of the catalytic site for *D. farinae* Grx1 and other mites

讨 论

Grx1 几乎存在于所有生物,调节细胞内的氧化还原平衡,最终起到抗凋亡、抗氧化等作用[10-11]。目前已

发现两种粉尘螨 Grx 的存在，包括 Grx1（XM_047062932.1）和 Grx3（XM_047052584.1）。Grx1 基因在秀丽隐杆线虫（*Caenorhabditis elegans*，NP_490812.1）、斑马鱼（*Danio rerio*，NP_001005942.1）、黑腹果蝇（*Drosophila melanogaster*，Q9W2D1）、深处古生球菌（*Archaeoglobus venefificus*，WP_013684320.1）、壁虎（*Gekko japonicus*，XP_015283682.1）和原鸡（*Gallus gallus*，NP_990491.1）等均有报道[12]。在梭状芽孢杆菌[13]、疟原虫[14]、细粒棘球绦虫[15]、布氏锥虫[16]和酿酒酵母[17]中已对该基因的结构和动力学进行分析，结果显示硫氧还蛋白家族结构中含有典型的保守催化位点 CXXC 活性位点和与 GSH 的结合位点（TVP、CXD、Lys 和 Gln/Arg），三维建模可观察到 3～5 个 α 螺旋包围 4 个 β 折叠。在孢子虫，Grx 1 基因缺乏会影响其生长[18]。此外，Grx1 在植物中对组织发育具有调控作用[19]。

生物信息学分析粉尘螨 Grx1 编码基因蛋白具有一定疏水性，无信号肽结构，主要分布于细胞核、线粒体和过氧化物酶体。该蛋白存在 11 个潜在磷酸化位点，氨基酸组成中含有半胱氨酸（19.7%）和甘氨酸（22.2%）。因其保守的活性催化中心的氨基酸序列为-Cys-Gly-Pro-Cys-（CXXC），所以氨基酸组成可能与其独有的活性催化位点相关。粉尘螨 Grx1 二级结构预测显示主要由 α-螺旋（45.19%）和无规则卷曲（31.73%）构成。本研究以粉尘螨 Grx1 基因为模板建立了粉尘螨 Grx1 三维空间结构，观察到该结构由 5 个 α 螺旋包围 4 个 β 折叠，与文献报道谷氧还蛋白的结构中 3～5 个 α 螺旋被 4 个 β 折叠所包围相一致[20]。

谷胱甘肽（$^{\gamma}$Glu-Cys-Gly，GSH）存在于细胞中，Grxs 主要依靠 GSH 催化巯基-二硫键转化反应来实现影响细胞的氧化还原状态，并且 Grxs 对与 GSH 相连接的二硫化物具有特异性[21]。谷氧还蛋白的经典活性位点为 CXXC 结构域，该结构主要位于 α 螺旋和 β 折叠的连接处并且通过其中的两个 Cys 催化还原蛋白质的二硫键，其是必不可少的关键催化位点[22]。此外，还具有其他保守位点，如 cis-Proline 的前面氨基酸（TVP）和 GG-motif（GGxdD）[8]。对粉尘螨 Grx1 编码基因进行 Blast 后选出相似度最高的序列为屋尘螨谷胱甘肽样 C8 样蛋白（*D. pteronyssinus* glutaredoxin-C8-like），高达 83.52%，确定未知序列为粉尘螨 Glrx1 的编码基因。此外还找出与其具由较高同源性的 5 种螨类，经 Clustal Omega 在线比对分析发现具有 25 个保守氨基酸，22 个半保守氨基酸。对序列保守位点进行分析，粉尘螨 Grx1 具有 Trx 家族的氧化还原酶蛋白质相似的典型活性催化位点和与 GSH 结合的保守位点，并且三维建模观察到大部分保守的残基位于活性位点的周围，可能与其催化作用相关。同样，同源序列比较出的 7 种螨类同样具有相似的结构组成和作用位点，说明不同螨种间催化位点相对保守。

本研究获得了粉尘螨 Grx1 编码基因及其原核表达质粒，生物信息学分析该蛋白含有潜在的磷酸化位点且主要分布于细胞核，为进一步探讨粉尘螨 Grx1 的生理功能奠定了基础。

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DOI:10.13350/j.cjpb.230611

·论著·

结核分枝杆菌重组 EspB 及 EspBN 蛋白的免疫原性研究*

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【摘要】 **目的** 构建结核分枝杆菌 EspB 及 EspBN 蛋白原核表达系统,表达并纯化重组蛋白,评价两种蛋白的免疫原性。 **方法** 构建 pGEX-4T-1-EspB 及 pGEX-4T-1-EspBN 重组质粒,转化大肠埃希菌,经 ITPG 诱导表达后纯化获得目的蛋白。分别取两种蛋白与等体积弗氏完全佐剂乳化后免疫 BALB/c 小鼠,在初次免疫后第 14、28 d 以相同剂量蛋白和弗氏不完全佐剂加强免疫。试验设 PBS 对照组。每鼠注射 100 μL PBS 溶液与等体积的弗氏不完全佐剂,末次免疫后第 14、28、42 d 小鼠取眼球采血,分离血清,采用 West ern blot 法检测抗血清的特异性,ELISA 法检测血清抗体效价及 IgG 亚型。分离免疫小鼠脾淋巴细胞,体外经相应抗原刺激后,CCK8 法检测脾淋巴细胞增殖水平,ELISA 法检测培养液上清中 IL-4、INF-γ 水平。 **结果** 成功构建了高效原核表达重组质粒 pGEX-4T-1-EspB 及 pGEX-4T-1-EspBN。重组质粒转化 DE3 后经 IPTG 诱导,稳定表达 EspB 融合蛋白和 EspBN 融合蛋白。分别用两种蛋白免疫小鼠,制备的抗血清均具有特异性,抗 EspB 蛋白血清与抗 EspBN 血清的总 IgG 及各亚型 IgG 效价均高于 PBS 组(均 $P<0.05$)。EspB 组和 EspBN 组脾淋巴细胞增殖刺激指数均高于 PBS 组(q 值分别为 7.17 和 3.47,均 $P<0.05$)。免疫小鼠淋巴细胞经抗原刺激后 IL-4 释放水平均呈降低趋势,INF-γ 释放水平均呈升高趋势(均 $P<0.05$)。 **结论** 结核分枝杆菌 EspB 蛋白及 EspBN 蛋白均具有较强的免疫原性,为进一步分析其动物免疫保护效应等研究奠定了基础。

【关键词】 结核分枝杆菌;ESAT-6 系统-1 分泌蛋白 B;免疫原性
【中图分类号】 R378.911 【文献标识码】 A 【文章编号】 1673-5234(2023)06-0677-06

[*Journal of Pathogen Biology*. 2023 Jun;18(6):677-682.]

Immunogenicity of recombinant EspB and EspBN proteins of *Mycobacterium tuberculosis*

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【Abstract】 **Objective** To construct the prokaryotic expression system of *Mycobacterium tuberculosis* EspB protein and EspBN protein, purify the recombinant proteins and evaluate the immunogenicity of the proteins. **Methods** The genomic DNA of EspB and EspBN were amplified by PCR and cloned into prokaryotic expression vector. The fusion proteins were induced by IPTG in *E. coli* BL21 (DE3) strains, purified by GST labeled protein purification kit and excised the GST labeled proteins to obtain the target proteins. BALB/c mice were immunized with two kinds of protein mixed with equal volume of Freund's incomplete adjuvant respectively. The same dose of protein and Freund's incomplete adjuvant were used to enhance the immunity on the 14th and 28th day after the first immunization. The mice in control group were only injected with 100 μL PBS solution and equal volume of Freund's incomplete adjuvant. On the 14th, 28th and 42th day after the last immunization, the eyeball blood was collected to prepare the antiserum. The specificity of each antiserum was detected by Western Blot, and the titer and IgG subtype of each antiserum were detected by ELISA. The proliferation of splenic lymphocytes was detected by CCK8 method, and the levels of IL-4 and INF-γ were detected by ELISA. **Results** The recombinant plasmids pGEX-4T-1-EspB and pGEX-4T-1-EspBN were successfully constructed, induced by IPTG and stably expressed fusion proteins in *E. coli*. After immunizing mice with the two proteins, the antisera obtained were specific. The total IgG and subtype IgG titers of anti EspB protein serum and anti EspBN protein serum were higher than those in PBS group. On the 28th and 42nd day after the last immunization, the proliferation stimulation index of splenic lymphocytes in EspB group were higher than that in PBS group. (q values were 7.17 and 3.47 respectively, with both $P<0.05$). On the 42nd day after the last immunization, the proliferation stimulation index of splenic lymphocytes in EspBN group were higher than that in PBS group ($q=3.47$, $P<0.05$). On the 42nd day after the last immunization, the proliferation stimulation index of splenic lymphocytes in EspB group were higher than that in EspBN group($q=3.70$, $P<0.05$). The release levels of IL-4 in EspB group and EspBN group showed a decreasing trend,

* 【基金项目】 四川省教育厅自然科学重点项目(No. 13ZA0217)。
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【收稿日期】 2022-12-20 【修回日期】 2023-03-05

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【收稿日期】 2022-12-28 【修回日期】 2023-03-08