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湖北钉螺硫氧还蛋白过氧化物酶全长基因克隆、表达与蛋白活性分析

马宪亮，刘琴，张仪 *

中国疾病预防控制中心寄生虫病预防控制所，卫生部寄生虫病原与媒介生物学重点实验室，世界卫生组织疟疾、血吸虫病和丝虫病合作中心，上海 200025

Cloning, Expression and Activity Analysis of Full-length Gene Encoding Thioredoxin Peroxidase from *Oncomelania hupensis*

MA Xian-liang，LIU Qin，ZHANG Yi*

National Institute of Parasitic Diseases，Chinese Center for Disease Control and Prevention；Key Laboratory of Parasite and Vector Biology，MOH；WHO Collaborating Centre of Malaria，Schistosomiasis and Filarasis，Shanghai 200025，China

摘要 目的 克隆湖北钉螺（*Oncomelania hupensis*）硫氧还蛋白过氧化物酶（TPx）全长基因cDNA，并分析其表达蛋白的抗氧化活性。 方法 抽提人工饲养的阴性湖北钉螺总RNA，采用逆转录PCR方法扩增TPx基因片段。使用快速扩增cDNA末端法（RACE）扩增TPx基因，获得全长基因cDNA，与质粒pGEM-Teasy连接后，转化大肠埃希菌（*E. coli*）DH5α，筛选、测序，并进行生物信息学分析。克隆质粒pGEM-Teasy/TPx和表达载体pET28a经双酶切后连接，构建重组表达质粒pET28a/TPx，转入*E. coli* BL21（DE3），以异丙基-β-*D*-硫代半乳糖苷（IPTG）诱导表达。十二烷基磺酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）分析表达产物，通过组蛋白标签镍离子（Ni-NTA）层析柱分离纯化可溶性蛋白。在体外过氧化氢（H_2O_2）还原试验中，分别加入不同浓度的TPx重组蛋白（10、20、30、40和50 μg/ml），各浓度均设二硫苏糖醇（DTT）平行对照，计算H_2O_2清除率。在DNA超螺旋保护试验中，分别加入2.5、5.0和10 μg/ml重组蛋白，观察其对DNA超螺旋结构的保护作用。 结果 TPx全长基因cDNA为992 bp，开放阅读框（ORF）为747 bp，GenBank登录号为JN831437，编码249个氨基酸，预期蛋白相对分子质量（M_r）为27 000。重组质粒pET28a/TPx构建成功，经诱导表达和纯化后获得了可溶性重组蛋白。SDS-PAGE结果显示，M_r为27 000。体外H_2O_2还原试验结果显示，含有DTT的反应体系H_2O_2清除率均显著高于不含DTT的体系（均$P<0.05$），各个浓度组间差异无统计学意义（均$P>0.05$）。DNA超螺旋保护试验结果显示，5.0 μg/ml组保护效果优于2.5 μg/ml组，但5.0 μg/ml组和10 μg/ml组的保护效果差异不明显。 结论 获得湖北钉螺TPx全长基因cDNA，且重组表达蛋白具有一定抗氧化功能。

关键词： 湖北钉螺 硫氧还蛋白过氧化物酶 克隆 表达

Abstract： Objective To clone and express full-length thioredoxin peroxidase (TPx) gene of *Oncomelania hupensis* and study on the peroxidase activity of the recombinant protein. Methods Total RNA was obtained from the cultivated *O. hupensis* and a cDNA sequence of the TPx gene was cloned by RT-PCR. The TPx cDNA ends were amplified by the SMARTer RACE cDNA Amplification Kit. After sequencing, blasting and matching, the full-length cDNA of the TPx gene was obtained. The TPx cDNA was ligated with the pGEM-Teasy and transformed into *E. coli* DH5α. After sequencing and blasting, the characteristics of biological information of the TPx gene was analyzed. The positive recombinants with pGEM-Teasy/TPx and expression vector pET-28a were digested by the double restriction enzymes, ligated each other, transformed into *E. coli* BL21（DE3）, and induced by IPTG for expression. The recombinant TPx was expressed as a histidine fusion protein and was purified with Ni chromatography and NTA cation exchange chromatography. The expressed and purified TPx was analyzed by SDS-PAGE. The different concentrations of TPx recombinant protein （10, 20, 30, 40， and 50 μg/ml）were added into hydrogen peroxide （H_2O_2） reduction test in vitro to calculate the clearance rate of H_2O_2， each concentration with parallel control of dithiothreitol sugar alcohol （DTT）. In the protection test of super-coiled DNA, the TPx protein was added with a concentration of 2.5, 5.0, and 10 μg/ml, respectively, to observe the protective effect of super-coiled DNA in metal-catalyzed oxidation （MCO）. Results The complate cDNA encoding TPx was 992 bp. ORF was 747 bp with GenBank accession number of JN831437. The ORF encoded 249 amino acids, and the relative molecular weight （M_r） of predicted protein was 27 000. The recombinant plasmid pET28a/TPx was built, and the soluble recombinant protein was obtained by induction and purification. The results of SDS-PAGE showed that the M_r was 27 000. H_2O_2 reduction test *in vitro* showed that the H_2O_2 clearance rate of reaction system containing DTT was significantly higher than the clearance rate without DTT （$P<0.05$）, the difference among various concentrations was not statistically significant （$P>0.05$）. The protection of super-coiled DNA showed that the protective effect of 5.0 μg/ml group was better than 2.5 μg/ml group, but there was no difference between the protective effect of 5.0 μg/ml group and 10 μg/ml group. Conclusion The full-length cDNA of the TPx gene of *O. hupensis* is obtained, and the recombinant TPx protein shows a certain antioxidant activity.

Keywords： *Oncomelania hupensis* Thioredoxin peroxidase Clone Expression

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