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白纹伊蚊唾液腺serpin1基因的可变剪接分析与原核表达

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Alternative splicing and expression of the Alboserpin-1 from salivary gland of Aedes albopictus

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摘要 参考文献 相关文章

摘要 目的 克隆并原核表达白纹伊蚊唾液腺丝氨酸蛋白酶抑制剂基因1(Aalbserpin-1)。方法 根据NCBI网站上白纹伊蚊Aalbserpin-1(AY826096.1)的序列设计引物, 用RT-PCR从白纹伊蚊广州株中获取Aalbserpin-1全长基因序列, 并进行生物信息学分析。将目的片段克隆到原核表达载体pET28a(+), 转化到大肠杆菌中诱导表达。利用实时荧光定量RT-PCR分析Aalbserpin-1_2基因在白纹伊蚊不同组织中的表达差异。结果 PCR扩增获得Aalbserpin-1基因两个转录本(Aalbserpin1_1 和Aalbserpin1_2), Aalbserpin1_1的基因序列为1 260 bp, 编码419个氨基酸;Aalbserpin1_2的基因序列为1 332 bp, 编码443个氨基酸, 均具有seprin结构域, Aalbserpin1_1和Aalbserpin1_2与Aalbserpin-1(AY826096.1)相比, 相似性分别为95%和90%。两个转录本比较, Aalbserpin1_2中含有24个氨基酸的可变剪接子正好位于其功能活性中心环(RCL)上。以Aalbserpin1_2序列为模板, 成功构建pET28a-Aalbserpin-1_2重组质粒。SDS-PAGE结果显示目的基因在Origami(DE3)中表达, 重组蛋白相对分子量(Mr)约为50 000 Da, 经亲和层析获得目的蛋白。组织表达谱显示Aalbserpin-1_2在唾

液腺(SG)、中肠(MG, $P>0.05$)丰富表达, 脂肪体低表达(FB, $P<0.05$)。结论 白纹伊蚊广州株Aalbserpin-1基因具有两个可变剪接子, 其中Aalbserpin-1_2在唾液腺、中肠丰富表达, 成功构建pET28a-Aalbserpin-1_2重组质粒并获得重组蛋白。
关键词： 丝氨酸蛋白酶抑制剂 可变剪接子 克隆表达 白纹伊蚊
Abstract： To clone and prokaryotically express the Aalbserpin-1 gene from the salivary gland of Aedes albopictus, the full-length cDNA sequence of Aalbserpin-1 gene was amplified by RT-PCR, then constructed into the prokaryotic expression vector pET28a(+) and expressed in E. coli BL21(DE3) with IPTG induction. The recombinant protein was detected by SDS-PAGE and purified by Ni-IDA affinity chromatography. An expression analysis was conducted by real-time RT-PCR. It was demonstrated that two alternative transcripts were obtained (Aalbserpin-1_1 and Aalbserpin-1_2). To compare with Aalbserpin-1 (AY826096.1), Aalbserpin1_1 and Aalbserpin-1_2 shared 95% and 90% similarity respectively. An alternative splicing exon (24 amino acids: NLLTNRIVSQSSYRRVAIYDFISG) in Aalbserpin1_2 was just located in the reactive centre loop (RCL) of SERPIN, a primary determinant of functionality. A recombinant Aalbserpin-1_2 protein was purified and obtained. In addition, we also found Aalbserpin-1_2 was expressed higher in salivary gland and midgut than in fat body ($P<0.05$). The results suggest that the Aalbserpin-1 gene from Ae. albopictus exists two alternative transcripts, and Aalbserpin-1_2 is expressed highly in salivary gland and midgut, indicating that the rAalbserpin-1_2 protein has been prokaryotic expressed and purified successfully.
Keywords： serine protease inhibitor alternative splicing cloning and expression Aedes albopictus
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