

## 蛇毒锯鳞蝰素基因Leu14-Lys15-Glu16的定点突变

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**摘要** 本研究的目的是利用蛋白质工程定点突变的方法,在蛇毒锯鳞蝰素基因分子上增加另一个保守序列RGD(14位精氨酸残基,15位甘氨酸残基,16位天冬氨酸残基),以其增加该分子的生物活性,并探讨蛋白质一级结构,空间结构和功能的关系。在质粒pJC264的基础上,利用PCR定点突变方法,对蛇毒锯鳞蝰素基因Leu14-Lys15-Glu16进行定点突变,使相应的DNA片段变成表达Arg14-Gly15-Asp16的核苷酸顺序,经酶切和DNA测序鉴定正确。CNBr裂解后,用反相HPLC分析,分离制备突变体蛇毒锯鳞蝰素,制备的突变体蛇毒锯鳞蝰素的N-末端10个氨基酸残基与天然蛇毒锯鳞蝰素的相同。在人的富含血小板血浆测活体系中,经10 $\mu$ mol/L的ADP诱导,突变体蛇毒锯鳞蝰素的IC<sub>50</sub>为3.0 $\times$ 10<sup>-7</sup>mol/L;重组野生型蛇毒锯鳞蝰素的IC<sub>50</sub>为4.0 $\times$ 10<sup>-7</sup>mol/L;天然蛇毒锯鳞蝰素IC<sub>50</sub>为2.8 $\times$ 10<sup>-7</sup>mol/L。此外,就蛇毒锯鳞蝰素中保守序列Arg-Gly-Asp以及其空间构象对蛇毒锯鳞蝰素抑制血小板凝集之间的关系进行了讨论。

**关键词** [蛇毒锯鳞蝰素](#) [PCR](#) [定点突变](#) [抑制血小板凝集活性](#)

分类号

## Site-directed Mutagenesis of the Gene of Echistatin Leu14-Lys15-Glu16

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### Abstract

In order to probe relationship of inhibition activity of platelet aggregation and RGD conformation beneficial to binding in Echistatin, we used the site-directed mutation technique to install another RGD sequence into one of irregular loops retaining a degree of conformational flexibility and substituting Leu-14, Lys-15, Glu-16 of (Leu-28) Echistatin. The mutant (Arg-14, Gly-15, Asp-16, Leu-28) Echistatin did not lose its inhibition activity of platelet aggregation; however, it showed at least as high activity as (Leu-28) Echistatin, or even a little higher than (Leu-28) Echistatin. This suggested that both RGD sequences inserted in one loop with a degree of conformational flexibility. The original RGD (Arg-24, Gly-25, Asp-26) motif projecting significantly from the surface of the scaffold or core might contribute synergistically to the function of inhibiting platelet aggregation induced by 10<sup>-7</sup> mol/LADP (final concentration). These results are useful in the elucidation of the relationship of structure and function of Echistatin-like disintegrins and GP IIb/IIIa-like integrins.

**Key words** [Echistatin](#) [PCR](#) [Site-directed mutagenesis](#) [Activity of inhibiting platelet aggregation](#)

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