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HIV-1 HXB2株蛋白酶的原核表达、纯化及其Gag蛋白CAP2NC的切割活性分析

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摘要：

目的：原核表达、纯化HIV-1型HXB2株蛋白酶(protease，PR)，用于对HIV-1 Gag P2/NC蛋白酶切割位点序列随机突变的噬菌体展示文库的切割筛选及构建新的蛋白酶抑制剂(protease inhibitor, PI)类药物体外筛选模型。方法：根据HIV-1 HXB2株PR DNA序列设计引物，在PR序列上游添加自切割位点MGTVSFNF 8个氨基酸编码获得PR107 DNA编码序列，将PR107 DNA克隆至原核表达载体pET-32a, 序列测定后转化大肠杆菌BL21 DE3进行诱导表达。表达产物经用Ni-NTA亲和柱纯化, 经复性后，进行靶蛋白CAP2NC切割试验，SDS-PAGE检测切割结果。结果：成功合成了使用大肠杆菌偏好密码子的HIV-1 PR107的DNA编码序列，序列测定显示其编码氨基酸序列与原始序列完全一致；成功构建了HIV-1 PR原核表达质粒pET32a-PR107，转化大肠杆菌BL21 DE3经IPTG诱导后，表达出相对分子质量为30 000的HIV-1 PR融合蛋白，纯化的目的蛋白浓度为2.54 mg/ml；纯化蛋白经复性后具有对底物蛋白CAP2NC的切割活性，该作用能被PI药物抑制。结论：成功构建大肠杆菌偏好密码子带自切割位点的HIV-1 PR原核表达载体pET32a-PR107，在大肠杆菌中表达，经纯化复性获得了具有切割活性的HIV-1 PR融合蛋白。

关键词：HIV 蛋白酶 基因表达 切割

Prokaryotic expression and purification of protease of HIV-1 HXB2 subtype and analysis of cleaving substrate protein Gag CAP2NC peptide

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Abstract:

Objective: To express, purify and identify the protease (PR) of HIV-1 HXB2 subtype in E.coli, so as to screen the HIV-1 Gag CAP2/NC protein phage displayed library with randomized P2/NC protease cleavage sites and establish a phage model for in vitro screening of PR inhibitors. Methods: The primers were designed according to the PR amino acid sequence of HIV-1 HXB2 subtype and the E.coli preferred codon, the additional 5′-nucleotide sequence encoding the eight peptide MGTVSFNF for autocleave sites was inserted into the upstream of PR sequence. Then the PR107 DNA sequence was cloned into pET-32a vector which was used as expression vector in E.coli. Expression of HIV PR was induced by IPTG in E.coli BL21-DE3 and the expressed PR protein was purified by the Ni-NTA affinity column. The purified PR protein was refolded by diluted with MES buffer and blended into substrate protein CAP2NC to test its cleaving activity and the result was identified by SDS-PAGE. Results: The HIV PR107 DNA fragment with E.coli preferred codon was synthesized and was successfully inserted into the expression vector pET-32a. HIV-1 PR was expressed in E.coli BL21 DE3 after the induction by IPTG with a relative molecular weight of 30 000. The purified PR protein has a concentration of 2.54 mg/ml, and after refolded it could cleave substrate protein CAP2NC and this effect can be blocked by PI agent. Conclusion: PR107 DNA fragment with E.coli preferred codon of HIV-1 HXB2 subtype has been successfully synthesized and the PR protein has been successfully expressed, which can cleave substrate protein CAP2NC.

Keywords: HIV protease gene expression cleave

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