

植物和微生物遗传学

装载RNA“病毒样”纳米颗粒表面巯基化表达载体的构建及荧光修饰

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摘要 以本实验室构建的能够装载外源RNA片段的MS2噬菌体“病毒样”颗粒表达载体为基础, 利用定点突变技术将衣壳蛋白基因的第15位密码子由编码苏氨酸突变为胱氨酸。表达产物在35%蔗糖密度梯度处有一目的产物, 目的产物在透射电镜下呈球形, 直径大小约为27 nm。用马来酰亚胺-5'-荧光素对表达产物进行化学修饰, 经光谱分析、SDS-PAGE分析和MALDI-TOF MS鉴定, 证明巯基在表达产物的外表面, 并能与马来酰亚胺-5'-荧光素进行反应。这种携带外源RNA片段的荧光修饰的天然纳米颗粒为制备各种功能性纳米材料提供了新途径。

关键词 “病毒样”颗粒; MS2噬菌体; 衣壳蛋白; 半胱氨酸残基; 荧光修饰

分类号

Construction of RNA-containing Virus-like Nanoparticles Expression Vector with Cysteine Residues on Surface and Fluorescent Decoration

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Abstract

Site-directed mutagenesis was performed at the codon 15 of the MS2 bacteriophage coat protein gene, which has been cloned to the virus-like particles expression vector containing non-self RNA fragment. The produced expression vector termed pARSC, was transformed to E.coli DH5α. The positive clones were selected and proliferated. The harvested cells were treated with sonication and the supernatant of sonication was then subject to linear sucrose density gradients centrifugation (15% to 60%) at 32 000 r/min for 4 h at 4°C. The virus-like particles, VLP-Cy, were collected at 35% sucrose density. The particles were examined by transmission electron microscopy, which revealed the presence of spherical viral particles of approximately 27 nm in diameter. The thiolated VLP-Cy was then chemically modified with fluorescein -5'-maleimide. The covalent fluorescent labeling was confirmed by absorption analysis, SDS-PAGE and MALDI-TOF mass spectroscopy. This is the first report of preparation of RNA-containing natural fluorescent nanoparticles. These studies highlight the versatility of MS2 bacteriophage capsids as building blocks for functional nanomaterials construction with a variety of applications.

Key words [virus-like nanoparticles](#) [MS2 bacteriophage](#) [coat protein](#) [cysteine residue](#) [fluorescent decoration](#)

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