生物物理学报(2003) 19(1), 13-17

PDF文档

# 质粒pUC18 DNA的螺线管型超螺旋结构

齐静、张臻峰、于春风、杨琴、黄志刚、侯亭、黄熙泰*
南开大学生命科学学院生化与分子生物学系

理论上，游离的超螺旋DNA可以采取两种结构形式：互缠式超螺旋和螺线管型超螺旋。前者早已被透射电子显微镜和原子力显微术的研究所证实，而后者却仍然缺乏足够的证据。使用温和的清亮裂解液法，从DNA拓扑酶野生型大肠杆菌HB101细胞抽提质粒pUC18 DNA。经CsCl-EB平衡密度梯度超离心分离获得超螺旋pUC18 DNA和松弛型pUC18 DNA (DNA Ⅱ)。纯化DNA分别用疏水性溶剂系统和亲水性溶剂系统的细胞色素单分子层展开技术制备电子显微镜标本。观察结果显示：在疏水性的甲酰胺-水展开系统中，DNA采取通常的互缠式结构；在含有1.5 mmol/L醋酸铵的水介质中制备的超螺旋DNA标本，DNA采取线圈型结构，测得pUC18 DNA(单体)分子这种结构的外直径约为43.8 nm，内直径约为2 nm。在相同亲水介质中松弛型pUC18 DNA采取典型的螺线管型结构，其单体平均外直径约为53.1 nm，内直径约为17.2 nm。表明：在疏水介质中超螺旋DNA趋向于采取互缠式结构，而在亲水介质中DNA则采取螺线管型结构。DNA链之间可能存在非共价相互作用以维持这种结构。螺线管型结构可能是水溶液中的超螺旋DNA分子普遍的存在形式。

# SOLENOIDALLY SUPERCILED pUC18 DNA AND SOLENOIDALLY RELAXED pUC18 DNA: THE EVIDENCE OF ELECTRON MICROSCOPY

The plasmid pUC18 DNA is isolated from Escherichia coli Cell by clear lysate method. The DNA samples are fractionated into "supercoiled DNA band" and "relaxed DNA band" by CsCl-EB equilibrium density gradient ultracentrifugation. The electron microscopic specimens of purified DNA samples are prepared by cytochrome c monolayer technique in dif-ferent solvent system. The results obtained by electron microscopic observation show that the supercoiled pUC18 DNA adopts a doughnut-like solenoidal structure with outside diameter about 43.8 nm and inside diameter about 2.0 nm in ammonium acetate-H2O medium. The re-laxed circular pUC18 DNA (DNA Ⅱ) also takes solenoidal structure with outside diameter about 53.1 nm and inside diameter about 17.2 nm in the same system. All of the results sug-gest that the solenoildal structure may be a favorable form of DNA in solution. Some of non-covalent interactions between the coils of DNA double strand may be involved in the solenoidal structure of DNA.

## 关键词

DNA拓扑结构(DNA topology); 螺线管型超螺旋DNA(Solenoidally supercoiled DNA); 螺线管型DNA Ⅱ (Solenoidally relaxed circular DNA); 电子显微镜(Electron microscopy)