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ELECTRON MICROSCOPY OF PLASMID DNA MOLECULES

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Col E1 plasmid DNA was micrographed using three different preparation techniques, e.g. protein monolayer, BAC diffusion and glow-discharge. The plasmid length was nearly 1.6μ in all the three techniques. But there was a wide variation in the DNA width and the conformation of the molecule. The glow discharge method was found to be the most suitable for DNA electron microscopy.

Key Words : Electron Microscopy; Plasmid DNA

INTRODUCTION

The extrachromosomal plasmid DNA molecules have been found to be responsible for the drug resisting property of bacterial calls in many diseases.[1,2] Attempts have been made to visualize the structural morphology of the plasmid molecules in different laboratories.[3–5] But the observations vary from one another, which may be due to variations in the techniques used. The present work is a comparative study of the conformation of Col E1 plasmid molecules prepared in three different electron microscopic techniques, e. g., protein monolayer technique,[6,7] BAC diffusion technique[8] and glow discharge method.[9]

MATERIALS AND METHOD

Col E1 plasmid DNA was obtained following the method of Lovett *et. al.*[10] Cytochrome C was obtained from Sigma Chemicals, U.S.A. BAC (Benzyldimethyl-alkylammonium chloride) was a gift from Bayer Co., Germany. The stock concentration of plasmid DNA was 1μg/ml in 10mM Tris, 1mM EDTA.

Specimens were prepared in Kleinschmidt's protein monolayer technique[6] modified by Davies *et al.*[7] The spreading solution contained 0.1μg/ml DNA, 0.1 per cent cytochrome C, 0.25M ammonium acetate and the hypophase was freshly triple distilled water. In BAC diffusion method[8] for specimen preparation, the final solution contained 0.05μg/ml DNA, 0.001 per cent BAC, 10mM Tris, 1mM EDTA.

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For specimen preparation using Dubochett's glow discharge method,[9] carbon coated grids were positively charged inside the glow discharge apparatus in presence of amylamine vapour. 50μl drops containing 0.1μg/ml DNA in *tris*-EDTA buffer were placed on the freshly charged grids and was allowed to stand for 2 to 3 minutes. Excess liquid was blotted dry. All grids were stained with 0.1 per cent uranyl acetate and rotary shadowed with 5Å platinum-palladium.

Micrographs were taken with Siemens Elmiskop 101B at an electronic magnification of 40,000 X. DNA widths were measured with a magnifier fitted with a micrometer scale. For length measurements, the micrographs were enlarged 10 times optically and the DNA contours were measured with a map measurer.

Results and Discussion

About 100 Col E1 covalently closed plasmid DNA molecules were micrographed in each technique. Plate IA and IB showed some Col E1 plasmid DNA molecules micrographed in protein monolayer technique[7] and BAC diffusion technique[8] respectively. Plate II represented a homogeneously distributed field of the same plasmid molecules micrographed by glow-discharge method.[9] The observations made on the plasmid molecules in the three techniques have been compared in Table I.

Table I

Visualization of Col E1 plasmid molecules

Technique used	Plasmid length in μ	DNA width in Å	Conformation observed
Protein Monolayer	1.60 ± 0.24	180 ± 9 Å	*tight relaxed*
BAC diffusion	1.56 ± 0.21	110 ± 5 Å	*highly kinky*
Glow Discharge	1.60 ± 0.19	50 ± 3 Å	*highly relaxed and flexible*

The much increased width of the molecule in Plate IA compared to actual DNA width of 20Å was due to the external agents cytochrome C, uranyl acetate and platinum-palladium alloy used during specimen preparation. Most of the plasmid molecules were of closed tight relaxed conformations with a few open molecules in this plate. Previously, Ghosh *et al.*[4] showed an increase in the relaxation of the plasmid molecule with decrease in salt molarity of the hypophase. This is in good agreement with our results since we have used only water as hypophase.

In Plate IB, BAC has replaced cytochrome C, and the DNA width has decreased by bout 70Å. This was due to low molecular weight of BAC (330) compared to that of cytochrome C (12,384). The plasmid length was slightly less in this case

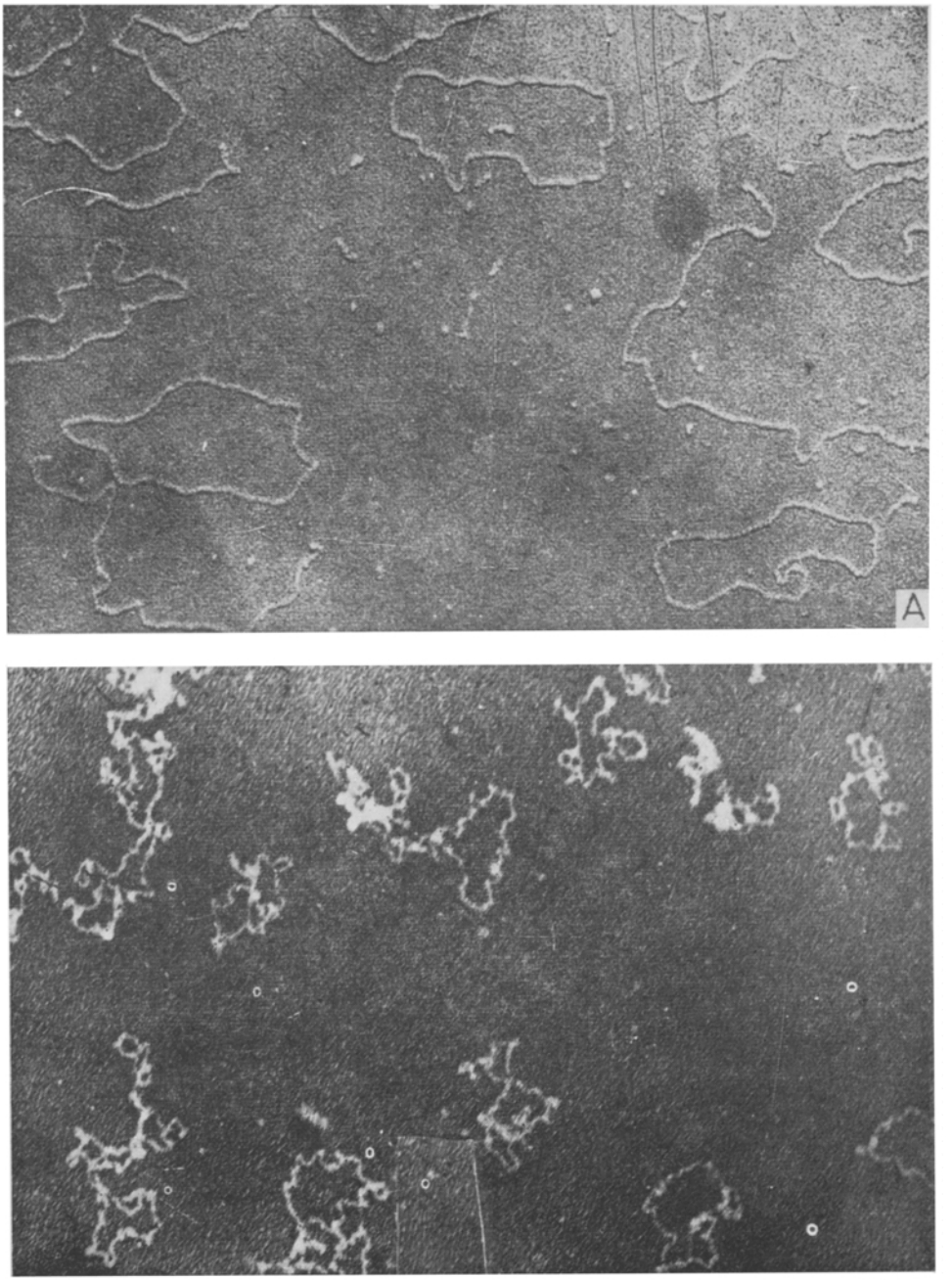

PLATE I Electron micrograph of Col E1 plasmid DNA molecules prepared in : (*A*) Protein monolayer technique; and (*B*) BAC diffusion method. magnification 60,000 X.

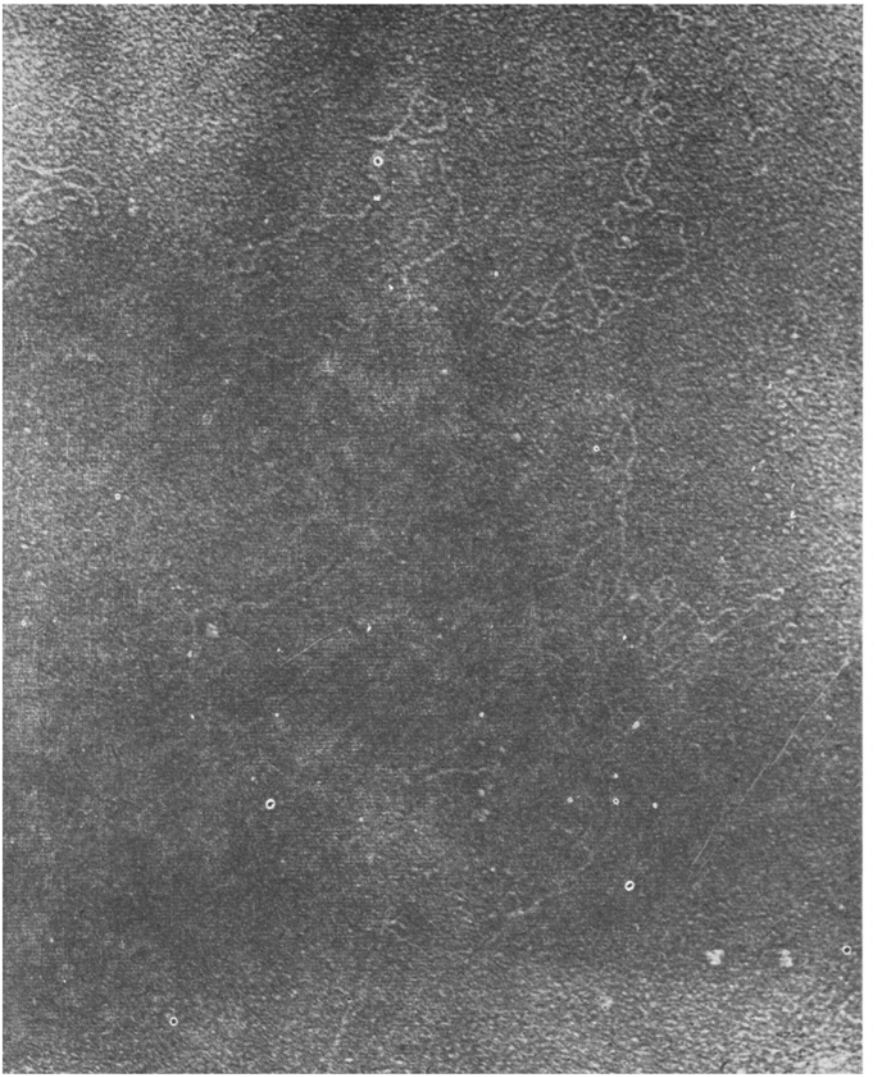

PLATE II Electron micrograph of Col E1 plasmid DNA molecules prepared in Glow-Discharge method, magnification 1,60, 000 X.

(Table I). This must be due to unavoidable length-measuring difficulties of the highly kinky nature of the molecules in this technique.

There was a sharp decrease in the width of DNA molecules observed in the glow discharge method (Plate II) although the plasmid length was the same (Table I). This was due to the fact that no external agent was used in this technique except the staining and shadowing materials. There was only an electrostatic interaction between the DNA molecule and the positively charged carbon coated

grids. Kunisada and Yamagishi[5] also avoided use of external agents in their mica-press-adsorption method and obtained similar DNA widths. But the molecule appeared stiff and stretched in their micrographs while the molecules observed in the glow-discharge method (Plate II) were relaxed and flexible. From the present results and the previous studies, the glow discharge method seems to be the most improved electron microscopic technique for visualizing small genetic materials like plasmid DNA.

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