Scanning Electron Microscopy of Agarose Beads during Degradation by a Gram-negative Bacterium

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Some bacteria can grow on agar as the sole source of carbon, and we have recently isolated a very efficient agar-degrading Gram-negative bacterium (v. Hofsten & Malmqvist, 1975). Colonies of this organism form large holes in agar plates, and an enzyme capable of hydrolysing agar solutions is found in cell-free media of stationary-phase liquid cultures.

We have tried to study bacterial agar degradation in the transmission electron microscope, but it is impossible to see an agar gel in thin sections due to its low contrast. This report describes how scanning electron microscopy may be used to study bacterial growth on agarose beads. Such beads are often used in chromatography and as supports for covalently coupled enzymes.

METHODS

The bacterium was grown on agarose beads (Sepharose 4B; Pharmacia Fine Chemicals, Uppsala, Sweden) suspended in salts medium B (v. Hofsten & Malmqvist, 1975). The beads were washed free of the bacteriostatic compound present in the commercial product, before being added to the sterile medium. Cultures were inoculated with a drop of an overnight culture grown on 0.2% Difco agar dissolved in medium B, and incubated on a rotary shaker at 37 °C. Glutaraldehyde was added to a final concentration of 2 % (v/v) to fix the bacteria, 1 h before samples were freeze-dried. To achieve rapid freezing, a few drops of the cultures were placed on to small circular coverslips and these were immersed in a propane-propene mixture liquefied by liquid nitrogen. The frozen samples were dried in a modified Glick-Malmström apparatus (Moberger, Lindström & Andersson, 1954) at a temperature of about −70 °C. Undegraded agarose beads were briefly washed in water before being treated in a similar way.

The coverslips were mounted on brass stubs and coated with gold in a high-vacuum evaporator while being rotated. Conductive silver paste was placed on the mounting stub surface around the edges of the coverslip to ensure good electrical contact. Specimens were observed in a Jeol JSM-S1 scanning electron microscope at a beam specimen angle of 45°, accelerating voltage of 4 or 10 kV, and final aperture of 200 μm.

RESULTS

Figure 1a is a low magnification electron micrograph of undegraded Sepharose beads. The diameter of the beads varied between approximately 15 and 100 μm. Their surface was distinctly furrowed as can be seen at high magnification (Fig. 1b), and some of the beads had slight depressions.

When a suspension of Sepharose beads in medium B was inoculated with the Gram-negative bacterium, light microscopic observations showed that after some hours bacteria
Fig. 1. Beads of Sepharose 4B. (a), (b) Before incubation with bacteria; (c), (d) after 16 h cultivation of the Gram-negative bacterium.
became attached to the beads. Figure 1c shows that apparently intact beads were still present after a culture had been grown for 16 h, but many of them had been degraded into slimy residues. Bacteria growing on the beads and residues could be seen at high magnification (Fig. 1d). Most cells were rod shaped and measured 2 to 3 μm, but some were distinctly curved.

No bead-shaped particles could be seen in cultures grown for two days, and the bacteria were slightly aggregated at this stage.

**DISCUSSION**

The Sepharose beads contain about 96% water and cannot be air-dried without collapsing. Critical point drying was also tried as a method of preserving their structure, but the freeze-drying method described here was found to give good results. Freeze-drying at low temperatures has given excellent results in the preparation of animal tissues for scanning electron microscopy (Boyde & Wood, 1969), and it is likely that it gives a true picture of the Sepharose beads.

Most efficient agar degradation occurs when bacteria are in direct contact with the gel, but agarase solutions alone can release reducing sugars when incubated with Sepharose beads (v. Hofsten & Malmqvist, 1975). The structure of the beads is changed when these are incubated with enzyme solutions, and we are at present studying this effect in the scanning electron microscope.

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**REFERENCES**

