SHORT COMMUNICATION

ATP-dependent Deoxyribonuclease Activity in Segregated Cell Types of Caulobacter crescentus

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Partially purified bacterial extracts were prepared from segregated stalked and swarmer bacteria of Caulobacter crescentus and assayed for ATP-dependent deoxyribonuclease activity. This activity was found to be very low in the swarmer bacteria compared with a pure population of stalked cells or an asynchronous population of C. crescentus.

INTRODUCTION

ATP-dependent deoxyribonucleases occur in various bacteria, in some of which they have been found to be involved in genetic recombination (Vövis & Buttin, 1970; Clark, 1973; Greth & Chevalier, 1973; Doly, 1978). A system of genetic exchange via conjugation has been reported in Caulobacter crescentus (Jollick & Tran, 1975; Newton & Allebach, 1975), a Gram-negative, stalked bacterium characterized by dimorphism of cell types that occur in a defined sequence in the life cycle (Shapiro, 1976). The stalked bacteria differentiate to become predivisional cells with a flagellum and pili. Asymmetric division then gives rise to a similar stalked bacterium and a motile swarmer bacterium. The stalked cell can enter another round of division immediately whereas the swarmer must first differentiate into a stalked bacterium (Shapiro, 1976). In terms of DNA synthesis activity the cell cycle in this organism can be divided into three distinct periods – G1, S and G2 – of which S is restricted to the stalked phase of the cycle.

The occurrence of a system of genetic exchange in Caulobacter prompted our interest in ATP-dependent deoxyribonuclease activity in this bacterium. Changes in the pattern of DNA-binding proteins during the cell cycle (Cheung & Newton, 1977; Z. Markiewicz, unpublished results) as well as the finding that stalked and not swarmer bacteria are recipients in plasmid transfer in Caulobacter vibrioides (Alexander & Jollick, 1977) led me to expect changes in the activity of this deoxyribonuclease in the separate cell types of C. crescentus.

METHODS

Bacterial strains and growth conditions. Caulobacter crescentus strains CB1, CB2 and CB15 were obtained from Dr L. Shapiro. Cultures were grown in complete peptone/yeast extract medium (Poindexter, 1964) or in minimal medium containing 0.2% (w/v) glucose (Shapiro et al., 1972).

Synchronization of cells. The method used was essentially that of Evinger & Agabian (1977) in which swarmer bacteria were purified by centrifugation in a density gradient of Ludox HS40 (Du Pont de Nemours, Düsseldorf, F.R.G.). One litre of a mid-exponential phase culture yielded $4 \times 10^{10}$ to $6 \times 10^{10}$ swarmer bacteria which differentiated synchronously as assessed by cell number count or by the incorporation of nucleic acid precursors.
Assessment of synchrony. (1) Samples (0.1 ml) were taken every 10 min from a synchronous culture in minimal medium and the cell number was determined in a model B Coulter counter. (2) Incorporation of [3H]deoxyadenosine into DNA was measured essentially as described by Iba et al. (1977).

Preparation of cell-free extracts. Bacteria in the mid-exponential phase of growth were harvested by centrifugation and resuspended in 50 mM-Tris/HCl buffer, pH 7-8, containing 10 mM-MgCl₂, 2 mM-2-mercaptoethanol and 0.1 mM-EDTA (TMS buffer). The bacteria were disrupted by ultrasonic treatment in an MSE disintegrator. Debris was removed by centrifugation for 30 min at 16000 g, and the supernatant was treated dropwise with streptomycin sulphate solution (30 μg per A₅₅₀ unit). Nucleic acids were removed by centrifugation for 20 min at 16000 g. The supernatant was retained and dialysed overnight against two changes of TMS buffer. All steps were carried out at 0 to 3 °C.

Assay of ATP-dependent deoxyribonuclease. Deoxyribonuclease activity was assayed by measuring the release of acid-soluble radioactivity from 3H-labelled DNA (Markiewicz & Kwiatkowski, 1975). The reaction mixture (0.4 ml) contained 3-5 nmol labelled DNA, 0.1 μmol ATP, 20 mM-MgCl₂, 50 mM-Tris/HCl buffer, pH 7-9, and partially purified extract. After 20 min incubation at 30 °C, 0.1 ml bovine serum albumin (0-5%, w/v) and 0-5 ml cold 10% (w/v) trichloroacetic acid were added. The mixtures were chilled on ice and centrifuged at 6000 g for 5 min. Radioactivity in the supernatant was counted in a toluene/Triton X-100 based scintillation fluid (Miller & Clark, 1976). A unit of enzyme converts 1 nmol DNA to acid-soluble products in 20 min at 30 °C.

Protein determinations were made by the Lowry method with bovine serum albumin as the standard.

RESULTS

ATP-dependent deoxyribonuclease activity was observed in all three C. crescentus strains examined; C. crescentus CB15 was chosen for further investigation because it is the most widely studied strain with respect to processes of genetic exchange.

Centrifugation of an asynchronous population of C. crescentus CB15 in a Ludox HS density gradient gave two bands of bacteria of which the lower contained 92 to 97% swarmer bacteria as determined by electron microscopy. These cells differentiated synchronously on resuspension in minimal medium (Fig. 1). The upper curve in Fig. 1 shows that the incorporation of [3H]deoxyadenosine into the DNA of C. crescentus followed the characteristic pattern outlined by Degnen & Newton (1972). Swarmer bacteria were either immediately used in further experiments or allowed to differentiate into stalked bacteria which were then harvested by centrifugation (10 min at 5000 g). Partly purified extracts of either swarmer or stalked bacteria were prepared. Extracts from both types of bacteria were adjusted to approximately the same protein concentration prior to assay for ATP-dependent deoxyribonuclease activity.

The specific activity of the ATP-dependent nuclease was much higher in the stalked cell extract than in that from the swarmer (Table 1). This difference was reproducible and the activity in swarmer cell extracts in a number of individual experiments ranged from 3-4 to 8-9% of the stalked cell extract activity. The possibility of the presence of an inhibitor of ATP-dependent deoxyribonuclease activity in the swarmer cell extract was excluded by measuring the activity of the enzyme(s) in a mixed extract control.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Specific activity [units (mg protein)⁻¹]</th>
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<tbody>
<tr>
<td>Stalked</td>
<td>1.590 (100%)</td>
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<tr>
<td>Swarmer</td>
<td>0.102 (6-4%)</td>
</tr>
<tr>
<td>Mixed*</td>
<td>1.140 (71-4%)</td>
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* Extracts were prepared from an asynchronous population of C. crescentus CB15.
DISCUSSION

Studies on protein synthesis in *C. crescentus* have demonstrated that the patterns of protein synthesis change sequentially during the life cycle (Cheung & Newton, 1977; Iba et al., 1978; Agabian et al., 1979). Differences in proteins associated with swarmer or stalked cell nucleoids have also been reported (Evinger & Agabian, 1977, 1979). The periodicity of DNA synthesis as well as the occurrence of the conjugational transfer of genetic material in *C. crescentus* stimulated our interest in DNA-binding proteins and particularly in deoxyribonuclease activities. In this study, ATP-dependent deoxyribonuclease activity was clearly much higher in the extracts of stalked cells than in extracts of the swarmers, and a similar pattern has been observed for some other DNA-binding proteins (unpublished results). In addition, the ATP-dependent deoxyribonuclease activity in extracts from asynchronous populations was inversely related to the number of swarmer bacteria in the population, confirming the results from separated cell types.

A conjugational system of genetic exchange in *C. crescentus* has been described (Jollick & Tran, 1975; Newton & Allebach, 1975). However, certain fundamental questions still remain unanswered, such as the role of the swarmer and stalked cell types in this process. Alexander & Jollick (1977) found that stalked and not swarmer bacteria were recipients in plasmid transfer in *C. vibrioides*. Since preliminary results obtained in our laboratory (Markiewicz & Kwiatkowski, 1980) point to the involvement of ATP-dependent deoxyribonuclease activity in conjugational transfer in *Caulobacter*, differences in the activity of this nuclease in the two cell types may reflect the unequal role of these cells in this process.

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REFERENCES


