DNA Binding and Deoxyribonuclease Activity in *Bacillus subtilis* During Temperature-induced Competence Development

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Rapid development of competence can be induced in cultures of *Bacillus subtilis* by incubation at 37 °C after previous growth at 42 °C. This temperature-induced competence was accompanied by an increase in DNA binding capacity and breakdown of donor DNA. Inhibition of protein synthesis prevented the rapid increase of competence. This is probably due to the inhibition of de novo synthesis of a constituent that enables the bacteria to bind DNA.

**INTRODUCTION**

Development of the physiological state in which bacteria are capable of irreversibly absorbing DNA from the medium (competence) is a prerequisite for the occurrence of DNA-mediated genetic transformation. Several reports have indicated that in *Streptococcus pneumoniae* the binding and entry of transforming DNA can be separated experimentally and genetically (Lacks, 1977). In this system, EDTA does not interfere with the binding of DNA, but prevents entry (Seto & Tomasz, 1974). Mutants have also been isolated which are unable to take up DNA but are proficient in DNA binding (Lacks et al., 1974). These mutants lack a membrane-located endonuclease (Lacks & Neuberger, 1975). In *Bacillus subtilis*, competent cells possess a loosely membrane-bound nuclease which can be released from the cells during protoplast formation (Joenje & Venema, 1975).

It was reported earlier (Joenje et al., 1973) that *B. subtilis* cells grown at 42 °C develop competence poorly, but do so rapidly when shifted to 37 °C. This has been ascribed to the possibility that the cells at 42 °C continue to enter the precompetent state characterized by Dooley et al. (1971), but they are unable to pass from precompetence into competence at 42 °C, whereas they can do so rapidly when shifted to 37 °C (Joenje et al., 1973).

In the present studies we have used a standardized system of temperature shift-induced competence to determine whether the low levels of transformation obtained at 42 °C are due to the absence of competence-specific nucleolytic activity, which is thought to be involved in the entry of transforming DNA into *B. subtilis* (Joenje & Venema, 1975), or to the inability of the cells to bind DNA. We found that the nuclease activity released during protoplast formation of competent cells is present in cells grown at 42 °C and that this activity is little affected by shifting the cells to 37 °C in the presence of chloramphenicol, whereas the binding of DNA is strongly inhibited after this shift in the presence of the drug. For these reasons, we presume that the poor transformability at 42 °C is due to the inability of the cells to develop DNA binding capacity at this temperature.

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METHODS

Strains. The highly transformable strain Bacillus subtilis 1G-20 (trpC2) was used as recipient. The thymine-requiring strain 1G-22 (thy) was used for the isolation of tritiated donor DNA; OG-1, a prototrophic strain, was employed for the isolation of unlabelled donor DNA.

Media and growth conditions. Bacteria were grown overnight at 37 or 42 °C in Spizizen's minimal salts medium (Spizizen, 1958) containing casein hydrolysate (200 μg ml⁻¹) and tryptophan (50 μg ml⁻¹), and diluted into WB medium (Wilson & Bott, 1968) to about 3 × 10⁷ colony-forming units (c.f.u.) ml⁻¹. The diluted cultures were either shifted to 37 °C or further incubated at 42 °C for the times indicated in Results.

DNA. Unlabelled DNA and tritiated DNA were isolated and stored as described earlier (Joenje et al., 1974). The specific radioactivities of the tritiated DNA preparations were approximately 1.5 × 10⁹ c.p.m. μg⁻¹.

Transformation. Culture samples (0.9 ml) were transformed with 0.1 ml unlabelled DNA (10 μg ml⁻¹) with gentle shaking at 37 or 42 °C for 15 min. Uptake of DNA was stopped by adding deoxyribonuclease I (20 μg ml⁻¹) and continuing incubation for a further 5 min. Under these conditions, the efficiency of transformation of cultures grown at 37 °C is the same at 37 and 42 °C (Joenje et al., 1973).

DNA binding to bacteria. Culture samples (0.9 ml) were incubated for 10 min with 0.1 ml of a solution containing 10 μg tritiated DNA ml⁻¹. Further increase in DNA binding was stopped by adding 1 ml of calf thymus DNA solution (1 mg ml⁻¹) and chilling on ice. The amount of cell-bound radioactivity was subsequently determined as previously described (Joenje & Venema, 1975) by layering each sample on top of a discontinuous sucrose gradient (5 to 10% w/v). Under these conditions, unbound DNA floated in the gradient. After centrifugation (10000 g, 20 min) the supernatant was discarded and the radioactivity associated with the pellet was counted. Control values for binding were obtained using the same method by adding the calf thymus DNA immediately after the labelled DNA. These values varied between 150 to 200 c.p.m. ml⁻¹ and were subtracted from each sample value.

Assay of acid-soluble DNA breakdown products. Samples (1 ml) were incubated with tritiated DNA (1 μg ml⁻¹) at 37 or 42 °C for 10 min unless otherwise stated. They were then centrifuged and the pellets were discarded. Each supernatant was mixed with 1 ml perchloric acid (6%, v/v) and 500 μg bovine serum albumin, chilled on ice for 15 min, centrifuged (10000 g, 15 min), and the radioactivity in 0.6 ml portions of the supernatant fluid was determined in a liquid scintillation counter after mixing with scintillation fluid [toluene/Triton X-100 (10:1, v/v) plus 5 g 2,5-diphenyloxazole and 0.05 g 1,4-bis-2-(5-phenyloxazolyl)-benzene per litre toluene].

Assay of nuclease activity in the supernatant fluid of protoplasts. Culture samples were concentrated 20-fold by centrifugation and resuspension in 0.05 M-potassium phosphate (pH 7.5) containing sucrose (20%, w/v) and 10 mM-MgSO₄. Lysozyme (100 μg ml⁻¹) was added and the samples were gently shaken at 37 °C. Incubation was continued until most cells had been converted to protoplasts (usually within 40 min), as revealed by light microscopy. Portions (2 ml) of the protoplast suspensions were layered on top of discontinuous sucrose (25 to 30%, w/v) gradients and centrifuged (15 min, 12000 g, 20 °C) to sediment the protoplasts; 1.4 ml portions of the supernatant were then pipetted off and incubated with tritiated DNA (1 μg ml⁻¹) for 60 min. After adding 0.2 ml perchoric acid (25%, v/v) the amount of acid-soluble radioactive products was determined as described by Joenje & Venema (1975).

RESULTS

DNA binding capacity and acid-soluble DNA breakdown products during temperature-induced development and loss of competence

During growth at 42 °C, B. subtilis cultures developed little competence compared with the level reached at 37 °C (Fig. 1; Joenje et al., 1973). When bacteria grown at 42 °C were shifted to 37 °C, competence increased rapidly (Fig. 1). The highest level of temperature-induced competence, about a 100-fold increase, was reached after 3.5 h incubation at 42 °C and subsequent shift to 37 °C for 60 min.

The temperature-induced rapid increase in transformability was paralleled by a similar increase in the capacity of the bacteria to bind DNA and to degrade DNA to acid-soluble material (Fig. 2). These three processes exhibited parallel curves at 42 °C, although the levels finally observed were very low (results not shown). In contrast, if highly competent cultures,
Competence in B. subtilis

Fig. 1. Competence development at 37 and 42 °C. Strain 1G-20 was grown overnight at 42°C, then diluted into WB medium (time 0). One portion was subsequently grown at 37 °C (○) and one portion at 42 °C (O). Competence was determined by transformation. After 3-5, 4 and 4.5 h, samples of the culture grown at 42 °C were shifted to 37 °C and competence development in these samples was determined during a 90 min period (□). No substantial changes in cell viability occurred during the incubation period at 37 °C after the shift of temperature. Generally the viable count during 90 min at 37 °C increased only from 3-0 × 10⁸ to 3.6 × 10⁸ c.f.u. ml⁻¹.

Fig. 2. Transformation (○), DNA binding (□) and DNA breakdown to acid-soluble products (○) by strain 1G-20 after a shift from 42 to 37 °C. Time 0 corresponds to 3.5 h in Fig. 1.

Fig. 3. Effect of a shift from 37 to 42 °C on transformation (a), DNA binding (b) and DNA breakdown (c). Control (○) transformation, DNA binding and DNA breakdown were assayed after 4 h growth at 37 °C at 30 min intervals. At the times indicated, samples were transferred to 42 °C and competence, DNA binding capacity and nuclease activity were measured after 1 h at 42 °C (□). Each point is the average of three experiments.
grown at 37 °C, were shifted to 42 °C, competence was rapidly lost with a concomitant loss of the capacity to bind and degrade DNA (Fig. 3).

**Effect of inhibition of protein synthesis on temperature-induced development of competence**

The increase in DNA binding capacity and DNA degrading activity during temperature-induced competence development could be due either to the activation of existing cell constituents having reduced activities at 42 °C, or to de novo synthesis of cell components engaged in these two processes. To distinguish between these two possibilities, we tested the effect of inhibiting protein synthesis on DNA binding and DNA degradation during temperature-induced competence development. As can be seen in Fig. 4(a), inhibition of protein synthesis by chloramphenicol (20 µg ml⁻¹) completely prevented the development of competence normally occurring after a shift from 42 to 37 °C. At the concentration used, chloramphenicol had no effect on the viability of the bacteria. The increase in DNA binding and DNA degradation subsequent to the shift of temperature was also prevented in the presence of chloramphenicol (Fig. 4b, c). We conclude that protein synthesis is required for the occurrence of temperature-induced competence development, rather than activation of a protein(s) which is inactive at 42 °C.

To examine whether the low level of competence at 42 °C was caused by the absence of competence-specific nuclease activity, we measured this activity by making use of the finding that this nuclease is released into the medium when the cells of a competent culture are converted to protoplasts (Joenje & Venema, 1975). After removal of the protoplasts by centrifugation, the nuclease activity in the supernatant was determined. Figure 5 shows the effect of inhibiting protein synthesis on the nuclease activity measured in supernatants of protoplasts from culture samples taken at different times during temperature-induced competence development. In contrast to the breakdown of DNA by whole cells (Fig. 4), the nuclease activity was hardly affected by inhibition of protein synthesis. This indicates that the protein synthesis-requiring step for development of competence after the shift of temperature is not related to the competence-specific nuclease, but possibly to the binding of transforming DNA.
Fig. 5. Effect of chloramphenicol on competence-specific nuclease activity in the supernatant fluid of protoplasts. A culture grown at 42 °C for 3.5 h was divided in two, chloramphenicol (20 µg ml⁻¹) was added to one part, and then both cultures were transferred to 37 °C (time 0). Competence was followed for 1 h: ○, without chloramphenicol; ●, with chloramphenicol. After 0, 30 and 60 min incubation at 37 °C, protoplasts were prepared from samples as described in Methods. DNA breakdown to acid-soluble products was measured in the protoplast supernatants: □, without chloramphenicol; ■, with chloramphenicol.

DISCUSSION

The results reported here strongly suggest that temperature-induced competence development of *B. subtilis* may be dependent on the de novo synthesis of a protein that enables the bacteria to bind exogenous DNA. Since the cell sites responsible for the binding of donor DNA in *B. subtilis* are most likely associated with the membrane of the competent cells (Joenje *et al.*, 1974; Joenje & Venema, 1975; Garcia *et al.*., 1978), the newly synthesized protein is possibly a DNA binding constituent of the membrane.

An alternative explanation may be that the required protein is an autolytic enzyme which unmasks existing DNA binding sites. This would agree with models proposed for competence in *B. subtilis* (Akrigg *et al.*, 1969), as well as in *Streptococcus pneumoniae* (Tomasz, 1973).

The breakdown of transforming DNA by competent *B. subtilis* cells is believed to be a consequence of the entry of the DNA (Davidoff-Abelson & Dubnau, 1973). The competence-specific nuclease liberated during protoplast formation is probably the enzyme responsible for this activity (Joenje & Venema, 1975). The synthesis of this nuclease is apparently not affected by growing the cells at 42 °C, and, since its activity is only slightly affected when the cells are shifted to 37 °C in the presence of chloramphenicol, the low transformability observed at this temperature in the presence of the drug does not appear to be due to a deficiency in the DNA entry system of the cells.

The observation that temperature-induced competence disappears quickly when the cells are shifted back to 42 °C is most easily explained by the assumption that continuous protein synthesis is required for DNA binding. This is in agreement with the finding that addition of chloramphenicol during the temperature-induced development of competence strongly decreases the cell’s transformability and capacity to bind DNA (results not shown).
interpret the low levels of transformability and of DNA breakdown by whole cells when the cultures are grown at or shifted back to 42 °C, as due to the inability of the cells to bind DNA at this temperature. This also agrees with the observation that DNA binding precedes the generation of DNA breakdown products (Joenje & Venema, 1975).

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REFERENCES


