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Transformation in *Escherichia coli*: Studies on the Role of the Heat Shock in Induction of Competence

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Escherichia coli can be rendered competent for DNA uptake by exposure to a heat shock in the presence of divalent cations. We have studied the influence of variations of the incubation temperature in the competence regimen on the efficiency of competence induction, i.e. the efficiency of uptake of DNA into a DNAase resistant form. For cells grown at 37 °C DNA uptake occurs (1) during a heat shock from 0 °C to temperatures between 15 °C and 42 °C (optimal, 30 °C) and (2) after a heat shock from 0 °C to temperatures between 20 °C and 42 °C (optimal, 32 °C) and a subsequent cold shock to 0 °C. Under the latter conditions DNA uptake occurs during incubation of the transformation mixture at 0 °C after the heat shock. In both cases the efficiency of DNA uptake increases as the incubation temperature during the heat shock increases from about 18 °C to about 32 °C. When recipient cells are grown at 22 °C instead of at 37 °C, the temperature range at which competence induction occurs is shifted by 5 °C to lower temperatures. These results indicate that phase transitions of membrane lipids may play a critical role in induction of competence. When recipient cells are shocked from high temperature to low temperature, leakage of the periplasmic β-lactamase occurs; the degree of leakage and the efficiency of competence induction are affected similarly by the temperature range of the shock. This observation indicates that phase transition of membrane lipids causes damage to the outer membrane, and that this damage may be essential for induction of competence.

INTRODUCTION

Escherichia coli can be rendered competent for DNA uptake by a heat shock in the presence of high concentrations of Ca^{2+} ions, as first described for the uptake of phage DNA (Mandel & Higa, 1970). Modifications of this procedure are frequently used to achieve uptake of plasmid or linear chromosomal DNA by *E. coli* (Bergmans *et al.*, 1981; Cohen *et al.*, 1972; Humphreys *et al.*, 1979, Kushner, 1978; Oishi & Cosloy, 1972; Wackernagel, 1973). The efficiency of DNA uptake by *E. coli* is still low; under optimal conditions only a small fraction of the cells (about 0·1–1·0%) is competent for DNA uptake (Bergmans *et al.*, 1980; Humphreys *et al.*, 1979). The mechanism of competence induction and DNA uptake is poorly understood.

We reported recently a modified transformation procedure (Bergmans *et al.*, 1981) which yields higher transformation frequencies for chromosomal donor DNA. In this method recipient cells, mixed with Ca^{2+} and Mg^{2+} ions, are subjected to a heat shock (from 0 °C to 42 °C) followed by a cold shock (from 42 °C to 0 °C) and incubated at 0 °C for 60 min. Recipient cells, treated in this way, appeared to take up DNA during the heat shock as well as after the cold shock during incubation at 0 °C.

In this paper we describe experiments that define more precisely the temperature changes required to render recipient cells competent for DNA uptake. The results show that induction of

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competence in *E. coli* occurs when the cells pass a critical temperature range (between 15–18 °C and 30 °C).

We have previously reported (Bergmans *et al.*, 1981) that leakage of β-lactamase, a periplasmic enzyme, occurs when recipient cells are subjected to a competence regimen involving a heat shock followed by a cold shock, in the presence of divalent cations. In this paper we show that there is a strong parallel in temperature dependency between induction of competence and leakage of β-lactamase. The possible relation between these phenomena is discussed.

METHODS

Bacteria. All strains used were *E. coli* K12 derivatives. PC0031 is a prototrophic strain. AM1095 is a transformable strain, described previously (Hoekstra *et al.*, 1976). AM1283 is a derivative of AM1170 (AM1095 *rpoB*) carrying plasmid R1*drd*19 (Ap^R) (Bergmans *et al.*, 1981).

Media and buffers. All media and buffers used were as described previously (Bergmans *et al.*, 1981).

DNA isolation. Donor DNA was isolated from strain PC0031 as described by Cosloy & Oishi (1973).

Transformation procedure. Cells of strain AM1095 were grown in phosphate-buffered minimal salts medium (Bergmans *et al.*, 1981) at 37 °C (and in some experiments, when indicated, at 22 °C) until cell density, measured in a Klett–Summerson photometer (650 nm filter) reached a value of 30 Klett units. The culture was chilled and kept at 0 °C during subsequent procedures unless otherwise indicated. The culture was harvested, washed once with 10 mM-NaCl and suspended in 0·05 vol. 20 mM-HEPES/NaOH buffer, pH 6·0. Transformation experiments were performed in two ways, both modifications of the transformation procedure described before (Bergmans *et al.*, 1981). *Method 1.* For DNA uptake during the heat shock a 0·3 ml portion of the cell suspension was mixed with 0·1 ml chromosomal DNA (final concentration 60 μg ml^{-1}) and 0·1 ml of a solution containing 150 mM-$CaCl_2$ and 130 mM-$MgCl_2$. This mixture was kept at 0 °C for 10 min, transferred to 42 °C and incubated for 6 min (heat shock). Subsequently, DNAase (400 μg ml^{-1}, Boehringer) was added and the mixture incubated for 15 min at 37 °C to terminate DNA uptake. *Method 2.* For DNA uptake after a heat and cold shock a 0·3 ml portion of the cell suspension was mixed with 0·1 ml of a solution containing 150 mM-$CaCl_2$ and 130 mM-$MgCl_2$. This mixture was kept at 0 °C for 10 min, transferred to 42 °C and incubated for 6 min (heat shock), then transferred to 0 °C (cold shock). After 2 min 0·1 ml DNA was added and the mixture was incubated for 60 min at 0 °C. Subsequently DNAase was added and the mixture was incubated for 30 min at 0 °C.

When indicated, the temperature of the heat shock (in both methods) or the temperature of the cold shock and the subsequent incubation period (in method 2) was varied. In all experiments transformation frequency was determined as the number of Leu^+ transformants selected on Tris-based minimal agar (Bergmans *et al.*, 1981) divided by the number of viable cells in the same sample.

Assay of β-lactamase leakage. Cells of strain AM1283 were treated in the same way as cells of AM1095 in transformation method 2 except that 0·4 ml samples of the cell suspension were used and no DNA was added. After the heat shock the cell mixture was postincubated for 60 min, centrifuged in an Eppendorf centrifuge and the β-lactamase activity was assayed in the supernatant (diluted 1:20 in assay buffer) essentially as described before (Bergmans *et al.*, 1981). Total β-lactamase activity was assayed in samples of spheroplasts prepared from the same culture, as described by Lugtenberg & Peters (1976).

RESULTS

DNA uptake during the heat shock

The influence of the temperature of the heat shock on transformation frequency was studied in a series of experiments in which transformation mixtures kept at 0 °C were subsequently incubated for 6 min at various temperatures between 0 °C and 42 °C. DNAase was then added and the mixtures were incubated for 30 min at the temperature of the heat shock (a modification of transformation method 1). The results (Fig. 1 A) show that appreciable transformation frequencies were only obtained when the transformation mixture was transferred from 0 °C to a temperature between 15 °C and 42 °C. Transformation proceeded most efficiently when a shock temperature of 30 °C was applied.

DNA uptake after a heat and cold shock

DNA uptake is possible when DNA is added to recipient cells that have first been heat shocked in the presence of Ca^{2+} and Mg^{2+} ions. In the experiments described by Bergmans *et al.* (1981) effective DNA uptake only occurred when heat shocked cells were returned to 0 °C, i.e. in cells that had been treated by a heat shock and a cold shock. For a more precise determination of

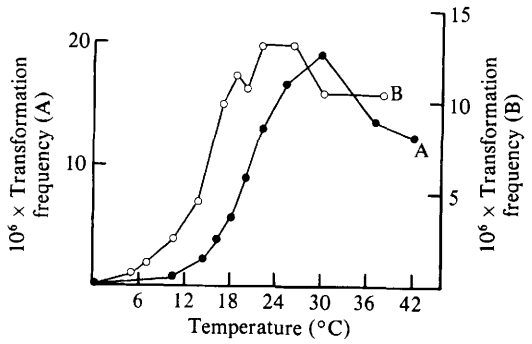

Fig. 1. Development of competence during a heat shock from 0 °C to various temperatures. Transformation mixtures, containing *E. coli* AM1095 recipient cells, Ca^{2+} and Mg^{2+} ions and DNA, were transferred from 0 °C to the temperatures indicated on the abscissa. After 6 min incubation at this temperature, DNAase was added and the mixtures were incubated for a further 30 min. Subsequently the frequency of Leu^+ transformants per viable cell was determined. Recipient cells were grown at 37 °C (●, curve A) or 22 °C (○, curve B). The results presented are the average of two independent experiments.

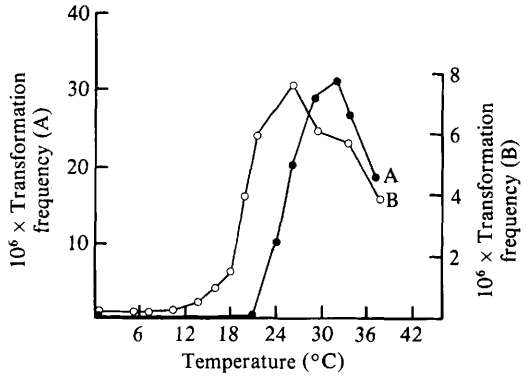

Fig. 2. Development of competence after a heat shock to various temperatures followed by a cold shock to 0 °C. Transformation mixtures, containing AM1095 recipient cells, Ca^{2+} and Mg^{2+} ions, were transferred from 0 °C to the temperatures indicated on the abscissa. After 6 min incubation at this temperature the mixtures were transferred to 0 °C. After 2 min DNA was added and the mixtures were incubated for 60 min at 0 °C. DNAase was then added and the mixtures were incubated at 0 °C for a further 30 min. Subsequently the frequency of Leu^+ transformants per viable cell was determined. Recipient cells were grown at 37 °C (●, curve A) or 22 °C (○, curve B). The results presented are the average of two independent experiments.

the conditions required for effective DNA uptake after the heat shock two experiments were performed (both modifications of transformation method 2): (A) recipient cells kept at 0 °C were incubated for 6 min at various temperatures between 0 °C and 42 °C and subsequently transferred to 0 °C for 60 min; (B) recipient cells kept at 0 °C were subjected to a heat shock of 6 min at 42 °C and subsequently postincubated at various temperatures between 0 °C and 42 °C. In both experiments DNA was added after the heat shock and cold shock. The results of these experiments are presented in Figs 2A and 3A, respectively. The results in Fig. 2A show that transformation occurred when a heat shock from 0 °C to a temperature between 20 °C and 42 °C, followed by a cold shock, was applied. Optimal transformation frequency was achieved after a shock temperature of 32 °C. The results in Fig. 3A show that DNA was most efficiently taken up when postincubation was at 0 °C. The results in Figs 2A and 3A can be interpreted by

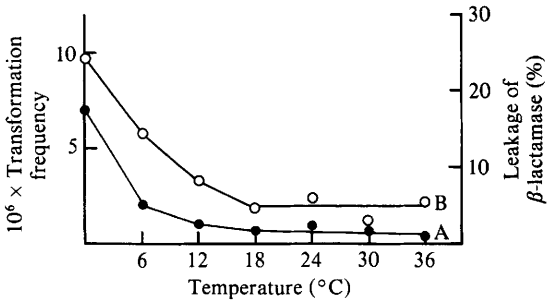

Fig. 3. Development of competence and leakage of periplasmic enzymes after a heat shock to 42 °C followed by a cold shock to various lower temperatures. In the transformation experiments mixtures of AM1095 cells and Ca^{2+} and Mg^{2+} ions were transferred from 0 °C to 42 °C. After 6 min the mixtures were transferred to the temperatures indicated on the abscissa and 2 min later DNA was added. After 60 min incubation DNAase was added and the mixtures were incubated for a further 30 min at 0 °C. Subsequently the frequency of Leu^+ transformants per viable cell (●, curve A) was determined. To determine leakage of β-lactamase a similar experiment was performed except that AM1283 cells were used and no DNA was added. After the 60 min postincubation period β-lactamase activity in the medium (○, curve B) was determined.

assuming that a cold shock alone, from a temperature between 20 °C and 42 °C to 0 °C, is sufficient to allow DNA uptake at 0 °C. Transformation experiments in which the heat shock was omitted were therefore performed. Cells were grown, harvested and washed at 37 °C, then HEPES buffer, $CaCl_2$ and $MgCl_2$ were added at 37 °C and cells were transferred to 0 °C, after which DNA was added. Under these conditions transformation indeed occurred, with a frequency of 10^{-6}–5×10^{-6}. Although this frequency is lower than obtained under standard conditions it is far above background (i.e. 10^{-8} Leu^+ revertants per viable cell; Bergmans *et al.*, 1981).

Lipid phase transitions

From the results presented in Figs 1 A, 2 A and 3 A it is clear that DNA uptake occurred most efficiently when the recipient cells had passed a critical temperature range between about 18 °C and 32 °C. As this is the temperature range in which membrane lipids change from solid phase to fluid phase and vice versa (Nichol *et al.*, 1980) phase transitions of membrane lipids could play an important role in the induction of competence.

Further evidence for the importance of phase transition of membrane lipids in the development of competence was obtained in transformation experiments with recipient cells grown at 22 °C instead of 37 °C. Under these conditions the relative amount of unsaturated fatty acids in the membrane lipids is increased, which causes a decrease of the transition temperature of the membrane (Nakayama *et al.*, 1980). The experiments described in Figs 1 A and 2 A were repeated with cells grown at 22 °C. The results (Figs 1 B and 2 B, respectively) show that the critical temperature range that had to be passed in order to get DNA uptake was lowered by about 5 °C when compared with cells grown at 37 °C. At the same time it is clear that the level of competence reached in the cell population appeared lower when cells were grown at 22 °C, as the highest transformation frequency observed was only 60% (Fig. 1) or 25% (Fig. 2) of the highest transformation frequency observed with cells grown at 37 °C.

Correlation between leakage of β-lactamase and competence induction

When cells were subjected to a competence regimen involving a heat shock from 0 °C to 42 °C, followed by a cold shock to 0 °C (method 2), leakage of β-lactamase from the periplasm occurred, indicating severe outer membrane damage. Extensive leakage of β-lactamase only occurs under conditions when DNA uptake is effective, e.g. when divalent cations are present during the heat shock and cold shock (Bergmans *et al.*, 1981).

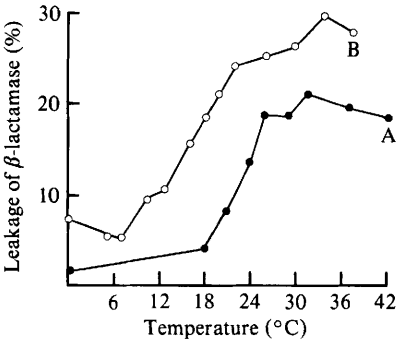

Fig. 4. Leakage of periplasmic enzymes after a heat shock to various temperatures followed by a cold shock to 0 °C. Mixtures of AM1283 cells and Ca^{2+} and Mg^{2+} ions were transferred from 0 °C to the temperatures indicated on the abscissa. After 6 min incubation at this temperature the mixtures were transferred to 0 °C. After a postincubation period of 60 min at 0 °C β-lactamase activity in the medium was determined. Cells were grown at 37 °C (●, curve A) or 22 °C (○, curve B). The results presented are the average of two independent experiments.

The correlation between leakage of β-lactamase and DNA uptake was further tested in experiments with strain AM1283, performed in a similar way as described for DNA uptake after the heat shock. The results, presented in Figs 4A and 3B show that leakage of β-lactamase is dependent on the shock temperature and on the postincubation temperature in a quite similar way to DNA uptake. Leakage of β-lactamase from the periplasm was also tested with AM1283 grown at 22 °C in a similar experiment to that described in Fig. 4A. The results (Fig. 4B) show that the temperature range at which leakage of β-lactamase occurs is lowered. These results indicate that the loss of integrity of the outer membrane after a heat shock and cold shock might be the consequence of lipid phase transitions in the membrane. However, contrary to transformation, leakage of β-lactamase occurs more extensively when the cells are grown at 22 °C than at 37 °C.

DISCUSSION

Escherichia coli cells can be rendered competent for DNA uptake by subjecting them to a temperature shock either from low to high temperature or from high to low temperature, in the presence of divalent cations. Efficient DNA uptake only occurs if a critical temperature range is passed during the heat shock or cold shock. The temperature dependency of both forms of DNA uptake is influenced by the growth temperature of the bacteria. On these observations we base our hypothesis that the physical state of membrane lipids plays a crucial role in the Ca^{2+}-mediated transformation process.

The results relating to competence induction in cells that are transferred from low to high temperature suggest that DNA uptake occurs during phase transition of the membrane lipids from solid to liquid. We suggest that the discontinuities which occur in membrane lipid packing at the edges of solid and fluid domains, and also at the edges of lipid and aggregated membrane protein (Verkleij, 1975), could function as sites where DNA can cross the membrane during phase transition. These discontinuities probably disappear as the membrane turns into its fluid state. In agreement with this, no more DNA is taken up at high temperature once the transition temperature range has been passed, as can be concluded from the results in Fig. 3A. Also, we have observed (results not shown) that during a heat shock from 0 °C to 42 °C all DNA uptake occurred within 20 s of the heat shock, when the incubation temperature rose from 0 °C to 30 °C. After that no more DNA was taken up, although excess DNA was used.

The results relating to competence induction in cells that are transferred from high to low

temperature show that DNA uptake can also occur during incubation at 0 °C, after cells have passed the phase transition temperature range. Possibly the discontinuities, generated during phase transition, are maintained at 0 °C, which facilitates DNA uptake over a prolonged period. The leakage of β-lactamase which occurs under these conditions indeed indicates that severe damage is inflicted on the outer membrane. For both forms of DNA uptake CA^{2+} ions are absolutely essential and Ca^{2+} or Mg^{2+} ions have to be present during the heat shock (Bergmans *et al.*, 1981). The role of these ions remains unknown although Ca^{2+} could be involved in the binding of DNA to the outside of the cells (Reynders *et al.*, 1979; Weston *et al.*, 1981) or act as electroneutralizers of the DNA molecules as hypothesized by Grinius (1980). On the other hand Ca^{2+} (and also Mg^{2+}) will have an effect in altering the physico-chemical behaviour of lipids. It is known that Ca^{2+} can enhance the phase transition of phosphatidylglycerol (Verkleij *et al.*, 1974) and lipopolysaccharide (Alphen *et al.*, 1980). Moreover, Ca^{2+} can induce non-bilayer structures in the total lipids of *E. coli* (Burnell *et al.*, 1980). The formation of such non-bilayer structures affects the stability of the bilayer (Gerritsen *et al.*, 1980; Mandersloot *et al.*, 1981).

Besides the integrity of the outer membrane, the permeability of the cytoplasmic membrane will also be affected by the competence regimen. Under normal culture conditions a cold shock induces permeability changes in the cytoplasmic membrane of *E. coli*, which results in release of cations and small non-electrolyte molecules from the cytoplasm, (Haest *et al.*, 1972; Hegarty, 1940; MacLeod & Calcott, 1976). These permeability changes have been explained as a consequence of the transition of membrane lipids from the liquid to the solid state (Haest *et al.*, 1972). We could not detect any leakage of β-galactosidase, however, which indicates that the cytoplasmic membrane had not been severely damaged (Bergmans *et al.*, 1981).

Competence induction and occurrence of outer membrane damage are apparently both dependent on lipid phase transitions. As we have argued before (Bergmans *et al.*, 1981) the correlation between the two phenomena is not straightforward. The fraction of the cells rendered competent (1% at most) is much lower than the fraction of periplasmic β-lactamase that leaks out of the cells (approx. 30%). The present experiments show that cells grown at 22 °C are not as competent as cells grown at 37 °C, but release more β-lactamase when subjected to the same competence regimen. The difference may be due to the fact that only competent cells have received some form of membrane damage without losing viability.

Possibly the influence of phase transitions on competence induction is restricted to the outer membrane. In spheroplast and revertible spheroplast systems, in which the outer membrane barrier has been destroyed, DNA can cross the cytoplasmic membrane without heat shock and divalent cations (Benzinger *et al.*, 1971; Guthrie & Sinsheimer, 1960; Suzuki & Szalay, 1979). How and when the cytoplasmic membrane is crossed in the Ca^{2+}-mediated transformation systems is not clear. If we assume that DNA has to cross the cytoplasmic membrane in order to be protected against externally added DNAase, our results imply that passage occurs even at 0 °C. DNA uptake then could be an energy-independent process (Sabelnikov & Domaradsky, 1981). If we assume that DNA is already protected against DNAase when it has only crossed the outer membrane, our results do not exclude the possibility that DNA crosses the cytoplasmic membrane when the cells are plated and incubated at 37 °C, possibly in an energy-dependent process.

The growth phase of batch cultures and also growth rate and nutrient limitation in chemostat cultures greatly influence competence in plasmid transformation (Brown *et al.*, 1979; Jones *et al.*, 1981). For both observations it was suggested that changes in competence could be due to some change in membrane constitution. These results may support our hypothesis, as changes in membrane constitution probably influence phase transition; moreover, we have observed that uptake of pBR322 DNA is dependent on the shock temperature in a similar way to the uptake of chromosomal DNA (results not shown). Therefore we assume that the interpretations of the results presented in this paper can be extended to plasmid DNA uptake also.

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