Gene, 6 (1979) 23—28

PROLONGED INCUBATION IN CALCIUM CHLORIDE IMPROVES THE COMPETENCE OF *Escherichia coli* CELLS

(Transformation with pBR322 plasmid DNA; recombinant DNA; ampicillin resistance)

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(Received January 8th, 1979)
(Accepted March 5th, 1979)

SUMMARY

Escherichia coli cells are 4—6 times more transformable and 20—30 times more competent after 24 h incubation in cold calcium chloride than immediately after calcium chloride treatment. With 24-h-old competent cells we obtained routinely $2 \cdot 10^7$ transformants per μg of pBR322 DNA, and transformed over 20% of viable cells.

INTRODUCTION

E. coli cells treated with calcium chloride solutions in an appropriate way become competent for DNA transformation. This was first described by Mandel and Higa (1970) who used phage λ DNA and was subsequently repeated with *E. coli* chromosomal (Oishi and Cosloy, 1972) and plasmid DNAs (Cohen et al., 1973).

The calcium chloride treatment generally used permits one to obtain *E. coli* competent cells which yield over 10^6 transformants per μg of pBR322 DNA (Bolivar et al., 1977). Recently, a somewhat more complex procedure, involving treatment of cells with rubidium, in addition to calcium chloride, has been reported (Kushner, 1978). Competent cells of *E. coli* strain SK1590, prepared in this way, yielded over 10^7 transformants per μg of pBR322 DNA. 3.7—5.5% of cells were transformed with saturating amounts of DNA (Kushner, 1978). Norgard et al. (1978) studied the factors influencing transforming efficiency of the disabled *E. coli* K-12 strain χ 1776, and were also able to obtain about 10^7 transformants per μg of pBR322 DNA.

We describe here a simple modification of the original calcium chloride

procedure, consisting essentially of prolonged (24 h) incubation of cells in calcium chloride. Competent cells, prepared in such a way, yield over 10^7 transformants per μg of pBR322 DNA, and over 20% of the cells can be transformed.

MATERIALS AND METHODS

The following method of inducing competence was used routinely. A single colony from an overnight L-agar plate was inoculated into 50 ml of L-broth and incubated, with shaking, at 37°C until the A_{650} reached 0.2 (we obtained about two times lower competence with cultures grown to A_{650} of 0.5, in agreement with the results of Norgard et al., 1978). The culture was then chilled for 10 min on ice, and the cells were harvested by centrifugation. The pellet was resuspended in 20 ml of cold (0°—4°C) 0.1 M $CaCl_2$ and incubated at 0°C for 20—25 min. Cells were harvested by centrifugation as above and resuspended in 0.5 ml of 0.1 M $CaCl_2$. They were then kept in ice until used.

Transformation was carried out by adding 0.01 ml of suitably diluted pBR322 DNA to 0.1 ml of cell suspension. The mixture was incubated on ice for 10 min, and then at 37°C for 5 min. It was diluted with 2 ml of L-broth and incubated for 1 h at 37°C with shaking. 10 μl of the culture was spread on an L-plate supplemented with 100 μg/ml of ampicillin, since spreading 100 μl gave reproducibly only 5 times more transformants. A similar effect was noted by Norgard et al. (1978). This difference was not observed when the selection was performed on L-plates supplemented with 15 μg/ml of tetracycline. When more than about 1000 transformants per plate were expected, a suitable dilution of the culture was plated and used to determine the number of transformed cells.

RESULTS

Transforming activity of pBR322 DNA on calcium-treated *E. coli* competent cells is shown in Fig. 1. About $3 \cdot 10^6$ transformants per μg were obtained with non-saturating amounts of DNA, when the cells were transformed immediately after calcium chloride treatment. However, $2 \cdot 10^7$ transformants per μg were obtained with cells incubated in calcium chloride for 24 h at 0°C prior to adding DNA. The six-fold difference persisted throughout the whole range of DNA concentrations, indicating that the cells are not only more efficiently transformed, but also that the fraction of competent cells is higher if the preparation is incubated for 24 h before use.

The effect of prolonged incubation in calcium chloride on the viability and transformability of *E. coli* cells (expressed as a number of transformants per μg of pBR322 DNA under standard conditions) is summarized in Fig. 2.

The viability decreases steadily throughout the period examined, to about 3% of the original value. Transformability, on the other hand, reaches a maximum after the first 24 h, and then decreases at essentially the same rate as the cell viability.

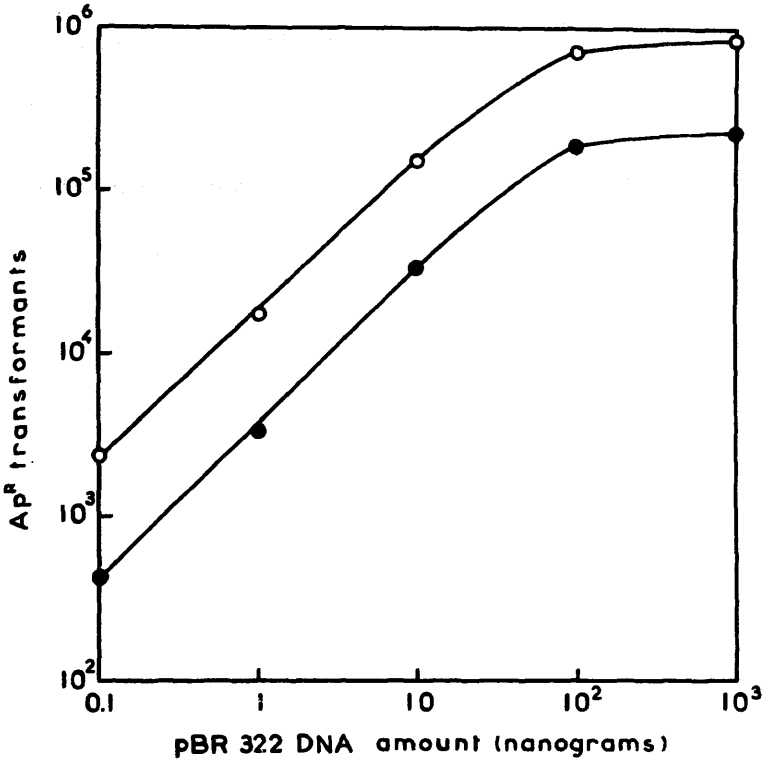

Fig. 1. Transforming activity of pBR322 DNA on *E. coli* SK1590 competent cells immediately after inducing competence (●) and after 24-h incubation in calcium chloride (○).

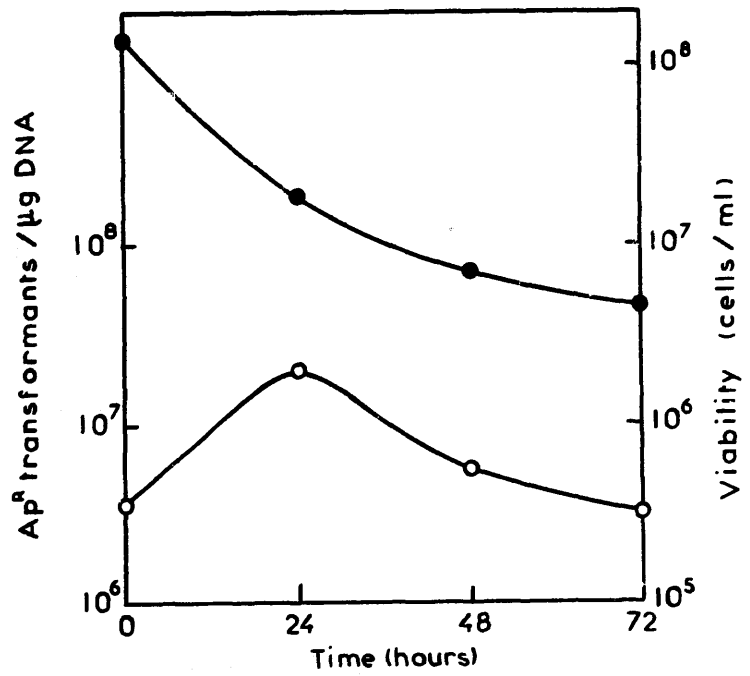

Fig. 2. Effect of incubation in calcium chloride on the viability (●) and transformability (○) of SK1590 competent cells.

The effect of incubation in calcium chloride on cell competence (expressed as a fraction of cells transformed at saturating DNA concentration) is shown in Fig. 3. Less than 1% of cells were transformed at zero time, while over 20% were transformed after an incubation of 24 h. No further change was observed, although, of course, the number of transformants decreased due to the decrease in cell viability.

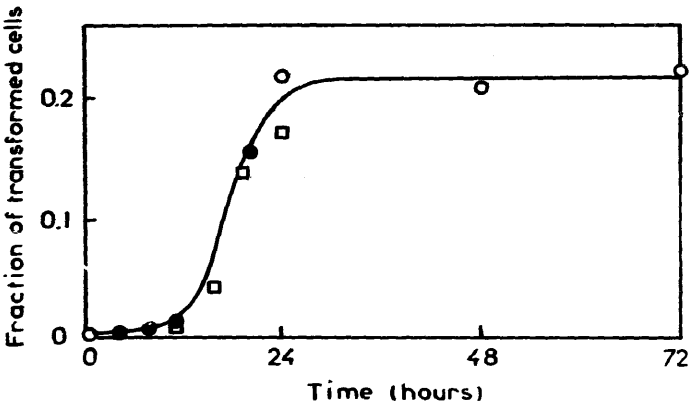

Fig. 3. Effect of incubation in calcium chloride on the competence of SK1590 cells. Different symbols refer to different experiments.

The level of competence induced by prolonged incubation of *E. coli* cells in calcium chloride appeared to be high enough to allow the detection of transformed cells without selecting for a plasmid-borne marker, provided that an appropriate screening method is available. This was tested in a model system, where 24-h-old SK1590-competent cells were transformed with pBR322 DNA, and spread on nonselective medium. Several hundred colonies thus obtained were tested by replica-plating on medium supplemented with ampicillin or tetracycline. About 10% appeared resistant to the two drugs which indicates that they harbored the pBR322 plasmid. Such a high fraction of transformed cells would readily allow the detection of a cryptic plasmid. In this case screening would be for the physical presence of extrachromosomal DNA, using perhaps the standard clear lysate technique rather than a genetic test as described for pBR322 DNA.

A discrepancy between the fraction of pBR322-transformed cells detected by screening as described above (10%) and that observed when the selection for ampicillin resistance after transformation was used (22%, see also Table I) is worth noting. The reasons are not clear at present, but may include plasmid loss from the transformed cells in the absence of the selective pressure, before it has become definitely established.

Several strains commonly used in our laboratory were tested to determine whether the prolonged calcium chloride treatment would affect their transformability and competence. The results are summarized in Table I.

All of the strains can be transformed more efficiently (4—6 times) if the competent cells are incubated for 24 h in calcium chloride, before being exposed to DNA. The highest levels, $2 \cdot 10^7$ transformants per μg of DNA

TABLE I

EFFECT OF 24 h INCUBATION IN CALCIUM CHLORIDE ON THE TRANSFORMABILITY AND COMPETENCE OF *E. coli* CELLS

Strain[a]	Transformants/μg of DNA[b]		Transformed cells[c]	
	0 h	24 h	Viable cells	
			0 h	24 h
C600	$4.1 \cdot 10^6$	$2.1 \cdot 10^7$	0.006	0.19
SK1590	$4.4 \cdot 10^6$	$2.3 \cdot 10^7$	0.007	0.22
HVC45	$3.9 \cdot 10^6$	$1.4 \cdot 10^7$	0.005	0.10
HB101	$2.6 \cdot 10^6$	$8.8 \cdot 10^6$	0.002	0.04
ts214	$1.9 \cdot 10^6$	$9.1 \cdot 10^6$	0.002	0.05

[a] C600 is *thr-1 leu-6 thi-1 supE44 lacY1 tonA2* (Bachman, 1972), SK1590 is *thi gal sbcB15 endA hsdR4 hsdM*$^+$ (Kushner, 1978), HVC45 is *pro leu thr thi supE44 lac tonA hsdR*$^-$ *hsdM*$^+$ Sm^R (our collection), HB101 is *thr leu thi recA hsdR*$^-$ *hsdM*$^-$ (from H. Boyer), ts214 is *thy his argG metB leu xyl lacY strA polAts214* (Kingsbury and Helinski, 1973).
[b] 1 ng of pBR322 DNA per 100 μl of competent cells was used.
[c] 0.5 μg of pBR322 DNA per 100 μl of competent cells was used.

were obtained with C600, SK1590 and HVC45 strains. The first two were also the most competent, since 19—22% of the cells could be transformed with saturating DNA concentrations. Somewhat lower transformability and competence were obtained with HB101 and ts214 strains. We do not know the reasons for this difference.

Transfection of the *E. coli* strain SK1590 with λ Charon 4 DNA (Blattner et al., 1977) gave a result consistent with the pBR322 transformation data: about 5 times more plaques were obtained when 24 h old rather than fresh competent cells were used, corresponding to over 10^7 plaques per μg of DNA.

DISCUSSION

The modified calcium chloride procedure described here does not require any additional manipulations, as compared with the standard method, and reproducibly yields cells which are about 5 times more efficiently transformed and are up to 20—30 times more competent. The increase in the time of preparation was not found to be a disadvantage. On the contrary, we were routinely able to test the competence of the cells immediately after calcium chloride treatment, and adjust the conditions of the experiments performed 24 h later accordingly.

ACKNOWLEDGEMENTS

We thank Dr. A. Goze for his involvement and continued interest in this work. M.D. was a recipient of a Universidad de Los Andes, Mérida, Venezuela

fellowship. S.D.E. is on the INSERM research staff. This work was supported, in part, by the grant from Université Paris VII.

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Communicated by P. Kourilsky.