

Ba²⁺-Induced Competence for Transfecting DNA

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Effect of alkaline earth metal ions on induction of the competence for DNA transfection was investigated. Unlike spheroplasts, the bulk of the bacteria treated with these ions retains colony-forming ability. The order of effectiveness for transfection of Φ A replicative-form DNA has been found to be Ba²⁺ > Ca²⁺ > Sr²⁺ > Mg²⁺. The competence of Ba²⁺-treated cells is 3 to 5 times higher than that of Ca²⁺-treated bacteria and about 40 times higher than that of lysozyme-EDTA spheroplasts. The Ba²⁺-dependent transfection is cryophilic and formation of the infective complex occurs very rapidly at 0 °C, but not at 37 °C.

Introduction

In 1970, Mandel and Higa¹ reported that cells of *Escherichia coli* treated with chilled calcium chloride were readily transfected by lambdoid phage DNA. The calcium chloride method is very simple and mild as compared with spheroplast- or helper phage-systems. Moreover, bulk of the cells remains viable after the Ca²⁺ treatment. Taking advantage of these properties of the Ca²⁺ system, not only transfection but also transformation by various DNA species has been achieved in *E. coli*²⁻⁶. In order to elucidate the DNA-uptake mechanism as well as to clarify various factors and conditions affecting the practical use, we have investigated the idiosyncrasy of the Ca²⁺-dependent transfection system in detail^{7,8}. In an extension of these studies, we have compared the competence-inducing effect of other alkaline earth metal ions with that of Ca²⁺. Present results show that the most effective ion for the competence development is Ba²⁺.

Materials and Methods

Unless otherwise specified, *E. coli* strain C was used throughout. The SS DNA and the RF of Φ A were prepared as described previously⁹. The infectivity of these DNA was calibrated by a lysozyme-EDTA spheroplast system^{2,10}. For induction of the competence, bacteria were grown in nutrient broth at 37 °C with shaking. At a density of $A_{660} = 0.7$

(measured by a Bausch & Lomb Spectronic 20 spectrophotometer), the culture was chilled in ice-water, sedimented, and suspended in 1/2 volume of chilled 0.1 M BaCl₂. After standing at 0 °C for 30 min the bacteria were collected by a centrifugation, resuspended in chilled 0.1 M BaCl₂ at a density of $A_{660} = 15$ and preserved at 0 °C. Transfection was carried out as follows: To the competent cell suspension (usually 0.1 ml), 1/2 volume of DNA in chilled 50 mM Tris-HCl, pH 7.5 was added and mixed at 0 °C. After chilling for 20 min, the infected complex was diluted with chilled 0.1 M BaCl₂ and plated with the indicator bacteria on nutrient agar. Treatment with other alkaline earth metal ion was performed similarly.

Results

In preliminary experiments, cells of *E. coli* C were treated with chilled 0.05 M solutions of CaCl₂, BaCl₂ or SrCl₂ and their competence for Φ A RF was compared. The relative efficiency of competence induction was found to be Ba²⁺ : Ca²⁺ : Sr²⁺ = 1 : 0.24 : 0.10. Consequently, several conditions for the Ba²⁺-dependent transfection were further examined. As shown in Fig. 1, concentration of BaCl₂ required to induce maximal competence for RF was nearly 0.1 M and that for SS was about 0.08 M. Competence of the bacteria treated with 0.1 M solutions of alkaline earth metal compounds is presented in Table I. Efficiency of SS transfection was

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Abbreviations: SS, single-stranded virus DNA; RF, double-stranded replicative form DNA.

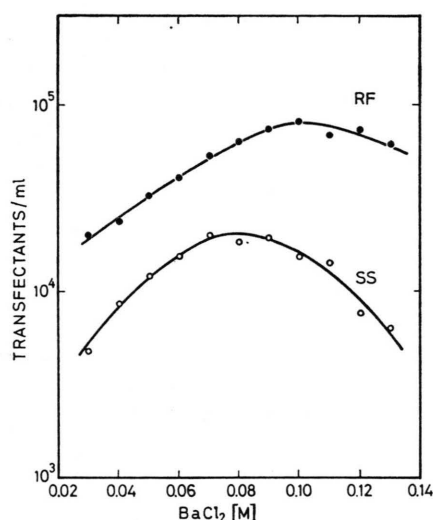


Fig. 1. Effect of BaCl₂ concentration on induction of the cellular competence. Bacteria were treated with various concentrations of chilled BaCl₂ and the competence for Φ A RF (●) or SS (○) was determined.

relatively high in CaCl₂-treated cells and considerably low in SrCl₂-treated bacteria. In production of competence for RF, however, BaCl₂ was 3 to 5 times efficient than CaCl₂ and the maximal competence of the Ba²⁺-treated cells was about 40 times higher

Table I. Competence of the cells treated with alkaline earth metal ions. Cells of *E. coli* C treated with each compound (0.1 M) were tested for their competence for Φ A SS and RF. Figures in parentheses indicate ratio of the cellular competence to that of the bacteria treated with 0.1 M CaCl₂.

DNA	MgCl ₂	CaCl ₂	Plaque yield			
			SrCl ₂	BaCl ₂	Ba(NO ₃) ₂	
SS	2.6 × 10 ⁶ (0.87)	3.0 × 10 ⁶ (1)	3.0 × 10 ⁵ (0.10)	2.0 × 10 ⁶ (0.66)	1.4 × 10 ⁶ (0.45)	
RF	1.3 × 10 ⁶ (0.026)	5.1 × 10 ⁷ (1)	2.6 × 10 ⁷ (0.51)	2.1 × 10 ⁸ (4.1)	1.2 × 10 ⁸ (2.4)	

than that of lysozyme-EDTA spheroplasts. It is clear that Ba²⁺ ion *per se* is the principal factor in competence induction, since Ba(NO₃)₂ as well is highly effective. As reported previously⁸ Mg²⁺, even at 0.1 M, was rather ineffective for RF transfection.

Colony-forming ability of the bacteria was not significantly affected by the treatment with alkaline earth metal ions (Table II). In chilled 0.1 M BaCl₂, the cellular competence could be stably maintained for 2 to 3 days. Fig. 2 shows a relationship between DNA concentration and the yield of transfectants. Over a wide range, the number of infective centers

Table II. Viability of cells suspended in alkaline earth metal salt solution. Bacteria were grown in nutrient broth at 37 °C with shaking and harvested at a density of $A_{660} = 0.7$. Ten ml aliquots of the chilled bacteria (1.5×10^9 viable cells/ml) were sedimented and suspended in 5 ml of the indicated media chilled at 0 °C. Viability of the treated cells was assayed 30 min or 24 h after suspension.

Media	1st suspension (after 30 min)	2nd suspension	
		(after 30 min)	(after 24 h)
0.1 M BaCl ₂	1.4×10^{10}	1.3×10^{10}	1.2×10^{10}
0.1 M Ba(NO ₃) ₂	1.4×10^{10}	1.2×10^{10}	1.1×10^{10}
0.1 M CaCl ₂	1.5×10^{10}	1.3×10^{10}	1.2×10^{10}
0.1 M SrCl ₂	1.5×10^{10}	1.3×10^{10}	1.1×10^{10}

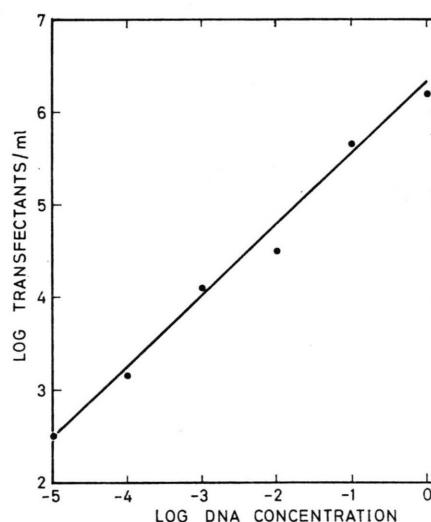


Fig. 2. Effect of DNA concentration. Φ A RF was serially diluted with 50 mM Tris-Cl, pH 7.5 and infectivity of each sample was assayed using the 0.1 M BaCl₂-treated hosts.

was directly proportional to the concentration of input DNA. In chilled BaCl₂ solution, formation of infective complex occurred very rapidly and within 20 to 30 sec after mixing, the yield of infective centers reached the plateau level (Fig. 3). The complex formed at 0 °C was stable in chilled BaCl₂ solution, but the infectivity was reduced by simple dilution into nutrient broth. Upon brief incubation at 37 °C, the complex became resistant to the interference by nutrient broth, though the heat pulse *per se* reduced yield of the transfectants by about 50%. (In practical use therefore, the infected complex has to be diluted with chilled BaCl₂ solution, without heat pulse.) As in Ca²⁺-dependent transfection, low temperature condition is essential at least for an early phase of Ba²⁺-dependent DNA-uptake. Thus, when DNA and the recipient cell suspension were mixed at 37 °C for 2 min and plated directly,

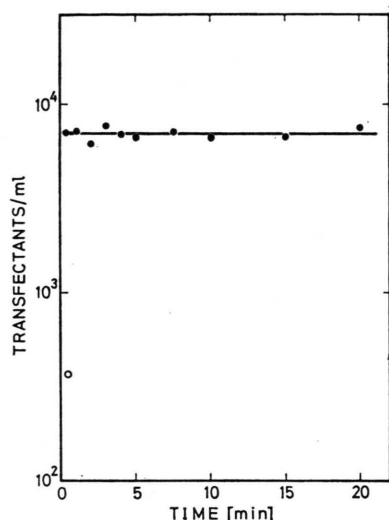


Fig. 3. Time course of infection at 0 °C. The competent cell suspension in 0.1 M BaCl₂ was mixed at 0 °C with Φ A RF. At the indicated time, aliquot was removed and plated directly (●) or after dilution with nutrient broth (○).

plaque yield was less than 5% of the control mixed at 37 °C and then cooled for 2 min at 0 °C before plating.

Discussion

Present data demonstrate that Ba²⁺ is more effective than Ca²⁺ in induction of the competence for transfection of Φ A RF. The Ba²⁺-treated *E. coli* was efficiently infected by Φ X 174 RF or double-stranded Φ T DNA as well (unpublished observation). In current experiments on DNA transformation in *E. coli*, the Ca²⁺-treated cells are generally used as the recipients. The transformation in *E. coli* is, however, less efficient and requires larger amounts of DNA as compared with transformation in other bacteria. Since the Ba²⁺-treated cells are mostly viable, this method may potentially be useful for transformation in *E. coli* or allied bacilli.

The Ba²⁺-dependent transfection system is similar to the Ca²⁺-dependent system in many properties. The Ca²⁺-dependent transfection is strangely cryophilic and this idiosyncrasy has led us to hypothesize that early phase of the DNA-uptake depends on crystallization of surface (phospho)lipids⁸. Although Ba²⁺-induced competence is consistent with the hypothesis, further work is needed to elucidate the complicated mechanism of DNA penetration through cell envelope.

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