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INHIBITION OF DNA-INDUCED TRANSFORMATION BY CONCANAVALIN A IN BACILLUS SUBTILIS

Cho-yam Chu and Kuo-chun Chen

Department of Biological Sciences
Wayne State University
Detroit, Michigan 48202

and

Botany School
University of Cambridge
Cambridge CB2 3EA, England

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SUMMARY

The use of concanavalin A (Con A) as a probe for studying the role of wall teichoic acid in bacterial transformation was investigated. The transformation of lysozyme-treated and untreated competent cultures of Bacillus subtilis strain 168 was found to be inhibited by treatment with Con A. The inhibitory action exerted by Con A was concentration-dependent. The minimum Con A concentration necessary to effect a measurable inhibition of transformation was much lower for the lysozyme-treated than for the untreated bacteria. It was postulated that the wall teichoic acid became more exposed as a result of the lysozyme treatment and, hence, was more accessible to Con A binding. The Con A-mediated inhibition was reversible by α-methyl-D-glucoside.

INTRODUCTION

Teichoic acid has been known as a major component of the cell wall of most gram-positive bacteria and the importance of its role in genetic transformation was first noted in pneumococci. Tomasz (7) reported that upon deprivation of choline, which is a normal constituent of the wall teichoic acid, pneumococcal bacteria showed a significant loss in the reactivity to competent factors and, hence, in the capacity for DNA binding. Moreover, a quantitative replacement of this choline component from the cell wall by its chemical analogues, such as ethanolamine or N-monoethylamine ethanolamine was found to impair the transformation of the organism.

Recently, Doyle and Birdsell (3) have found that the wall teichoic acid of B. subtilis 168 interact selectively with the phytohemagglutinin, concanavalin

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A (Con A) in a reversible manner. This chemical interaction involving Con A and the bacterial cell wall led the authors to investigate the Con A effect and its use as a probe for studying the basic function of the wall teichoic acid in B. subtilis transformation.

MATERIALS AND METHODS

An auxotrophic mutant of B. subtilis strain 168 (trp C2) characterized by its inability to synthesize tryptophan was used as recipient and the prototrophic wild-type strain (SB 19) was employed for the extraction of transforming DNA.

The media for growing culture and transformation tests are as described in a previous publication (2). Spizizen's minimal medium supplemented with 50 μg/ml of nine stimulatory amino acids was used for growing a culture rendered to competence for transformation. The make-up of transformation medium was essentially the same as that of the minimal medium, except for the addition of only 5 μg/ml of the nine amino acids plus L-tryptophan.

Innoculum of the recipient bacteria originally maintained in nutrient agar was transferred to 20 ml Penassay broth in a flask and allowed to grow for 15 h at 37°C with vigorous shaking. Cells were collected by centrifugation and resuspended in growing medium of the same volume. The growing cells were allowed to divide for a period of 4 to 5 h at 37°C and then transferred to transforming medium for development of competence. The prepared competent culture was immediately used in transformation experiments.

The method of Marmur (6) was adopted in the extraction of transforming DNA. It consisted of the following steps: lysis of cells with lysozyme and dupanol, deproteinization of cell lysate with a 4:1 chloroform/N-butanol mixture, precipitation of DNA with absolute ethanol, and removal of the contaminated RNA with RNase treatments.

In the transformation experiment, competent culture of B. subtilis 168 try C2 was exposed to 1.4 μg/ml of SB 19 DNA for 20 min at 37°C with vigorous aeration. The cells/DNA reaction was terminated by adding 10 μg/ml of Mg^{++}-activated DNase into the reaction mixture and the reaction products were allowed to incubate for 10 min. The transformation reaction mixture was then suitably diluted and plated onto minimal and nutrient agar plates for determination of the number of Trp^{+} transformants and the total viable counts.

The experiments with Con A were carried out by treating 0.9 ml of competent culture with 0.05 ml of various concentrations of Con A for 10 min at 37°C. The Con A pretreated bacteria were then subjected to transformation assays in the usual manner. In those cases where cell agglutination was noted following the Con A treatment, cells were immediately dispersed with the aid of a Vortex mixer. This procedure was found to be effective for cell dissociation and, hence, was adopted as a general procedure in the present study.

RESULTS AND DISCUSSION

Inhibition of Transformation by Con A When competent cultures of B. subtilis 168 with a cell density of about 10^8/ml were treated with 500 μg/ml of Con A

at 37°C, there was only a slight microagglutination but no detectable macro-agglutination during a period of 10 minutes incubation. If the Con A administered was in excess of the above threshold dose (500 μg/ml), both micro- and macro-agglutinations were then observed.

To examine the effects of Con A on B. subtilis transformation and cell viability, Con A at the threshold dose level was added into a series of six tubes each of which contained 0.9 ml of recipient bacteria. The controls consisted of a series of three tubes containing the same amount of recipient cells, but without addition of Con A. These two series of tubes were incubated at 37°C for 10 min with constant shaking.

Subsequently, the agglutinated bacteria in one half of the Con A-treated tubes were thoroughly dispersed and the cells in all the other tubes were left undisturbed. All the Con A pretreated bacteria as well as the control cells were then subjected to transformation tests.

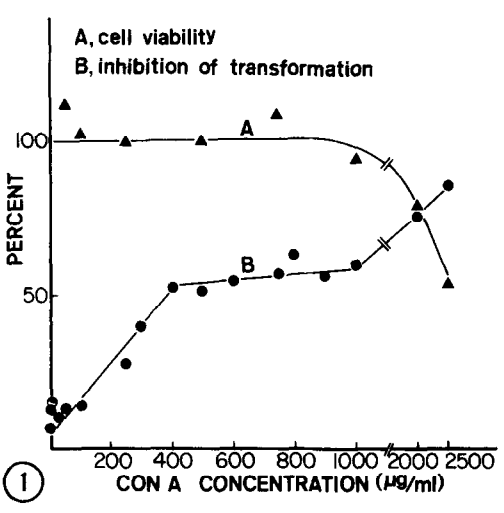
The results, summarized in Table 1, show that Con A treatment resulted in a 16-32% decrease in the number of Trp^+ transformants. However, there was no appreciable effect on the viability of the recipient bacteria following the Con A treatment. The observation that a minimum of 16% reduction in transformation efficiency was found with the Con A-treated cells after thorough dissociation, and its significance were further examined by the experiments discussed below.

Enhanced Inhibition by Lysozyme Treatment It was previously reported (4) that cell wall preparation isolated from B. subtilis 168, when treated with lysozyme or autolysin, would bind twice the amount of isotopically labeled Con A found in the control. This suggests that approximately one-half of the

Table 1 Inhibitory Effect of Con A on Genetic Transformation of B. subtilis

Con A Addition (μg/ml)	Cell Agglutination	Cells Dispersed prior to DNA Exposure	Trp^+ Transformants (No./ml) x 10^4	Colony Forming Unit (x 10^8)	Inhibition %
0	-	None	8.14	1.51	0
500	+	Yes[1]	6.83	1.46	16.1
500	+	None	5.52	1.54	32.2

[1]Cells thoroughly dispersed by a Vortex mixer.



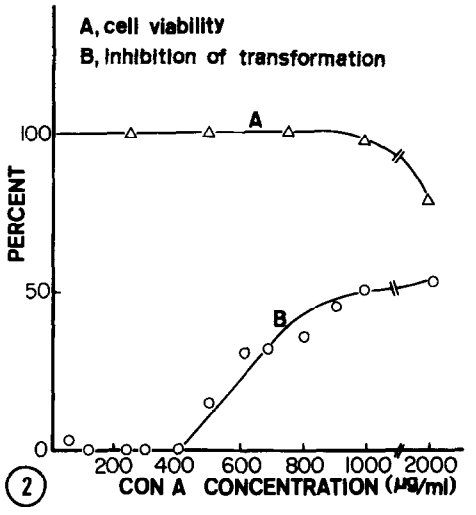


Figure 1 Effect of Concanavalin A on Lysozyme-Treated Competent Culture of B. subtilis.

Figure 2 Effect of Concanavalin A on Competent Culture of B. subtilis without pretreatment with lysozyme.

teichoic acid molecules are exposed at the wall surface, whereas the remainder are embedded within the peptidoglycan matrix. Therefore, a study was made using lysozyme treatment to ascertain the involvement of cell wall teichoic acid in the Con A-mediated inhibition of B. subtilis transformation. Figure 1 shows the results obtained with lysozyme-treated cells. In the range of Con A concentrations tested, an appreciable amount of inhibition was found at a Con A dose as low as 0.5 µg/ml. It is of interest to note in Figure 1 that at Con A concentration below 400 µg/ml, the number of Trp^+ transformants produced is inversely proportional to the Con A dose. The inhibitory action appears to level off above a concentration of 400 µg/ml, indicating saturation of receptor sites on the bacterial surface by Con A at the high concentrations.

The results shown in Figure 2 indicate that competent cultures without prior lysozyme treatment did not show any inhibition until a Con A concentration of 400 µg/ml was reached. Since at 400 µg/ml of Con A a typical microagglutination was produced without affecting transformation frequency of the Trp^+ marker, the cell agglutination is not a contributing factor responsible for the inhibitory effect observed after the Con A treatment. However, above a Con A concentration of 500 µg/ml, inhibition of transformation increases with the amount of Con A added up to a concentration of about 1,000 µg/ml, beyond which the

Table 2 Effect of α-Methyl-D-Glucoside (α-MG) on Cell Competence

α-MG Concentration (μg/ml)	Number of Trp^+ Transformants/ml With Preincubation[1]	Without Preincubation[2]
0	0.52×10^4	1.92×10^4
5	0.50×10^4	---
10	0.66×10^4	---
25	0.47×10^4	2.04×10^4

[1]Each sample preincubated for 10 min and then incubated for 30 min after α-MG addition.

[2]Samples treated with α-MG for 10 min without preincubation.

inhibitory effect begins to level off. These findings suggest that some of the teichoic acids buried within the peptidoglycan matrix have great affinity for Con A binding, once they become more readily accessible as a consequence of lysozyme treatment.

As also seen in Figures 1 and 2, no detectable effect on cell viability was found up to a Con A concentration of 1,000 μg/ml. Above this Con A dose, there was a decline in cell viability, accompanied by a corresponding reduction in the number of transformed bacteria produced in the lysozyme-treated as well as the untreated cultures.

Reversibility of the Con A Action The hapten inhibition studies made by Goldstein et al. (5) have shown that α-methyl-D-glucoside is one of the most active molecules among a number of monosaccharides and oligosaccharides that interfere with interaction of Con A with polysaccharides. Electron microscopic examinations have further shown that the ability of Con A to bind to the cell wall of B. subtilis 168 becomes ineffective in the presence of α-methyl-D-glucoside. In addition, α-methyl-D-glucoside has been shown also to be capable of reversing the Con A inhibition of phage attachment to the cell walls of B. subtilis 168 (1). Consequently, a study was made using α-methyl-D-glucoside to determine the reversibility of the Con A effect.

The results summarized in Table 2 show that the cell competence as measured by transformation frequency was not affected by α-methyl-D-glucoside over the range of concentration studied, regardless of whether or not there was preincubation before the introduction of α-MG. As shown in Table 3, there was no inhibition in Trp^+ transformation regardless of whether Con A and α-MG

Table 3 Reversibility of Con A-Mediated Inhibitory Effect on Transformation

α-MG Concentration (μg/ml)	Separate Addition of Con A and α-MG[1]		Simultaneous Addition of Con A amd α-MG[2]	
	Trp^+ Transformants (No. /ml)	Inhibition[3] (%)	Trp^+ Transformants (No. /ml)	Inhibition[4] (%)
0	0.23×10^4	55.77	0.58×10^4	69.79
10	0.67×10^4	None	---	---
25	0.53×10^4	None	3.29×10^4	None

[1] Cells incubated with Con A (1 mg/ml) for 10 min, and then for another 30 min following the α-MG addition.

[2] Cells incubated for 10 min after simultaneous addition of Con A (1 mg/ml) and α-MG.

[3] Based on an assay of 0.52×10^4 Trp^+ transformants/ml for the control.

[4] Based on an assay of 1.92×10^4 Trp^+ transformants/ml for the control.

were added at the same time or α-MG was introduced after the addition of Con A. In fact, in the absence of α-MG, a reduction in transformation frequency of about 56-70% was noted, an observation normally expected following exposure of cells to saturating amount of Con A. This greater reduction in the number of Trp^+ transformants after the treatment with 1,000 μg/ml of Con A cannot be explained as due solely to a loss in cell viability, because the percentage of survival for the Con A-treated recipients remains essentially the same (90-95%) for both lysozyme-treated or untreated experiments -- see Figures 1 and 2.

In conclusion, the present results demonstrate that Con A significantly inhibits B. subtilis transformation via its interaction with the surface-exposed teichoic acid of the bacterial cell walls. The extent of this inhibitory effect appears to depend on the Con A concentration and can be effectively reversed by a chemical inhibitor α-methyl-D-glucoside. The exact mechanism whereby the wall teichoic acid is involved in the process of B. subtilis transformation is being investigated.

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