Short Communication

THE BEHAVIOR OF THE BACTERIOPHAGE SPO1 IN ADSORBING TO *BACILLUS SUBTILIS*

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Most of the bacteriophages of *Bacillus subtilis* have been reported as requiring α -D-glucosylated teichoic acid for attachment to the bacterial cell (1–3). The necessity of glucosylated teichoic acid for phage attachment was mainly determined using glucosylated teichoic acid-deficient mutants of bacteria and measuring the unadsorbed phage and plating efficiency (2–7). Using a similar method, YASBIN et al. (5) determined that glucosylated teichoic acid was required for the adsorption of bacteriophages SPO1 and ϕ 29 by a similar method.

In contrast to these reports, we have found that SPO1 does not depend on glucosylated teichoic acid for initial attachment to bacterial cells. In this study we employed a different method from those mentioned above, using concanavalin A, a plant lectin. This substance is reported to bind specifically to glucosylated teichoic acid and to inhibit the attachment of bacteriophage ϕ 25, which requires glucosylated teichoic acid for attachment (8, 9). Figure 1 shows the results. Attachment of SPO1 to the cell of *Bacillus subtilis* strain SR22 (spoA₁₂, trpC₇) (10) was not inhibited by concanavalin A up to a concentration of 2 mg per ml. This was four times the concentration that inhibited ϕ 25 attachment (8). In contrast to the above result with SPO1, adsorption of bacteriophage M2 was completely inhibited by concanavalin A at 0.5 mg per ml. The bacteriophage M2 is very closely related to ϕ 29 (10). The fact that SPO1 was adsorbed to two glucosylated teichoic acid-deficient mutants of bacteria, IA 105 (gtaB) and IA 107 (gtaC), and developed lysis zones on the lawns of these two glucosylated teichoic acid-deficient mutants cells (though less efficiently than the control) also indicates that glucosylated teichoic acid is not essential for the attachment of SPO1 (Both mutant strains were gifts from the Bacillus Stock Center of Ohio State University).

Fig. 1.

Fig. 1. Effect of concanavalin A on the attachment of SPO1 and M2.

The cells precultured at 30°C or 48°C for three generations were centrifuged and resuspended in 1 M Tris HCl buffer (pH 7.0) containing 10^{-3} M $MgCl_2$ and 10^{-5} M $MnCl_2$ at 1×10^7 cells/ml and distributed into small tubes to which indicated concentrations of concanavalin A and phage at moi of 1 were added. Thereafter, these samples were all treated similarly as described in Table 1.

○: cells grown at 30°C with SPO1, △: cells grown at 48°C with SPO1, ●: cells grown at 30°C with M2, ▲: cells grown at 48°C with M2.

Fig. 2.

Fig. 2. Alteration of SPO1 adsorbing ability of the cell with growth temperature.

Experimental procedures and methods were essentially the same as described in Table 1. Initially, the cells precultured at 30°C were transferred to 48°C, and at indicated times, a portion of the cells was taken up to examine SPO1 adsorption (A). The remaining cells at 48°C were returned to 30°C and recovery of SPO1 adsorbing ability was examined at indicated times (B).

Another interesting phenomenon, seen in Fig. 1, is that when cells grown at 48°C were used as the host cells, the unadsorbed fraction of SPO1 was increased compared to the fraction when the cells were grown at 30°C. Such a phenomenon was not observed in the adsorption of M2. Reduction in the SPO1-adsorbing ability of the cells seemed to depend not on the temperature at adsorption but on the temperature of the bacterial growth itself (Table 1).

The presence of chloramphenicol or mitomycin C during incubation of the cells grown at 48°C inhibited changes in the SPO1 adsorbing ability of the cells (Table 1). Unlike SPO1, the other bacteriophages, $\phi 105$ and SPPI in addition to M2 and $\phi 29$ shown in Table 1, were not thermo-sensitive. Among the bacteriophages cited, SPPI was reported to be initially attached to sites other than glucosylated teichoic acid, and these sites were also different from the sites for SPO1 (7). It is more clearly seen in Fig. 2 that the SPO1-adsorbing ability of the cells is correlated with the temperature at which the cells are grown. The bac-

Table 1. Phage-adsorbing ability of the bacteria grown at different temperatures and the effect of inhibitors on that ability.

Phage	Temperature (°C) of		No. of unadsorbed phages ($\times 10^5$ /ml)
	growth	adsorption	
SPO1	30	30	1.63
	30	48	2.20
	48	30	28.31
	48	48	31.40
	48 ^a	48	0.59
	48 ^b	48	4.35
ϕ 29	30	30	8.12
	48	48	5.50
M2	30	30	10.00
	30	48	8.83
	48	30	7.05
	48	48	7.31
	48 ^a	48	6.63

^a Chloramphenicol (20 μ g/ml) (product of Sankyo Ltd. Co.) was added.

^b Mitomycin C (25 μ g/ml) (product of Nakarai Chemicals Ltd.) was added.

A portion of the bacterial cells of strain SR22 (10) growing logarithmically in TY medium (10) at 30°C was taken up and its concentration was adjusted with fresh TY to 1×10^7 cells/ml. After distributing the diluted cells into several tubes, each tube was allowed to grow for two generations at 30°C or 48°C with or without inhibitor. At the end of incubation, the concentration of the cells in each tube was again adjusted with fresh TY to 1×10^7 cells/ml in order to put the cells into contact with phage at moi of 1. After 10 min adsorption at the indicated temperature, the cultures were centrifuged (5,000 rpm, 15 min) and the supernatants of the respective samples were assayed for unadsorbed phage. The method used for plaque assay was as described in the previous paper (10). Through all the experiments, *B. subtilis* strain SR22 was used as the host cells for growth of the phage, adsorption experiments and plaque assay, unless otherwise mentioned.

terial cells precultured at 30°C gradually lost the SPO1-adsorbing ability after generations were transferred 48°C. Meanwhile, if the bacterial cells growing at 48°C were changed to 30°C and allowed to grow at that temperature, the SPO1-adsorbing ability was recovered increasingly with each succeeding generation.

From these results, it can be concluded that α -D-glucosylated teichoic acid may not have a direct role in the initial attachment of SPO1 to the bacterial surface. At present, the detailed structure of the phage receptor is not known. However, from the reports on the structure of the phage receptor of *Escherichia coli* (11, 12), we can assume that the phage receptor of *Bacillus subtilis* is similarly complex and constructed with various materials such as proteins, polysaccharides and teichoic acids. It seems likely that the targeted element of thermosensitivity in the SPO1 receptor would be a protein substance.

REFERENCES

- 1) H. E. HEMPHILL and H. R. WHITELEY, *Bacteriol. Rev.*, **39**, 257 (1975).

- 2) F. E. YOUNG, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 2377 (1967).
- 3) F. E. YOUNG, C. SMITH, and B. E. REILLY, *J. Bacteriol.*, **98**, 1087 (1969).
- 4) E. D. JACOBSON and O. E. LANDMAN, *J. Bacteriol.*, **124**, 445 (1975).
- 5) R. E. YASBIN, V. C. MAINO, and F. E. YOUNG, *J. Bacteriol.*, **125**, 1120 (1976).
- 6) J. LEPESANT-KEJZLAROVA, J. A. LEPESANT, J. WALLE, A. BILLAULT, and R. DEDONDER, *J. Bacteriol.*, **121**, 823 (1975).
- 7) M. A. SANTOS, H. DELENCASTRE, and L. J. ARCHER, *J. Gen. Microbiol.*, **129**, 3499 (1983).
- 8) D. C. BIRDSELL and R. J. DOYLE, *J. Bacteriol.*, **113**, 198 (1973).
- 9) R. J. DOYLE and D. C. BIRDSELL, *J. Bacteriol.*, **109**, 652 (1972).
- 10) J. TAKAGI and H. HIROKAWA, *J. Gen. Appl. Microbiol.*, **25**, 315 (1979).
- 11) A. A. LINDBERG, *Ann. Rev. Microbiol.*, **27**, 205 (1973).
- 12) V. BRAUN and K. HANTKE, *In Microbial Interactions*, ed. by J. L. REISSIG, Chapman and Hall Inc., London (1977), p. 101.