Blocking of Bacteriophage Receptor Sites by Concanavalin A

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Teichoic acids form part of the bacteriophage receptor sites in the walls of several Gram-positive bacteria (Young, 1967; Coyette & Ghuyssen, 1968; Chatterjee, 1969). Concanavalin A interacts with teichoic acids that possess α -linked glucosyl or *N*-acetyl-glucosaminyl substituents (Archibald & Coapes, 1971; Reeder & Ekstedt, 1971) and so might be expected to prevent access of bacteriophages to their receptor sites in walls which contain such teichoic acids. We have examined the influence of Concanavalin A on the binding of bacteriophage K (Ralston, Baer, Lieberman & Kreuger, 1957) to *Staphylococcus aureus* strains 3528, A1 and H, the walls of which contain exclusively α -, exclusively β - and predominantly β -, with some α -, *N*-acetyl-glucosaminyl substituents respectively (Baddiley, Buchanan, Martin & RajBhandary, 1962; Nathenson & Strominger, 1962; Davison, Baddiley, Hofstad, Losnegard & Oeding, 1964). The action of Concanavalin A on the binding of bacteriophage SP-50 (Foldes & Trautner, 1964) to *Bacillus subtilis* strains 3610 and w-23, the wall teichoic acids of which contain respectively α - and β -linked glucosyl substituents (Chin, Burger & Glaser, 1966; Doyle & Birdsell, 1972) has also been studied. Additionally we have studied the effect of Concanavalin A on the infection of *B. subtilis* strains w-23 and 3610 by bacteriophage SP-50 and of *S. aureus* strains 3528 and H by the typing bacteriophages 53 and 52A respectively.

METHODS

Bacteriophages. Bacteriophage-SP-50, obtained from Dr T. A. Trautner, was propagated on *Bacillus subtilis* w-23; bacteriophage K, obtained from Dr E. Asheshov, was propagated on *Staphylococcus aureus* 9318; bacteriophages 52A and 53, obtained from Mr G. Fenwick, were propagated on *S. aureus* strains 52A and 3528 respectively.

The bacteriophages were re-isolated from single plaques and then propagated by the soft agar overlay method (Adams, 1959). The bacteriophages were eluted from the plates with tryptic soy broth, filtered through a 0.45 μ m Millipore filter and assayed in soft agar layers containing the propagating strain (2×10^7 bacteria/ml) grown for 5 h at 37 °C. The yield of plaque-forming units (p.f.u.) was between 5×10^8 /ml and 2×10^{10} /ml.

Antiserum. Rabbit antisera were prepared against the above bacteriophages and diluted so that under the test conditions over 99.9 % of the bacteriophages were inactivated.

Growth of bacteria. Bacteria were grown in NBS medium containing Oxoid Nutrient broth (2.5 %, w/v), sodium succinate (1 %, w/v) and magnesium sulphate (0.01 M). Flasks (50 ml) containing this medium (25 ml) were inoculated with 0.2 ml of an overnight culture, grown in the same medium, and incubated with aeration at 37 °C for 5 h when the bacilli attained 10^9 organisms/ml and the staphylococci 2×10^9 /ml. Preliminary studies showed that this medium gave only a slight precipitate with Concanavalin A and did not inhibit the precipitation of glycogen by the lectin.

Concanavalin A. Water-soluble Concanavalin A was prepared by the method of Olson & Leiner (1967). A stock solution was prepared containing Concanavalin A (1 g/10 ml buffer of the following composition: Na_2HPO_4 , 7 g; KH_2PO_4 , 3 g; NaCl , 5 g; 0.1 M- MgSO_4 , 10 ml; 0.01 M- CaCl_2 , 10 ml; water, 1 l). This stock solution was diluted into NBS medium before use.

Action of Concanavalin A on the binding of bacteriophages by *Bacillus subtilis* and *Staphylococcus aureus* strains. Samples (0.1 ml containing approximately 7×10^7 bacteria) of the 5 h cultures suitably diluted in NBS medium at 37 °C were placed in plugged sterile tubes (16 × 150 mm) held in a water bath at 37 °C. Portions (0.1 ml) of Concanavalin A solution (0.5 % or 1 %) were added and the mixtures incubated at 37 °C for 5 min before being mixed with bacteriophage (0.1 ml containing approximately 7×10^5 p.f.u.). The mixture was incubated at 37 °C for a further 15 min before adding a sample (0.1 ml) to 9.9 ml of NBS medium at 37 °C. It was filtered through a 0.45 µm Millipore filter to remove bacteria and adsorbed bacteriophage and samples (0.1 ml) of the filtrate and of a tenfold dilution of the filtrate were assayed for plaque-forming units.

In control experiments bacteria were first incubated with 0.1 ml of Concanavalin A (1 %) plus α -methyl glucoside (1 %, Sigma Chemical Co., London) or with NBS medium (0.1 ml). Further controls were set up to determine the number of bacteriophage and bacteria added to the mixtures. Plaque and colony counts were determined in duplicate, about 200 plaques or colonies were counted per plate except where more than 90 % of the bacteriophage were adsorbed. The experiments were repeated giving closely similar results. Under the above conditions incubation with Concanavalin A for 20 min had no significant effect on the number of colonies formed, showing that agglutination was not detectable in this time at the low cell densities used.

The action on Concanavalin A on the infection of *Bacillus subtilis* and *Staphylococcus aureus* strains by bacteriophages. Samples (0.1 ml containing 7×10^7 bacteria) of 5 h cultures diluted into NBS medium at 37 °C were placed in sterile plugged tubes and mixed with samples (0.1 ml) of warm medium alone or with 0.5 % Concanavalin A plus and minus α -methyl glucoside as above. After incubation at 37 °C for 5 min, bacteriophage (0.1 ml containing approximately 10^6 p.f.u.) was added followed, after incubation for 5 min, by diluted anti-serum (0.7 ml). After incubation for a further 5 min to inactive unadsorbed bacteriophage samples (0.1 ml) were serially diluted into NBS medium at 37 °C and duplicate portions (0.1 ml) were assayed for infectious centres by the soft agar overlay method.

In control experiments the numbers of bacteria and bacteriophage added to the mixtures were determined. Pre-incubation with Concanavalin A did not inactivate any of the phages studied and again none of the bacteria were agglutinated by incubation with Concanavalin A under these conditions. Repeat experiments gave closely similar results although the values obtained were influenced by slight differences in the concentrations of bacteria and bacteriophage in separate experiments.

RESULTS AND DISCUSSION

All three strains of staphylococci adsorbed over 90 % of the bacteriophage originally present in the mixtures in 15 min of incubation. Pre-incubation with Concanavalin A had little effect on the adsorption of bacteriophage K by *Staphylococcus aureus* A1 but markedly reduced the adsorption by *S. aureus* 3528 (Table 1). α -Methyl glucoside prevented this effect of Concanavalin A. The blocking is therefore due to the specific interaction of Concanavalin A with the sugar residues on the bacterial surface. These groups are almost certainly the α -N-acetyl-glucosaminyl substituents of the wall teichoic acid which is the only

Table 1. *Influence of Concanavalin A on the interaction of bacteriophage K with Staphylococcus aureus and bacteriophage SP-50 with Bacillus subtilis*

Samples (0.1 ml, containing approximately 7×10^7 bacteria) were incubated in medium with Concanavalin A or Concanavalin A plus α -methyl glucoside as shown and then mixed with bacteriophages (0.1 ml). After incubation for 15 min bacteria and adsorbed bacteriophages were removed by filtration and unadsorbed bacteriophages were counted. Conditions were as described in text.

Bacteria	Pre-incubation in medium with additions shown	No. ($\times 10^{-4}$) phage in:		Phage adsorbed (%)
		Incubation mixture	Filtrate	
<i>Staphylococcus aureus</i> A1	None	66 ± 3	0.96 ± 0.27	99
	0.25 % Con A	66 ± 3	5.9 ± 0.3	91
	0.50 % Con A	66 ± 3	8.9 ± 0.4	87
	0.50 % Con A + α -methyl glucoside	66 ± 3	0.92 ± 0.27	99
<i>S. aureus</i> 3528	None	63 ± 3	3.7 ± 0.2	94
	0.25 % Con A	63 ± 3	48 ± 2	24
	0.50 % Con A	63 ± 3	59 ± 3	6
	0.50 % Con A + α -methyl glucoside	63 ± 3	3.4 ± 0.2	95
<i>S. aureus</i> H	None	63 ± 3	4.2 ± 0.3	93
	0.25 % Con A	63 ± 3	8.2 ± 0.3	87
	0.50 % Con A	63 ± 3	27 ± 3	57
	0.50 % Con A + α -methyl glucoside	63 ± 3	3.9 ± 2	94
<i>Bacillus subtilis</i> w23	None	67 ± 3	30 ± 2	55
	0.25 % Con A	67 ± 3	29 ± 2	57
	0.25 % Con A + α -methyl glucoside	67 ± 3	30 ± 2	55
<i>B. subtilis</i> 3610	None	67 ± 3	38 ± 3	43
	0.25 % Con A	67 ± 3	61 ± 3	9
	0.25 % Con A + α -methyl glucoside	67 ± 3	42 ± 2	37

known surface component which can interact with Concanavalin A. This conclusion is strengthened by the failure of Concanavalin A to affect markedly the adsorption by strain A1, the wall teichoic acid of which is reported to contain only β -*N*-acetyl-glucosaminyl substituents. This organism may however contain a small proportion of α -linked *N*-acetyl-glucosaminyl substituents since pre-incubation in 0.5 % Concanavalin A reduced bacteriophage binding by approximately 10 %, an effect which was reversed by the presence of α -methyl glucoside indicating that the blocking is not due to non-specific adsorption of Concanavalin A. Approximately 15 % of the *N*-acetyl-glucosaminyl substituents of the wall teichoic acid of *S. aureus* H have the α -anomeric configuration. Pre-incubation in 0.5 % Concanavalin A blocked adsorption of bacteriophage by this organism by approximately 40 %, a value which is intermediate between that of 10 % obtained with strain A1 and that of approximately 90 % obtained with strain 3528, the wall teichoic acid of which contains exclusively α -linked *N*-acetyl-glucosaminyl substituents. Under the conditions described the action of Concanavalin A on adsorption of bacteriophage by these three strains of *S. aureus* correlates well with what would be forecast on the basis of the known structures of the wall teichoic acids of the organisms. Similar results were obtained with *Bacillus subtilis* strains w-23 and 3610. Pre-incubation with Concanavalin A had little effect on bacteriophage adsorption by strain w-23 but blocked adsorption by strain 3610 by 80 %. This blocking was also reversed by α -methyl glucoside (Table 1) and so was specifically due to the α -glucosyl substituents of the wall teichoic acid; in strain w-23 the wall teichoic acid contains only β -glucosyl substituents. Doyle & Birdsell (1972) have recently referred to

unpublished results which show that Concanavalin A blocks the phage receptor sites in walls of *B. subtilis* 168, the teichoic acid of which is similar to that of strain 3610.

The influence of Concanavalin A on bacteriophage infection was examined directly by neutralizing unadsorbed bacteriophage by the addition of antiserum and counting the infected organisms. In these experiments the bacteria were incubated with bacteriophage for only 5 min and the percentage of bacteriophage causing infection varied in different systems from 43 % to 92 %. On incubation with *Bacillus subtilis* strain 3610, 49 % of the added bacteriophage SP-50 gave rise to infective centres but this value was reduced to 3 % after pre-incubation with 0.25 % Concanavalin A, whereas after pre-incubation with Concanavalin A plus α -methyl glucoside 33 % of the added bacteriophage gave rise to infective centres. The corresponding values for strain W-23 were 49 %, 44 % and 44 % showing that Concanavalin A does not block infection in this organism. On incubation with *Staphylococcus aureus* 3528, 43 % of the added bacteriophage 53 gave infective centres. This value was reduced to 6 % on pre-incubation of the cells with Concanavalin A and to 32 % on incubation with Concanavalin A and α -methyl glucoside. With *S. aureus* H and bacteriophage 52A the corresponding values were 92 %, 69 % and 78 %. Thus in the presence of Concanavalin A infection of strain 3528 by bacteriophage 53 is specifically inhibited by over 50 % whereas infection of strain H is inhibited by less than 10 %. These results correlate well with the adsorption studies and both methods show that Concanavalin A specifically blocks adsorption of the bacteriophage to those organisms with wall teichoic acids in which the sugar substituents have the α -anomeric configuration but has no action on adsorption of phage to organisms in which these substituents have the β -configuration. The results obtained with the three strains of *S. aureus* suggest that these procedures might form the basis for a correlation between the bacteriophage and serological typing of staphylococci. After removal of excess Concanavalin A, determination of the increase in infection or phage binding by cells coated with Concanavalin A might be used to study synthesis of new wall material.

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