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Note

Mechanism of the formation of concanavalin A–teichoic acid complexes

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A variety of microbial polysaccharides, including arabinogalactans, dextrans, glycogens, and levans readily give a precipitate with concanavalin A (con A) in neutral, aqueous solutions[1–3]. We have used con A to probe structural features of the cell wall of *Bacillus subtilis.* For example, con A inhibits adsorption of bacteriophage to the D-glucosylated teichoic acid of *B. subtilis* 168 by competing for the D-glucosyl groups on the teichoic acid[4]. In addition, con A has been utilized as a morphological marker to locate the teichoic acid in cell walls[5]. Moreover, we have successfully used insolubilized con A to prepare large quantities of teichoic acid from autolyzates of *B. subtilis*[6]. Mauck and Glaser[7] used con A to study the synthesis and turnover of D-glucosylated teichoic acids in *B. subtilis.*

This report details some of the conditions necessary for occurrence of con A–teichoic acid interactions.

EXPERIMENTAL

Concanavalin A was prepared by the Sephadex adsorption method of Agrawal and Goldstein[8]. The con A was dialyzed overnight against cold, distilled water, the suspension was centrifuged to remove aggregated protein, and the supernatant liquor was stored at −20° until used. No additional aggregation occurred during storage.

Cell walls of *Bacillus subtilis* 168 were prepared as described previously[9]. The cell walls were extracted with 10% trichloroacetic acid (TCA) at 60° according to Strominger and Ghuysen[10]. The TCA-soluble teichoic acid was precipitated by the addition of 4:1 acetone–ethanol at −20°. The precipitated teichoic acid was washed twice with acetone–ethanol, freeze-dried, and stored at −20°.

All salts were of reagent grade. Methyl α-D-mannopyranoside (Me α-Man*p*) was obtained from Calbiochem, La Jolla, California.

The coprecipitation of con A with teichoic acids was conducted by the methods of Reeder and Ekstedt[11]. Usually, to con A (100 μg) and the teichoic acid (100 μg) in distilled water (500 μl) was added sufficient buffer to make a final volume of 1.5 ml and a 33mM concentration of buffer. After 1 h at room temperature, the con A–teichoic acid precipitate was sedimented by centrifuging for 20 min at 8000 *g*. The precipitate was washed by suspending it in 1.5 ml of 33mM buffer at the same pH, agitating with a Vortex mixer, centrifuging, and draining. Finally, the precipitate was dissolved in 1.0M sodium chloride–0.05M Me α-Man*p*. Aliquots were removed for analyses for protein[12]. At pH 3.1, phthalate buffer was used, and, at pH 7.0, phosphate buffer.

RESULTS AND DISCUSSION

The teichoic acid of *Bacillus subtilis* 168 is a poly(glycerol phosphate) having α-D-glucopyranosyl groups attached to O-2 of the glycerol residues (D-glucose: phosphorus = 0.8:1.0). Doyle and Birdsell[9] have shown that the teichoic acid readily gives a precipitate with con A in acidic (pH 2.5–4.0) and neutral (pH 6.5–7.5) solutions. Con A will not precipitate neutral polysaccharides in acidic media[2,9]. The present study was designed to determine whether the con A–teichoic acid reaction is similar under acidic and neutral conditions.

One qualitative means of detecting differences between polymer complexes is to determine their solubility. The solubility of the con A–teichoic acid complex at pH 3.1 and 7.0 was determined as shown in Fig. 1. At pH 7.0, the solubility of con A was calculated to be 10 μg/ml, whereas, at pH 3.1, it was 13 μg/ml. The data indicate that the complex at pH 7.0 is the stronger, and that it may be formed by a different mechanism.

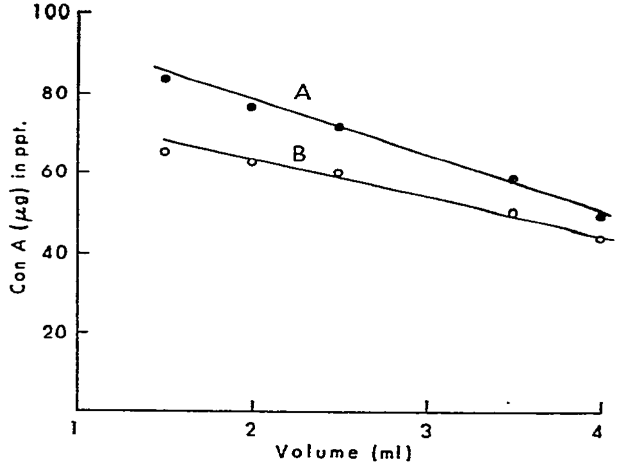

Fig. 1. Solubility of the concanavalin A–teichoic acid complex. [Concanavalin A (100 μg) and teichoic acid (100 μg) were mixed in various volumes of buffer. After incubation for 18 h at 4°, the protein content of the precipitates was determined, and the solubilities were calculated from the slopes of the curves. A, at pH 3.1; B, at pH 7.0.]

If con A binds to sugar residues of the teichoic acid, the interaction should be inhibited[9] by Me α-Man*p*. At pH 7.0, Me α-Man*p* readily inhibited precipitate formation (see Fig. 2). At pH 3.1, inhibition by Me α-Man*p* was not detected. It is known that maximal formation of a precipitate between neutral polysaccharides and con A occurs near neutrality, and that, below pH 4, precipitation does not occur[2]. On the basis of the data given in Fig. 2, it would appear that the complex at pH 3.1 is not mediated through con A–carbohydrate interactions. Previously[9], we had shown a slight inhibition of complex formation of con A–cell-wall autolyzate by Me α-Man*p*. The present study differs in that purified teichoic acids were used, and an analysis of the precipitate for protein was performed. (Turbidimetric assays were previously conducted[9].)

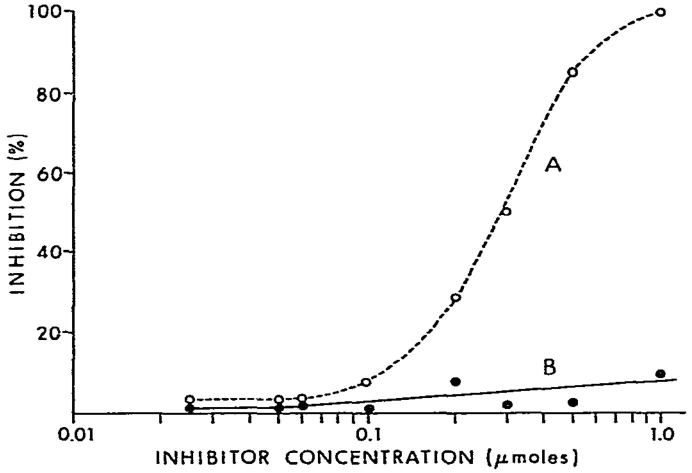

Fig. 2. Inhibition of formation of concanavalin A–teichoic acid complex by methyl α-D-mannopyranoside. [Reactions were conducted in 33mM buffer containing con A (100 μg) and teichoic acid (100 μg) in a total volume of 1.5 ml. A, at pH 7.0; B, at pH 3.1.]

Several workers have reported that salts tend to inhibit interaction of con A with teichoic acids[9,11,13]. Doyle and Birdsell[9] noted that solutions of high ionic strength inhibit complex formation of con A–*B. subtilis* cell-wall autolyzate. Under the same conditions, sodium chloride had no effect on con A–glycogen complexes[9]. As shown in Fig. 3, we have examined the effects of sodium chloride on formation of the con A–teichoic acid complex at pH 3.1 and 7.0. At the lower pH, the interaction is markedly sensitive to increasing ionic strength. Sodium chloride at a concentration of 0.1M inhibited precipitation of con A by ~85% at pH 3.1, but at pH 7.0, only 22% inhibition occurred. We have recently shown that teichoic acids undergo a loss of secondary structure in aqueous solutions of high ionic strength[14]. Thus, the inhibition by sodium chloride at pH 7.0 is probably not the result of competition by ions, but a reflection of the availability of D-glucosyl groups of teichoic acid for interaction with con A.

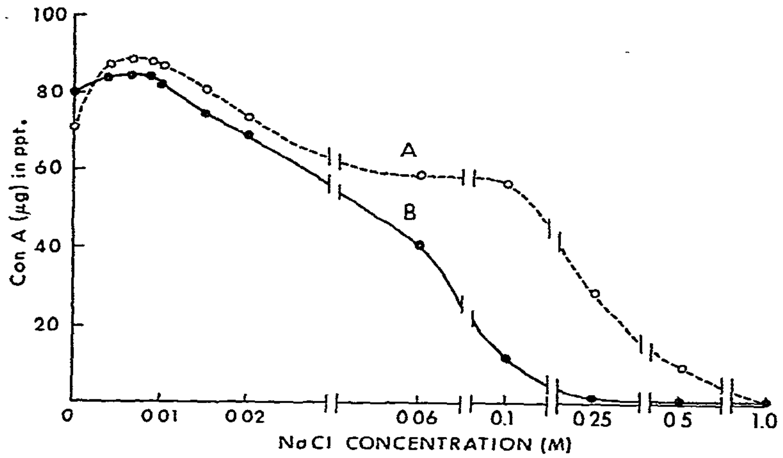

Fig. 3. Effect of sodium chloride on the interaction between concanavalin A and teichoic acid. (Reaction mixtures contained 100 µg each of con A and teichoic acid in 33mM buffer, plus the amount of sodium chloride indicated, in a total volume of 1.5 ml. A, at pH 7.0; B, at pH 3.1.)

Teichoic acids could serve as useful models for examination of the interactions between con A and other lectins with carbohydrate-containing polyelectrolytes.

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