The Interaction of Concanavalin A with Teichoic Acids and Bacterial Walls

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The lectin concanavalin A has been studied for several years. Its specificity is now well defined and Goldstein and his associates (Goldstein, Hollerman & Smith, 1965; Goldstein & Iyer, 1966; Goldstein, Iyer & Smith, 1967) have shown that it will interact with polysaccharides containing α -D-glucopyranosyl or α -D-mannopyranosyl substituents in which hydroxyl groups at positions 3, 4 and 6 are unsubstituted. The hydroxyl group at position 2 can be substituted or replaced by an acetamido group without loss of affinity for concanavalin A. Many teichoic acids possess α -D-glucopyranosyl or α -D-N-acetylglucosaminyl residues, which have free hydroxyl groups at positions 3, 4 and 6. These teichoic acids should interact with concanavalin A, which would therefore be a valuable reagent for use in the study of the structure and cellular location of these polymers.

We have found that the intracellular or 'membrane' teichoic acid isolated by extraction with phenol or trichloroacetic acid from the cytoplasmic contents fraction of disrupted cells of *Streptococcus faecalis* 8191 gives a precipitin reaction with concanavalin A, which is readily observed by double diffusion in agar gel. This teichoic acid contains kojibiosyl- and kojitriosyl-glycerol residues (Wicken & Baddiley, 1963) and so has the appropriate chemical determinants for interaction with the lectin. Addition of α -methyl glucoside to the system leads to the disappearance of the precipitin lines, showing that the reaction is specific. Appropriately labelled concanavalin A should therefore be of value for the determination, purification and detection of this teichoic acid and so should be useful in studying the nature of the association of the teichoic acid with the cell membrane.

Certain wall teichoic acids also give precipitin lines on double diffusion in agar gel against concanavalin A. Samples of these teichoic acids were prepared by extraction of walls with cold trichloroacetic acid or with dilute alkali (Archibald, Coapes & Stafford, 1969). Plates for agar-gel diffusion studies were prepared as follows. A 2% (w/v) solution of Noble agar (Difco Laboratories, Detroit, Mich., U.S.A.) in 0.9% sodium chloride, buffered with 0.1M-sodium phosphate buffer, pH 7.3, was heated at 100°C for 20 min, and then sodium azide was added, to a final concentration of 1%.

A sample (5 ml) of the resulting solution was quickly pipetted on to a glass slide (2 in $\times$ 2 in) and allowed to cool. Wells were cut in the gel with a Feinberg cutter, giving one central well (capacity 0.10 ml) and six hexagonally arranged peripheral wells (capacity 0.05 ml). Concanavalin A solutions (3-25 mg/ml) were prepared in 0.01 M-sodium acetate buffer, pH 5.4, half-saturated with sodium chloride. Solutions of teichoic acids (1-10 mg/ml) were prepared in water. Concanavalin A solution was placed in the central well and the teichoic acid solutions in the peripheral wells. The glass slides were then placed in Petri dishes over damp filter paper and incubated at room temperature. Zones of precipitation were normally visible after incubation for 16 h and the slides were washed with several changes of water, drained and photographed. Wall teichoic acid from *Lactobacillus plantarum* C106 (Adams, Archibald, Baddiley, Coapes & Davison, 1969) gave a single sharp precipitin line readily observable by using concentrations of teichoic acid of 1-10 mg/ml and concanavalin at 3 mg/ml. A sharp precipitin line was similarly observed with wall teichoic acid of *Strep. faecalis* 8191, which also contains α -D-glucopyranosyl substituents (A. R. Archibald & F. Miller, unpublished work). Addition of α -methyl glucoside (0.96 mg/ml; 0.05 or 0.10 ml) to the well containing concanavalin A or to the wells containing teichoic acid led to the disappearance of the precipitin line, as did the addition of sodium metaperiodate (0.14 M; 0.10 ml) to the well containing concanavalin A. No precipitin lines were obtained with the wall teichoic acid, containing β -glucopyranosyl substituents, from *Staphylococcus epidermidis* 12 (Archibald, Baddiley & Shaikat, 1968), so that precipitation appears to depend specifically on the presence of α -glucopyranosyl substituents in the teichoic acids. Wall teichoic acids of *L. plantarum* 17-5 (Archibald, Baddiley & Buchanan, 1961) and *Lactobacillus buchneri* N.C.I.B. 8001 (Shaw & Baddiley, 1964) also contain α -glucopyranosyl substituents. However, these teichoic acids did not give an observable precipitin line at any of the concentrations studied until the agar gel was washed with water. The lines which then developed appeared normal though rather diffuse. These lines did not develop on slides to

Table 1. *Agglutination of walls by concanavalin A*

Experimental details are given in the text. The extent of agglutination is classified as follows: 0, homogeneous suspension; 1+, milky suspension plus some aggregated walls; 2+, marked aggregation; 3+, walls starting to settle; 4+, walls settled, supernatant clear.

Organism	Glycosidic substituent of teichoic acid	Degree of agglutination at					
		2 min	5 min	7 min	9 min	12 min	15 min
<i>L. plantarum</i> 17-5	α -D-Glucopyranosyl-	0	1+	3+	3+	4+	4+
<i>L. plantarum</i> C106	Kojibiosyl-	1+	2+	3+	4+	4+	4+
<i>L. buchneri</i> N.C.I.B. 8001	α -D-Glucopyranosyl-	0	1+	1+	2+	2+	2+
<i>Strep. faecalis</i> 8191	α -D-Glucopyranosyl-	0	1+	2+	3+	4+	4+
<i>B. subtilis</i> W 23	β -D-Glucopyranosyl-	0	0	0	0	0	0
<i>B. licheniformis</i> A.T.C.C. 9945	β -D-Glucopyranosyl-	0	0	0	0	0	0
<i>Staph. epidermidis</i> I2	β -D-Glucopyranosyl-	0	0	0	0	0	0

which α -methyl glucoside had been added, so that specific interaction of concanavalin A with the α -glucopyranosyl substituents of the teichoic acids is apparently involved. We do not understand this phenomenon, but it may be that the highly ionic nature of the teichoic acids hinders normal precipitation. Thus those teichoic acids that precipitate normally have a relatively high sugar/phosphorus ratio (2.8:1 in *Strep. faecalis* membrane teichoic acid, 1.78:1 in *L. plantarum* C106 wall teichoic acid and approx. 6:1 in *Strep. faecalis* 8191 wall teichoic acid) whereas the wall teichoic acids from *L. plantarum* 17-5 and *L. buchneri* N.C.I.B. 8001 have relatively low sugar/phosphate ratios (1:1 and 0.26:1 respectively) and may form complexes with concanavalin A, which are soluble in the buffer systems used. A related observation has been reported by Reeder & Ekstedt (1971). These authors have shown that concanavalin A forms precipitates with suitably substituted staphylococcal teichoic acids but precipitation of ribitol teichoic acids from *Staphylococcus aureus* is inhibited by phosphate buffer. Precipitin bands were observed in agar containing only sodium chloride, but these were rather diffuse. Under similar conditions we have also observed very diffuse bands with the wall teichoic acids from *L. plantarum* 17-5 and *L. buchneri* N.C.I.B. 8001, but these were less sharply defined than those obtained on washing agar plates that had contained phosphate buffer.

Walls of *L. plantarum* 17-5 and *L. buchneri* N.C.I.B. 8001 did, however, agglutinate in the presence of concanavalin A, and this agglutination reaction appears to be specific for walls carrying the correct determinants for interaction with the lectin. For these studies a solution of concanavalin A (14.7 mg/ml) was prepared in 0.1 M-sodium acetate buffer, pH 5.2, half-saturated with sodium chloride, and walls were suspended (3-4 mg/ml) in the same buffer. Samples (0.2 ml) of the various wall suspensions were added to the concanavalin solution (0.2 ml) in clear polystyrene test tubes (5 mm $\times$

50 mm). The mixtures were placed in racks at 22°C. Agglutination was observed at intervals and progressed through flocculation to the settling out of the precipitate, leaving a clear supernatant solution. The progress of the reaction was followed by using a magnifying glass and extent of agglutination was recorded as shown in Table 1.

Controls were set up with wall samples and these contained samples (0.2 ml) of the various wall suspensions and sodium acetate-sodium chloride buffer (0.2 ml). None of the wall samples agglutinated in the absence of concanavalin A. Hapten inhibition studies were performed by adding α -methyl glucopyranoside (1.0 mg/ml; 0.1 ml) to the concanavalin A solution (0.2 ml) and incubating this mixture at 22°C for 2 h before addition of walls. In no case was agglutination observed in the presence of α -methyl glucopyranoside.

All wall samples that contained teichoic acids having α -glucopyranosyl substituents (*L. plantarum* C106, *L. plantarum* 17-5, *Strep. faecalis* 8191 and *L. buchneri* N.C.I.B. 8001) underwent agglutination reactions and these were inhibited by the presence of α -methyl glucopyranoside. Walls containing β -glucopyranosyl substituents (*Bacillus subtilis* W23, *Staph. epidermidis* I2) did not undergo agglutination, nor did walls of *Bacillus licheniformis* A.T.C.C. 9945 (Burger & Glaser, 1966), which contain a teichoic acid having α -D-glucopyranosyl residues that carry phosphate groups on position 6 and so are unable to interact with concanavalin A.

Concanavalin A thus interacts specifically with walls containing the appropriate glycosidic substituents on their teichoic acids. It also interacts with several isolated teichoic acids that possess such substituents, and this has already proved of value in the study of the teichoic acids from walls of *L. plantarum* C106, since it has been found (A. R. Archibald & H. E. Coapes, unpublished work) that the precipitate formed with this teichoic acid contains only kojibiosylglycerol residues. The glucosylglycerol and nigerosylglycerol residues also

found in this teichoic acid do not have the appropriate determinants for interaction with concanavalin A, since in the teichoic acid these residues carry phosphate substituents at position 6 of their terminal glucose moieties. The kojibiosylglycerol residues are similarly substituted with phosphate, but the non-terminal glucose moiety is substituted only at position 2 and so has the necessary free hydroxyl groups at positions 3, 4 and 6 for interaction with concanavalin A. The composition of the precipitated teichoic acid shows that the kojibiosylglycerol residues occur in chains that do not contain the other two residues.

The present results are fully in accord with the reported specificity of concanavalin A, and this lectin should be of considerable value as indicated above for studies on the purification, determination, structural analysis and cellular location of both wall and membrane teichoic acids.

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- Adams, J. B., Archibald, A. R., Baddiley, J., Coapes, H. E. & Davison, A. L. (1969). *Biochem. J.* **113**, 191.
Archibald, A. R., Baddiley, J. & Buchanan, J. B. (1961). *Biochem. J.* **81**, 124.
Archibald, A. R., Baddiley, J. & Shaukat, G. A. (1968). *Biochem. J.* **110**, 583.
Archibald, A. R., Coapes, H. E. & Stafford, G. H. (1969). *Biochem. J.* **113**, 899.
Burger, M. M. & Glaser, L. (1966). *J. biol. Chem.* **241**, 494.
Goldstein, I. J., Hollerman, C. E. & Smith, E. E. (1965). *Biochemistry, Easton*, **4**, 876.
Goldstein, I. J. & Iyer, R. N. (1966). *Biochim. biophys. Acta*, **121**, 197.
Goldstein, I. J., Iyer, R. N. & Smith, E. E. (1967). *Biochemistry, Easton*, **6**, 2373.
Reeder, W. J. & Ekstedt, R. D. (1971). *J. Immun.* **106**, 334.
Shaw, N. & Baddiley, J. (1964). *Biochem. J.* **93**, 317.
Wicken, A. J. & Baddiley, J. (1963). *Biochem. J.* **87**, 54.