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INTERACTION OF CONCANAVALIN A WITH BACTERIAL LIPOPOLYSACCHARIDES IN AGAROSE GEL

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Binding of fluorescein isothiocyanate-labeled concanavalin A to a series of molecular species of lipopolysaccharide (LPS), purified from pathogenic bacteria, was studied via agarose gel precipitation experiments and the results were compared with available structural data.

The LPS species could be divided into ConA-reactive and non-reactive ones. Reactivity resided in the O-specific chain of LPS, and binding to the lipid A or core moieties of LPS could not be demonstrated by the present methods. The α-D-glucose or α-D-mannose residues of the repeating O-specific oligosaccharide units appeared to be recognized by ConA, except when blocked by steric hindrance. Specificity of the reaction was verified by inhibition with 2% D-glucose. Binding by bacterium-specific sugar-residues could not be demonstrated.

For precipitation to occur, polyvalency was required both for LPS and ConA, and the resulting precipitation appeared to be promoted by hydrophobic interactions between the lipid A moieties of LPS molecules. The LPS species were differently retained by the agarose gel, which can be explained by differences in their micellar structure in aqueous solution. *E. coli* O83 LPS did not readily diffused in 1% agarose gel, but its precipitation with ConA could be demonstrated either at elevated temperature or mixing it previously with molten agarose (Mancini's arrangement).

Keywords: lipopolysaccharide, O-specific chain, ConA, *Escherichia coli, Salmonella, Shigella*

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Introduction

Lectins of the horseshoe crabs help their survival in bacterium-infested waters: namely tachylectins [1, 2] and limulin [3]. Lectins of plant origin are widely used as specific carbohydrate reagents, but they rarely react with bacteria [3], except ConA [4–6]. This latter lectin targets several lipopolysaccharide species in the bacterial outer membrane [7], although other reactive components cannot be excluded. ConA is primarily an α-glucose/α-mannose-specific lectin with lesser affinity to N-acetyl-glucosamine [8].

In preliminary experiments we examined the precipitation of a series of LPS species by ConA in the wells of a hemagglutination plate, but with unclear solutions the evaluation was ambiguous. The reaction in agarose gel gave better results, except the LPS species of low diffusibility. In the present study we enhanced the sensitivity of the detection by using FITC-labelled ConA, and compared the results with the known molecular structures of the respective LPS species.

Material and Methods

Salmonella enterica sv. *Minnesota* R595 was a gift of H. Mayer (Freiburg, Germany), *Shigella sonnei* phase I and rough mutants were isolated by Kocsis and Kontrohr [9]. All other strains were supplied by the Hungarian National Collection of Medical Bacteria (National Institute of Hygiene, Budapest, Hungary).

The LPS of *Salmonella enterica* sv. *Minnesota* R595 and two *Shigella sonnei* R-mutants were extracted with phenol/chloroform/petrol-ether (PCP) according to Galanos et al. [10], whereas all the other lipopolysaccharide species were extracted by phenol/water [11]. All the LPS preparations were freeze-dried and stored frozen until use. ConA and its derivatives were Sigma products.

The O-specific chain was isolated according to Vinogradov [12]. *E. coli* O83 LPS was partially hydrolysed in 1% acetic acid at 100°C for 90 min. Precipitated lipid A was removed by centrifugation (10,000 *g* for 15 min), the oligosaccharide was separated on a Sephadex G50 column and freeze-dried.

One % agarose L (Pharmacia) was solidified on glass slides. Wells of 2.5 mm diameter were cut 1 to 2 mm apart from each other. LPS and FITC-ConA was used in 10 mg/cm^3 concentration and pipetted into separate wells according to the double diffusion method of Ouchterlony [13]. Specificity of the lectin/LPS reaction was examined by inhibition with 2% D-glucose included into the agarose [8].

In another experimental series the dissolved LPS was mixed with 1% molten agarose in 4 mg/cm^3 concentration and left to solidify. The lectin was pipetted into a well. This arrangement resembled that of Mancini et al. [14].

The reactants were left to diffuse in the agarose in a humid chamber at room temperature. Next day the gel was covered with several layers of filter paper soaked in water or 2% glucose solution (control) and left to dry. After removal of the paper precipitation arcs were evaluated under a Leica DMR HC fluorescence microscope with a 5x objective and photos were taken with a Leica camera.

Unlabelled ConA was allowed to react with LPS in 1:1 or 5:1 ratio. The native gel was evaluated by the naked eye and photos were taken by hand.

Results

A panel of purified LPS species was tested against FITC-ConA in agarose gel. *Shigella dysenteriae* 2 (Figure 1A), and *Shigella flexneri* 3 (Figure 1C) produced highly fluorescent precipitates between the reagent-wells, whereas the precipitation was less defined with *Salmonella Minnesota S* (Figure 1D) and *E. coli* O83 LPS (Figure 1G) and the isolated O-specific chain of the latter (Figure 2D). Other LPS species formed fluorescent arcs immediately at the edge of the well, namely *Yersinia enterocolitica* 09 (Figure 1E) and *Salmonella enterica* sv. *Urbana* O30 (Figure 1F). The formation of all these fluorescent spots and arcs were inhibited by the inclusion of 2% D-glucose into the agarose (Figure 1B). Several LPS species also formed well-visible precipitate with unlabelled ConA (Figure 1H), but after drying the gel, the complex stained poorly with Coomassie blue. No precipitate could be detected with unlabelled succinyl-ConA.

When the *E. coli* O83 LPS was included into the agarose (Mancini's arrangement (Figure 2A, B) a marked and uniform precipitation zone was formed around the well of the FITC-ConA, which could be inhibited by 2% D-glucose (Figure 2C). The width of the zone depended on the concentration of the lectin (Figure 2A, B).

In the Ouchterlony arrangement several LPS species showed no reaction with FITC-ConA or unlabelled ConA (Table I), namely *Morganella morganii* O34, *E. coli* O55, O111, O157, *Shigella sonnei* and its mutants, *Salmonella enterica* sv. *Adelaide* O35 and *Minnesota* R595 (Figure 1H). These same LPS species were also negative in Mancini's arrangement (not shown).

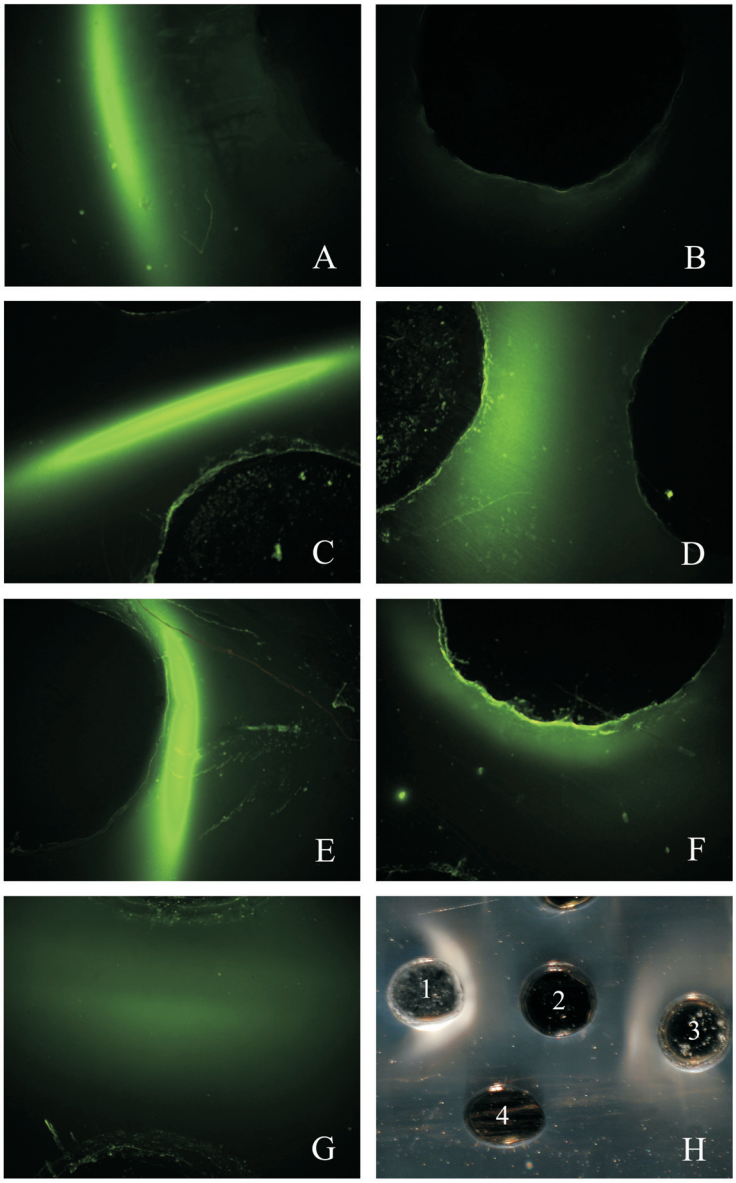

Figure 1. Precipitation of various LPS species with FITC-labelled or unlabelled ConA in agarose gel (Ouchterlony's arrangement). FITC-ConA to LPS 1:1, diameter of the wells: 2.5 mm

Photos: *Shigella dysenteriae* 2 (A) and the same in presence of 2% glucose (B); *Shigella flexneri* 3 (C); *Salmonella enterica* sv. *Minnesota* smooth (D); *Yersinia enterocolitica* 09 (E); *Salmonella enterica* sv. *Urbana* O30 (F); *Escherichia coli* O83 (G). Unlabelled ConA (H) at 5:1 ratio to LPS. Native agarose gel, without drying. Wells: *Shigella flexneri* 3 (1), FITC-ConA (2), *Shigella dysenteriae* 2 (3), *Salmonella enterica* sv. *Minnesota* R595, the latter without reaction (4)

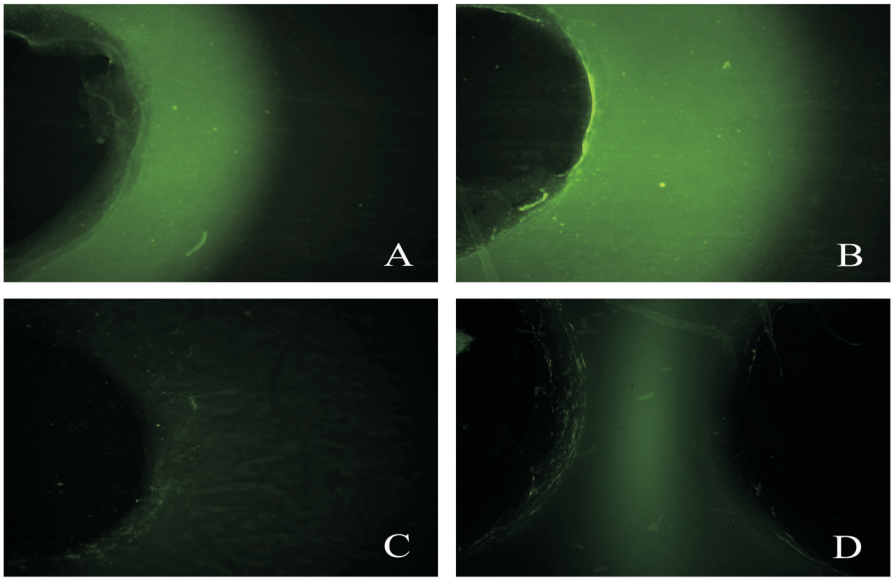

Figure 2. Reaction of *E. coli* O83 LPS with FITC-ConA in agarose gel. Diameter of the wells: 2.5 mm

LPS included into the agarose gel (Mancini's arrangement): 2.5 µl (A) and 5 µl (B) FITC-ConA solution and control with 2% glucose (C). Reaction of the isolated O-specific chain of *E. coli* O83 LPS with FITC-ConA in Ouchterlony's arrangement (D)

Table I

Reaction of ConA with different lipopolysaccharides

Positive reaction at least in one of the experimental arrangements	Little or no reaction
Escherichia coli O83 and its O-specific oligosaccharide	*Escherichia coli* O55, O111, O157
Salmonella enterica sv. Urbana O30 and sv. Minnesota S	*Shigella sonnei* and a series of its R mutants
Shigella dysenteriae 2	*Salmonella enterica* sv. Minnesota R595 and sv. Adelaide O35
Shigella flexneri 3 and 6	*Morganella morganii* O34
Yersinia enterocolitica O9	

Discussion

LPS is an amphiphilic molecule composed of a hydrophobic lipid A moiety, hydrophilic inner and outer core oligosaccharides and an O-specific part made up by repeated oligosaccharide units. In water, the hydrophobic intermolecular interactions result in micellar aggregates of different sizes [15] in connection with their membrane-forming abilities. The aggregates may restrict the diffusion of some LPS species in the agarose (Figure 1, D and E).

ConA and its derivatives diffused readily in the agarose gel and they were efficiently extracted into the Whatman-1 filter paper during drying, except if precipitated.

Using FITC-ConA the examined LPS species could be divided to reactive (Figure 1) and non-reactive ones (not shown). Specificity of the binding was tested by the inhibition of the lectin-carbohydrate reaction with 2% D-glucose (Figure 1A, B and Figure 2B, C).

The reaction of readily diffusible LPS species could be demonstrated with both labelled and unlabelled ConA (Figure 1A, C, D, H). However, LPS species which were efficiently retained by the agarose, produced precipitation arcs only immediately at the edge of the wells (Figure 1E, F). The bulk of *Escherichia coli* O83 LPS was unable to diffuse in the gel, but it appeared to contain a smaller, diffusible fraction (Figure 1G). Elevation of gel-temperature to 50ºC facilitated the diffusion of this LPS (unpublished). The other option may be the application of Mancini's method, when the immobile LPS is included into the gel and the FITC-ConA diffuses out of the well (Figure 2A, B, C). The width of the precipitation zone depended on the concentration of both the lectin and LPS. Principally this could provide a basis for the development of a quantitative determination of LPS. However, in contrast to Mancini's original method developed for serum proteins [14], the spreading of the zone of the LPS-ConA precipitate was lacking a stable end-point.

The LPS of both *Salmonella Minnesota* R595 and *Shigella sonnei* absolute R-mutants consist of lipid A and a part of the core. These very similar LPS species did not form precipitate with FITC-ConA in agarose gel, neither in Ouchterlony's, nor in Mancini's arrangement. The lack of reactivity of these mutants indicates that the ConA binds to the O-specific part of the molecule, also shown by the use of the isolated O-specific chain of *E. coli* O83 LPS (Figure 2D). However, this chain produced a blurred precipitation spot, indicating that the lipid A moiety of the molecule may contribute to the compactness of the precipitate.

The results of FITC-ConA precipitation experiments were compared with the known composition of LPS O-specific chains. *Yersinia enterocolitica* O9, containing α-D-mannose residues [16] reacted with FITC-ConA. Several other LPS species containing α-D-glucose, namely *Shigella dysenteriae* type 2 [17], *Shigella flexneri* type 3 [18], *Salmonella enterica* sv. *Urbana* O30 [19], *Salmonella Minnesota* S [20] and *E. coli* O83 [21] were also reactive. These findings are in agreement with the known α-glucose/α-mannose specificity of ConA [8]. Bacterium-specific sugar residues, namely colitose in *Escherichia coli* O55 and O111 [22] and N-acetyl-amino-altruronic acid [23] were not recognized by the lectin.

Recognition of D-glucose by ConA depends on the configuration of hydroxyl-groups on C1, C3 and C4 carbons [24], allowing C1 and C4 to participate in the formation of glycosidic bonds. In the LPS of *Salmonella enterica* sv. *Adelaide* O35 and *Escherichia coli* O111 four hydroxyl-groups of each α-D-glucose residue (C1, C3, C4 and C6) forms glycosidic linkage [25]. The lack of reactivity with FITC-ConA may be due to steric hindrance of the binding ("caging").

The repeating oligosaccharide structure containing lectin-reactive moieties confer polyvalency onto the O-specific region toward the tetravalent FITC-ConA. Although the LPS of *Shigella sonnei* contains three glucose-residues (two of them in α-configuration) in the outer core [26–28], but none in the repeating units of the O-specific region, and this LPS did not produce precipitation reaction with FITC-ConA. A similar situation exists in *Escherichia coli* O157 LPS [29]. Succinylation decreases the valency of ConA from four to two [30]. This dimeric lectin did not produce precipitation with the LPS of *Shigella dysenteriae* 2 or *Shigella flexneri* 3 in the present experiments. These findings indicate that polyvalency of both ConA and the O-specific chain is required to the formation of a 3D precipitation network.

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