LIPOPOLYSACCHARIDE IMMUNOADSORBENTS AND THEIR APPLICATION TO AFFINITY CHROMATOGRAPHY OF O-ANTIBODIES AND SPECIFIC PHAGES

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1. Introduction

Affinity chromatography has been already employed in the purification of many classes of naturally occurring polymers, like proteins, nucleic acids, polysaccharides. The application of affinity chromatography to cell wall lipopolysaccharides isolated from Gram negative bacteria seems also to be of interest for immunochemical purposes. The present communication describes the preparation of immobilized *Shigella sonnei* lipopolysaccharides (LPS) and their degradation products on Sepharose matrix. Such affinity products have been used for purification of homologic antisera and for studying the interaction of bacteriophage–lipopolysaccharide receptor.

2. Materials and methods

Pure lipopolysaccharides from *Sh. sonnei* phase I and phase II bacteria and respective rabbit immune sera were obtained as described earlier [1,2].

2.1. *Preparation of* Sh. sonnei *immunoadsorbents*

For coupling to CNBr-activated Sepharose 4B or epoxy-activated Sepharose 6B (Pharmacia) the following preparations were used: (a) O-specific polysaccharides obtained after 1% acetic acid hydrolysis (100°C, 1.5 h) of phase I or phase II LPS [3,4] (phase I PS or phase II PS), (b) phase II LPS treated with 2% hydroxylamine in 4% NaOH (63°C, 3 min, Phase II LPS_H); hydroxylaminolyzed LPS is devoid of ester linked fatty acids, (c) phase II LPS treated with 0.25 N NaOH (37°C, 2 h), then sonicated and neutralized with 2 N HCl (phase II LPS_A).

The coupling procedures were essentially the same as those given by Pharmacia for use with CNBr-activated Sepharose 4B and epoxy-activated Sepharose 6B respectively.

The antibody titer of fractions eluted from the affinity columns was estimated by passive hemolysis test [2].

Protein was determined according to the method of Lowry et al. [5].

Bacteriophage: Phage designated as SKVI (Kallings collection, National Bacteriological Laboratory, Stockholm) propagated on phase II *Sh. sonnei* was used in the experiments. The agar layer technique of Adams [6] was used for plaque assay. Inactivation of the phage (10^3 plaque-forming units/ml nutrient broth) by LPS (previously treated with 0.25 N NaOH at 37°C for 2 h, then sonicated and neutralized) at concentration 0.1–1000 μg/ml after incubation at 37°C for 1 h was examined.

3. Results and discussion

The *Sh. sonnei* LPS or PS preparations containing 0.2–1.5% free amino groups could be coupled to CNBr-activated Sepharose. Amino groups are those from ethanolamine present in the lipopolysaccharides and phase II polysaccharide. In the case of phase I-specific polysaccharide, low content of free amino groups originate probably from N-unsubstituted amino sugar components.

The coupling efficiencies of CNBr-activated and epoxy-activated Sepharoses for various LPS and PS preparations are shown in table 1.

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Table 1
Coupling of *Sh. sonnei* phase I- and phase II-specific preparations to activated Sepharose

Ligand	Amount of ligand added to reaction mixture (mg)	Amount of ligand coupled (mg)
	CNBr-activated Sepharose 4B (10 ml gel)	
Phase I PS	16	3.1–3.7
Phase II PS	22.5	5.2
Phase II LPS_H	42	13.6
Phase II LPS_A	50	24
	Epoxy-activated Sepharose 6B (10 ml gel)	
Phase II LPS_A	200	44.6

3.1. *Affinity chromatography of* Sh. sonnei *anti-sera*

The results of affinity chromatography of anti-*Sh. sonnei* sera are presented in table 2. After one-step fractionation the antibodies eluted with 3 M KCNS were purified 20–40 times with 85–100% yield. The purity and characterization of anti-phase I antibodies as immunoglobulins G was proved in the immunoelectrophoretic experiments (detailed as in [2]). The same grade of purification of anti-phase II antibodies was obtained when affinity chromatography of phase II antiserum on phase II PS-Sepharose 4B or on phase II LPS_A coupled to epoxy-activated Sepharose 6B was used.

The antibodies coupled to CNBr-activated Sepharose 4B can be used in turn for purification of *Sh. sonnei* antigenic or haptenic materials.

The attempts to specifically bind anti-lipid A antibodies (lipid A isolated from *Sh. sonnei* LPS) to Phase II LPS_H or Phase II LPS_A columns failed. This indicates that lipid A immunodeterminants are not exposed after coupling LPS ligands to Sepharose gel.

3.2. *Affinity chromatography of* Sh. sonnei *phage SKVI*

Sh. sonnei phage SKVI lyses phase I and phase II cells. Phase II LPS_A is a strong inhibitor of the phage: 50% phage inactivation was obtained by $\leqslant 0.1$ μg of the LPS. Phase I LPS_A and phase II LPS_H (devoid of ester-linked fatty acids) were 100 times less active inhibitors. Phase II PS was almost inactive in blocking the phage. The above results seem to prove that phase II-specific polysaccharide is the receptor of phage SKVI although the complete structure of phase II LPS is necessary to inactivate the phage.

The data on the specific binding of phage SKVI to phase II LPS_A-Sepharose 4B column are presented

Table 2
The affinity chromatography of anti-*Sh. sonnei* phase I and phase II sera

Immunoadsorbent (10 ml gel)	Sample of homologic serum[a]		Eluents			
			0.9% NaCl in 0.01 M phosphate buffer pH 7.3 (40 ml)		3 M KCNS[b]	
	Protein (mg)	Serological activity[c]	Protein (mg)	Serological activity[c]	Protein (mg)	Serological activity[c]
Phase I PS-Sepharose 4B	75	5000	70.3	0	2.5	5000
Phase II PS-Sepharose 4B	75	2560	71.2	0	2.5	2400
Phase II LPS_H-Sepharose 4B	71.5	2300	67	0	3.6	1920
Phase II LPS_A-Sepharose 4B	71.5	2300	62.3	0	4.0	1920
Phase II LPS_A-Sepharose 6B (epoxy-activated)	112.5	2400	110	0	2.2	2200

[a] The sample (immune serum, 1–1.5 ml) was diluted 5 times with 0.9% NaCl in 0.01 M phosphate buffer pH 7.3 before applying to column.
[b] 3 M KCNS eluent was dialyzed 3 times against 0.9% NaCl in 0.001 M phosphate buffer, pH 7.3.
[c] Serological activity: effluent volume in ml × reciprocal titer in passive hemolysis test.

Table 3
The specific binding of phage SKVI on phase II LPS_A-Sepharose 4B

Temperature of phage binding (°C)	Time of binding (min)	Number of phage particles added in 10 ml buffer[a]	Number of phage particles attached	Phage attached (%)
0	5	$1 \cdot 10^6$	$< 2 \cdot 10^3$	< 0.1
	30	$1 \cdot 10^6$	$1 \cdot 10^5$	10
	120	$6.75 \cdot 10^5$	$1 \cdot 10^5$	15
	60[b]	$6.75 \cdot 10^5$	$2.5 \cdot 10^5$	37
8–10	120	$1.68 \cdot 10^6$	$1.56 \cdot 10^6$	93
20	90	$3.35 \cdot 10^6$	$2 \cdot 10^6$	63

The gel (10 ml) was equilibrated with 0.9% NaCl in 0.01 M phosphatebuffer, pH 7.3.
[a] Phage SKVI lyzate containing about 10^9 particles/ml was diluted 10^4 times with the buffer mentioned above.
[b] 0.01 M $MgCl_2$ and 0.003 M $CaCl_2$ was present in the reaction mixture.

Table 4
The elution of active phage SKVI attached to phase II LPS_A-Sepharose 4B

Temperature of phage binding (°C)	Number of phage particles attached	Effluent	Temperature of phage elution (°C)	Number of phage particles eluted	Active phage eluted (%)
0	$1 \cdot 10^5$	2 M NaCl in 0.5 M borate buffer, pH 6.0	25	$6.2 \cdot 10^4$	62
0	$2.5 \cdot 10^5$[a]	0.05 EDTA in 0.01 M phosphate buffer, pH 7.3	25	$1.28 \cdot 10^5$	52
0	$1.06 \cdot 10^6$[a]	0.05 M EDTA in 0.5 M borate buffer, pH 7.3	20	$4 \cdot 10^5$	40
8–10	$1.56 \cdot 10^6$	2.5 M NaCl in 0.2 M glycine buffer, pH 4	10	$2.4 \cdot 10^4$	1.5
20	$1 \cdot 10^6$ – $1.7 \cdot 10^6$	2.5 M NaCl in 0.01 M phosphate buffer, pH 7.3 2.5 M NaCl in 0.2 M glycine buffer, pH 4 0.05 M EDTA in 0.01 M phosphate buffer, pH 7.3 0.9 M borate buffer, pH 6.0	20	$< 2 \cdot 10^3$	0

The gel (10 ml) was eluted with approx. 60 ml effluent added in 3 portions during 45–60 min. The eluents were dialyzed 3 times against 0.9% NaCl in 0.001 M phosphate buffer, pH 7.3, before the determination of phage activity.
[a] The phage binding was carried out in the presence of 0.01 M $MgCl_2$, 0.003 M $CaCl_2$ and 0.003 M $MnCl_2$.

in table 3. For the binding at least 30 min contact between phage and the column was required. Phage particles attached to the receptor column better at room temperature than at 0°C; the presence of divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}) increased the number of phage particles bound. CNBr-activated Sepharose treated with ethanolamine (control gel) gave negative results in attachment of the phage. Phage SKVI was also not bound to phase II LPS_H-Sepharose 4B indicating that the specific binding of the phage occurs only to a complete LPS structure.

The conditions of the elution of active phage SKVI attached to phase II LPS_A-Sepharose are given in table 4. The elution of active phage particles, up to 60%, was possible when the adsorption was carried out at 0°C. At higher temperature (8–20°C) the binding of the phage was irreversible. The best elution results were obtained in 0.5 M borate buffer, pH 7.3, containing 0.05 M EDTA.

The binding and elution of phage SKVI was studied also on phase II LPS_A coupled to epoxy-activated Sepharose 6B. Although coupling efficiency was twice as high as in the case of CNBr-activated Sepharose 4B (see table 1), only $0.5 \cdot 10^6$ phage particles were attached to 10 ml of the gel at 0°C and 30% of them were eluted from the column.

The affinity chromatography of the anti-serum or the phage can be repeated several times on the same column. In every case before use, the affinity column should be eluted with 3 M KCNS to remove completely all adsorbed materials, and then equilibrated with 0.9% NaCl in 0.01 M phosphate buffer pH 7.3.

In conclusion the present results show the following possibilities:

(1) Lipopolysaccharide or its degradation products can be immobilized on Sepharose gel and used for purification of bacterial antisera.

(2) Coupling of lipopolysaccharide to Sepharose matrix yields a receptor which can adsorb bacteriophage specifically. When the binding of the phage is reversible (at 0°C), the significant portion (up to 60%) of the active phage particles can be eluted.

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