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Affinity chromatography of human anti-dextran antibodies Isolation of two distinct populations

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Abstract

Affinity chromatography is a very efficient method for antibody purification. Two affinity chromatography supports were prepared to analyze the specificity of anti-dextran antibodies. Silica beads were grafted with native dextran or with functionalized dextran. The anti-dextran antibodies present in some human sera were analyzed by enzyme-linked immunosorbent assay method. These antibodies play an important role in severe dextran-induced anaphylactic reactions in humans by forming immune complexes with clinical dextran. The results indicated that two distinct populations of anti-dextran antibodies were purified from human serum, using dextran-coated silica beads. Elution from this support with an oligo-dextran of 4000 g/mol allowed the isolation of one population that only recognized native dextran as antigen. Functionalized dextran coated on dextran silica beads led to the purification, with a glycine–HCl buffer, of another subclass of antibodies that recognized substituted dextran derivatives. Furthermore, these antibodies could be useful tools for in vitro and in vivo investigations using dextran derivatives as bio-active polysaccharides.

Keywords: Antibodies; Anti-dextran antibodies; Dextran

1. Introduction

Dextrans are polysaccharides synthesized from sucrose by bacterial species and constituted of linear chains of α(1,6)-linked D-glucopyranosyl units with occasional branching of the chain due to α(1,3) linkages [1]. Commercial dextrans are produced by sucrose fermentation using a strain of *Leuconostoc mesenteroides* and have no animal source [2]. The molecular weights ranging from 10^4 g/mol to 10^6 g/mol are controlled by altering the fermentation conditions. Dextran are interesting polysaccharides since they are used in medicine as plasma volume expanders or carrier molecules [3]. Dextran gels have also been elaborated as drug release matrices [4,5]. Recently, dextrans were used to improve preservation of large organs including the kidney, liver, lungs and pancreas [6,7]. However, anaphylactoid and anaphylactic reactions to dextran may occur [8]. These reactions are developed shortly after the beginning of the infusion with clinical dextrans of $4 \cdot 10^4$ to $7 \cdot 10^4$ g/mol [9]. In fact, the pathogenic mechanism of this anaphylactic reaction has been identified as an immune complex-mediated reaction caused by naturally occurring dextran-reactive antibodies [10–13]. Dextran-reactive antibodies of the IgG class played the most important role in the induction of severe dextran-induced anaphylactic

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reactions that is classified in four grades of severity [12]. Such antibodies are even present in sera from healthy humans who have never received infusion of clinical dextrans. Different studies showed that such antibodies resulted from antigenic stimulation during the first year of life. The appearance of antibodies directed against dextran is also influenced by the type of feeding [14]. A cross-reaction was also considered with antibodies induced by bacterial polysaccharides, e.g., pneumococci, streptococci. Other published studies pointed out that reaction to dextran seems to occur in patients suffering from rheumatoid arthritis, gastro-intestinal ulcers, systemic lupus erythematosus, nephropathy and cirrhosis of the liver since antibodies directed against dextran are frequently elevated in these people's sera [11–15]. Nevertheless, these dextran reactive antibodies are poorly characterized and the specificity of such antibodies remains under investigation.

In this context, the use of antibodies whose specifity is well-defined could be very useful to diagnose some diseases where dextran reactive antibodies are implicated.

Moreover, chemically modified dextrans were demonstrated to exhibit different biological properties [16]. In fact, according to the chemical nature and the distribution of the substituents, dextran derivatives exhibit anticoagulant activities [17] and inhibit complement activation in vitro and in vivo [18]. Some modified dextrans inhibit smooth muscle cell proliferation [19–21] and others inhibit the growth of human breast cancer cells [22]. These substituted dextrans have also been reported to develop specific interactions with HIV-1 envelopes [23] and other types of viruses [24]. Thus, various investigations involving dextran reactive antibodies are necessary to perform medical applications with the bio-active dextran derivatives. Dextran antibodies could also be used as immunological tracers for in vivo and in vitro experiments using dextran derivatives.

In the present study, human dextran specific antibodies were analyzed by an enzyme-linked immunosorbent assay (ELISA) method in which dextran was coated to wells in a microtiter plate [10]. The purification of human anti-dextran antibodies by affinity chromatography using two types of silica beads coated with dextran or dextran derivatives is reported. Finally, the distinct specificities of the purified fractions were studied.

2. Experimental

2.1. Sources of sera

Human IgG anti-dextran serum named MPC was provided by Dr. M.P. Carreno (Broussais Hospital, Paris, France). Normal human sera as negative controls were obtained from 3 healthy volunteers of our laboratory.

2.2. Antigen preparations

Heparin (H 108) was purchased from Sanofi-Recherche (Centre Choay, Gentilly, France). This material exhibited a specific anticoagulant activity of 173 IU/mg. A fraction of a sulfated polysaccharide from brown seaweed with a chromatographic molecular mass (M_r) of 18 000 was obtained from Ifremer (Nantes, France). Oligo-dextran (M_c=4000) batch OD1 860901 was a gift from Bio-Europe (Toulouse, France). Dextran sulfate (M_r=8000) was purchased from Sigma (La Verpillière, France) and dextran T 40 (M_r=43 900) was provided from Pharmacia (Bois d'Arcy, France). All water-soluble dextran derivatives were prepared from dextran T 40 and characterized by acidimetric titrations and elemental analyses as previously reported [16]. Briefly, carboxymethyl dextrans (CMD) were synthesized from native dextran (D) by random substitution of glucosyl units with carboxymethyl groups (CM). In a second step, benzylamine was coupled to some carboxylic groups along the CMD backbone to form Carboxymethyl Dextran Benzylamide (CMDB). Finally, some benzylamide aromatic rings were sulfonated in order to obtain the Carboxymethyl Dextran Benzylamide Sulfonate (CMDBS). The chemical structure of these substituted dextrans is shown in Fig. 1. The chromatographic molecular mass (M_c) of the dextran derivatives were determined by high-performance liquid steric exclusion chromatography in 0.2 *M* sodium chloride, using a Licrospher Si 500 Diol column (Merck, France) calibrated with pullulans standards (Polymer Laboratories, Montluçon,

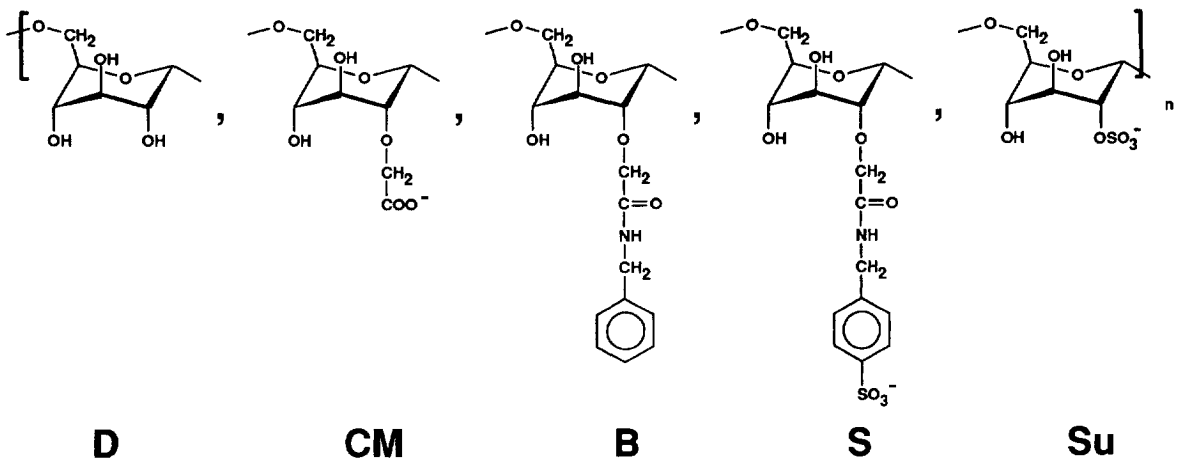

Fig. 1. Chemical structure of substituted dextrans. Carboxymethyl dextran (CMD), carboxymethyl benzylamide dextran (CMDB), carboxymethyl benzylamide sulfonate dextran (CMDBS) and carboxymethyl sulfate dextran (CMD Su) were synthesized from native dextran T 40 (D).

France). Substitution levels and the chromatographic molecular mass are indicated in Table 1.

2.3. Chromatographic supports

2.3.1. Dextran-coated silica beads (SiD)

The synthesis of coated silica beads with dextran was performed as originally described [25]. Silica beads (particle size 15–25 μm, 1000 Å porosity), kindly provided by Biosepra (Villeneuve la Garenne), were coated with dextran T 70 (Pharmacia) carrying a calculated amount of diethyl aminoethyl (DEAE) groups to neutralize negatively charged silanol groups bearing by silica. The percentage of dextran units carrying DEAE groups was 4%. The coating of silica beads with the DEAE-dextran polymer was performed using a batch method followed by a cross-linking reaction with 1,4-butanediol diglycidyl ether (Sigma).

2.3.2. Derivatized dextran grafted upon dextran-coated silica beads (SiD–CMDBS)

Dextran-coated silica beads were functionalized by grafting carboxymethyl dextran benzylamide sulfonate (CMDBS) as previously described [25–28] using 1,4-butanediol diglycidyl ether as a coupling agent.

2.4. Chromatographic procedures

2.4.1. Dextran-coated silica support (SiD)

A 1.0-ml quantity of the affinity sorbent was packed into a chromatographic column (7×0.2 cm I.D.). After equilibration with 15 ml of phosphate buffered saline (PBS) pH 7.2, a 1-ml aliquot of human anti-dextran IgG prepared as an ammonium sulfate precipitate of whole human serum was loaded on the column. Unadsorbed antibodies were collected by washing off the column with 4 ml of the initial buffer solution. To desorb the adsorbed antibodies, different buffers were later used: 5% (w/v) dextran (M_c=4 000 g/mol) in PBS (pH 7.2), 0.1 *M* glycine–HCl (pH 2.6) and NaCl concentrations of 1, 2 and 3 *M*. The desorbed proteins were detected at 280 nm and the collected chromatographic fractions were dialyzed at 4°C against glycine–HCl (pH 2.6) and then against 0.01 *M* Tris buffer (pH 7.2). The dialyzed fractions were analyzed by ELISA.

2.4.2. Derivatized dextran-coated dextran silica support (SiD–CMDBS)

A 1.0-ml volume of the derivatized dextran-coated dextran silica support was packed into the chromatographic column as described above and equilibrated in PBS. The same buffers described above and 0.1 *M* glycine–NaOH (pH 10.5) were used to desorb the

Table 1
Characterization of polysaccharides

Polysaccharides	Composition (%)					Chromatographic molecular mass M_c (g/mol)
	D	CM	B	S	Su	
Dextran 4	100	0	0	0		4 000
Dextran T 40	100	0	0	0		43 900[a]
CMD 1	49	51	0	0		nd
CMD 2	39	61	0	0		61 700
CMD 3	24	76	0	0		nd
CMD 4	16	84	0	0		nd
CMD 5	0	110	0	0		nd
CMDB 1	0	70	30	0		67 600
CMDB 2	0	60	45	0		60 000
CMDBS 1	7	73	5	15		42 700
CMDBS 2	0	80	4	16		100 000
CMDBS 3	0	61	19	26		60 300
CMDBS 4	0	75	24	19		50 000
Dextran sulfate DxS	0	0	0	0	230	8 000[a]
CMD Su	54	33	0	0	13	52 500
Fucan						18 000
Heparin						21 000

Percentage of glucosidic dextran (D), Carboxymethyl (CM), benzylamide (B), sulfonate (S) and sulfate (Su) units. Carboxymethyl dextran (CMD), carboxymethyl benzylamide dextran (CMDB), carboxymethyl benzylamided sulfonate dextran (CMDBS) and carboxymethyl sulfate dextran (CMD Su) were synthesized from native dextran T 40. Total percentage of D, CM, B, S and Su units could be higher than 100 if more than one hydroxyl group per glucosidic unit was substituted. Fucan and heparin, two other sulfated polysaccharides were used as controls.

[a] Average molecular mass (M_r). nd, not determined.

anti-dextran antibodies. The chromatographic fractions were collected and dialyzed before analysis by ELISA.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The assay was carried out in polystyrene 96-well microplates (Dynatech Immulon 4 or Costar 3590, Guyancourt, France). Microplate wells were coated in duplicate with 100 μl of antigen preparations (native dextran, substituted dextrans and other polysaccharides) at a concentration of 10 μg/ml in carbonate–bicarbonate buffer, (pH 9.6). The plates were left overnight at 4°C. The wells were washed 3 times with phosphate-buffered saline containing 0.05% Tween (PBS-Tween), pH 7.2 using an automatic washer (Dynatech). Overcoating was performed with 100 μl/well of 0.5% teleostean gelatin (Sigma) in PBS for 90 min incubation. Then, 50 μl of diluted unpurified or purified human anti-dextran antibodies prepared in PBS-Tween were incubated for 90 min at room temperature. The wells were washed 3 times with PBS-Tween and incubated again for 90 min at room temperature with 100 μl of Fc specific monoclonal anti-human Ig or IgG conjugated biotin (Sigma) at 1:5000 in PBS-Tween. After washing 3 times with PBS-Tween, 100 μl of streptavidin-labeled peroxydase at 1:50 (Sigma) were added to each well. The plates were further incubated at room temperature for 90 min and washed 3 times with PBS–Tween and 3 times with 0.1 *M* citrate buffer (pH 6). Finally, 100 μl of citrate buffer containing 0.1% 3,3′,5,5′-tetramethyl benzidine (Sigma) and 0.05% hydrogen peroxide at 30% (Sigma) was added to each well. After stopping the reaction with 100 μl/well of 1 *M* H_2SO_4, absorbance was measured at 450 nm using an automated microplate reader (Model EL 311, Bio-Tek, Winooski, VT, USA).

3. Results

3.1. Study on the specificities of unpurified human serum

We first investigated the presence of anti-dextran antibodies from the sera of human donors. Fig. 2 shows the recognition of the IgG class anti-dextran antibodies by means of ELISA, using dextran T 40 as antigen. All sera were tested at dilutions ranging from 1:100 to 1:4000 or 1:8000. A positive response was observed for the human MPC serum. A sigmoid curve was obtained and the titer of this serum was approximately 800. In contrast, other human sera originating from other donors did not recognize the dextran T 40, whatever the dilution used and the low optical density values corresponded to the background noise of the test. The MPC anti-serum also reacted with some dextran derivatives as shown in Fig. 3. However, these antibodies presented different affinities towards each tested antigen (Table 2). A positive response for some CMDBS and CMDB

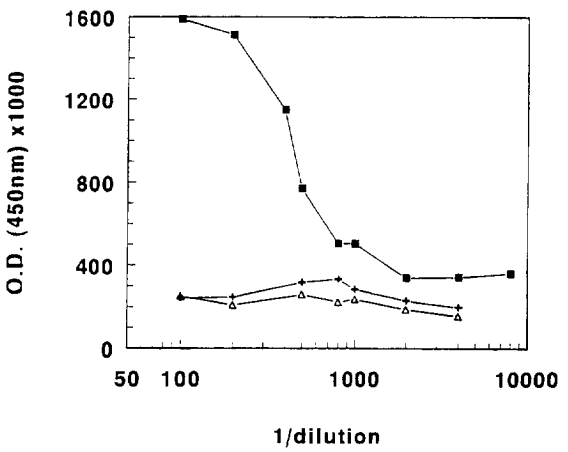

Fig. 2. Assesment of anti-dextran antibodies. The human antiserum MPC (■) and two normal human sera, NS-1 (+) and NS-2 (△), were tested by ELISA using wells coated with 1 μg of native T 40 dextran. Fc specific monoclonal anti-human IgG conjugated biotin–peroxydase (100 μl/well) at 1:5000 in PBS–Tween and 3,3′,5,5′-tetramethyl benzidine as substrate were used and the reaction was measured at 450 nm. The human MPC serum contains antibodies that react with T 40 dextran and has a titer of about 800. Tested normal human sera NS-1 and NS-2 did not respond to dextran and were considered as negative controls.

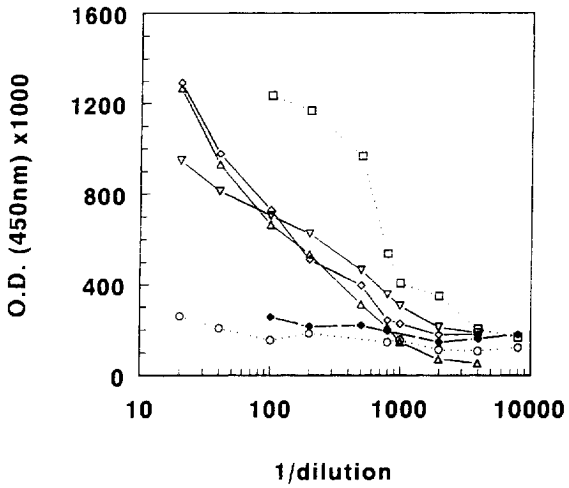

Fig. 3. Specificities of unpurified human MPC serum for various derivatized dextrans. Wells were coated with 10 μg/ml (100 μl/well) of CMD 5 (○), CMDB 1 (△), CMDB 2 (▽) CMDBS 1 (□), CMDBS 3 (◇) and dextran sulfate (DxS; ♦). For chemical compositions see Table 1. The tested CMDB and CMDBS polymers gave a positive response. DxS and CMD polymers gave low density values corresponding to the background of the test.

Table 2
ELISA analysis of some dextran derivatives by the unpurified human IgG anti-dextran antibodies

Polysaccharides	Recognition by anti-dextran antibodies (MPC serum)
Dextran T 40	+++
CMD 1	–
CMD 2	–
CMD 3	–
CMD 4	–
CMD 5	–
CMDB 1	++
CMDB 2	+
CMDBS 1	+++
CMDBS 2	++
CMDBS 3	+
CMDBS 4	+
Dextran sulfate (DxS)	–
CMD Su	–
Fucan	–
Heparin	–

+++: Very high recognition; ++: high recognition; +: moderate recognition; –: no recognition. Composition of derivatized dextrans (CMD, CMDB, CMDBS, DxS, and CMDSu) are reported in Table 1. Fucan and heparin were used as control.

polymers was detected, whereas no response was obtained for the CMD polymers, whatever the percentage of CM units. No recognition was found for the MPC anti-serum towards dextran sulfate (Fig. 3). It should be noted that no recognition of the other tested sulfated polysaccharides such as fucan, a poly(L-fucose-4 sulfate) or heparin (constituted of partially sulfated alternating D-glucosamine and glucuronic acid residues) was detected for the MPC anti-serum (Table 2). Thus, the specificity of the antibodies from the MPC serum was directed against specific structures upon native dextran and some dextran derivatives.

3.2. Purification of the human anti-dextran antibodies by affinity chromatography

Two different polysaccharides, CMDB and CMDBS, were recognized by the MPC anti-serum. These data suggested that the MPC serum contained several populations of antibodies which recognized different epitopic groups upon the tested polysaccharides. In order to test this hypothesis, affinity chromatography experiments were performed to purify these antibodies.

3.2.1. Dextran-coated silica support (SiD)

The human anti-serum MPC was subjected to affinity chromatography on a column of dextran-coated silica beads bearing native dextran as ligand. Elution was performed with PBS (pH 7.2), 0.1 *M* glycine–HCl (pH 2.6), 1 *M* NaCl, 2 *M* NaCl and then 5% (w/v) dextran (4000 g/mol) in PBS (pH 7.2). Fig. 4 reports the analysis by ELISA of the collected and dialyzed fractions. Fig. 4A represents the results towards the native dextran T 40 as antigen. Both unpurified fraction and fraction eluted with the PBS buffer reacted with dextran T 40. This result means that unadsorbed antibodies on the SiD support were recognized by human anti-serum MPC. Fractions eluted with the non-specific buffer as glycine–HCl (pH 2.6), 1 *M* NaCl and 2 *M* NaCl did not respond to native dextran as antigen. Anti-dextran antibodies could not be eluted with these non-specific buffers. However, as indicated in Fig. 4B, antibodies were eluted using a specific buffer, dextran (4000 g/mol) in PBS solution. The chromatographic fractions eluted with the oligo-dextran solution recognized, after dextran removal by dialysis, the native dextran as antigen. We also studied the specifity of these purified anti-dextran antibodies against derivatized CMDB 1 and CMDBS 1. Affinity-purified human anti-dextran antibodies were only detected when the wells of the microplates were

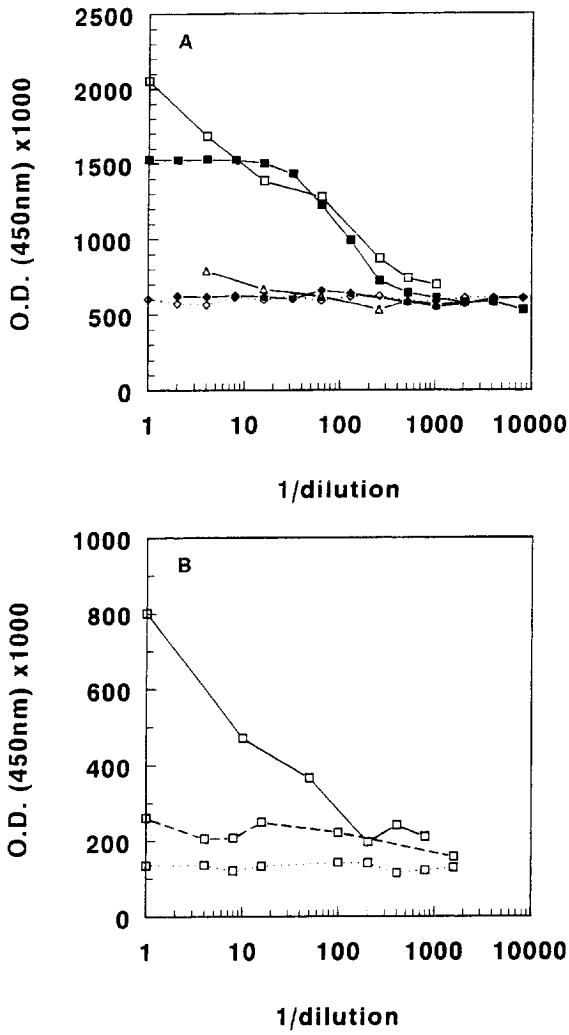

Fig. 4. Analysis by ELISA of the MPC fractions purified on dextran-coated silica beads (SiD support). (A) Native T 40 dextran as antigen, coated at 1 μg/well. The unpurified fraction (■) and the fraction eluted with the PBS buffer (□) recognized the T 40 dextran. Fractions eluted with glycine–HCl, pH 2.6 (♦), 1 *M* NaCl (◇) and 2 *M* NaCl (△). (B) Fractions eluted with 5% (w/v) dextran (4000 g/mol) in PBS were tested on different polysaccharides coated at 1 μg/well: T 40 dextran (□—□), derivatized CMDB 1 (□– – –□), and CMDBS 1 (□···□).

coated with native dextran. The tested CMDB and CMDBS did not respond to the SiD-purified antibodies.

3.2.2. Dextran derivative coupled with dextran-coated silica support (SiD–CMDBS)

To verify if several subclasses of anti-dextran antibodies from the MPC serum were present, an affinity chromatography experiment was performed, using the dextran derivative CMDBS as bound ligand on dextran-coated silica beads (SiD–CMDBS). The following buffers were used to determine the optimum conditions for anti-dextran antibodies desorption: PBS (pH 7.2), 5% (w/v) dextran 4000 g/mol in PBS (pH 7.2), glycine–HCl (pH 2.6), glycine–NaOH (pH 10.5) and 1 to 3 *M* NaCl. After being collected and dialyzed, the fractions were analyzed by ELISA and the results are indicated in Fig. 5. Fig. 5A reports the results using dextran T 40 as antigen. The unpurified fraction and fractions eluted with 5% (w/v) dextran 4000 g/mol and PBS buffer, respectively, showed that these fractions contained antibodies that positively reacted with native dextran. Results of the ELISA test towards a dextran derivative named CMDB 1 are reported in Fig. 5B. Unpurified antibodies from the MPC serum recognized the CMDB 1 antigen. Antibodies in the fraction eluted with the initial buffer solution (PBS, pH 7.2) also recognized the CMDB 1 antigen, although the titer was lower. This suggested that a major part of antibodies present in the MPC serum had been adsorbed on the SiD–CMDBS support. We concluded that the SiD–CMDBS column presented some specific epitopic groups recognized by one population of antibodies. It is interesting to note that the adsorbed antibodies that positively reacted with CMDB 1 were eluted with the glycine–HCl (pH 2.6) buffer. The others buffers, such as 3 *M* NaCl (Fig. 5) and glycine–NaOH (data not shown), did not allow desorption of the antibodies from the SiD–CMDBS support. Competitive elution from this column with soluble CMDB 1 was not performed since its high molecular mass prevented the further dialysing steps. The data indicated that two populations of antibodies were present in the serum and could be purified on the SiD–CMDBS column, using two different desorption solutions. Other ELISA analyses demonstrated that anti-dextran

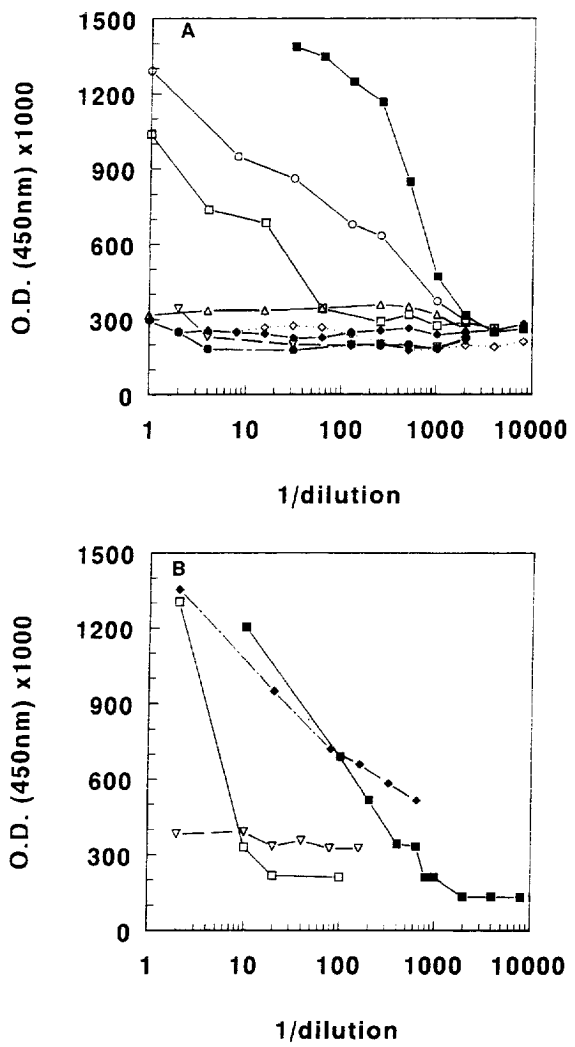

Fig. 5. Analysis by ELISA of the MPC purified fraction on CMDBS as ligand coupled to the SiD support (SiD–CMDBS support). (A) Native dextran as antigen coated at 1 μg/well. The unpurified fraction (■) and the fraction eluted with the PBS buffer (□) reacted positively with native dextran. Fractions eluted in 5% (w/v) dextran 4000 g/mol in PBS solution (○), 1 *M* NaCl (◇), 2 *M* NaCl (△), 3 *M* NaCl (▽) and glycine–NaOH, pH 10.5 (●). (B) ELISA with CMDB 1 (1 μg/well) of the unpurified fraction (■), the fraction eluted on the SiD–CMDBS support with the PBS buffer (□) and the glycine–HCl, pH 2.6 (♦) reacted with CMDB 1. In contrast, fractions eluted in 3 *M* NaCl (▽) did not react with CMDB 1.

antibodies that reacted with the derivatized dextran CMDBS 1 could be purified with CMDBS grafted on dextran coated silica beads (SiD–CMDBS support).

These antibodies had been eluted with the glycine–HCl buffer and not with the basic or higher molarity buffers (data not shown).

4. Discussion

Two compounds as immobilized ligands on silica beads were used for affinity chromatography of human anti-dextran antibodies: native dextran (D) and dextran derivatized with carboxymethyl, benzylamide and sulfonate groups (CMDBS). Anti-dextran antibodies were present in some human sera and were detected using an ELISA protocol. Others have reported that these antibodies belong mainly to the IgG class [10]. Among several possibilities to explain the natural occurrence of anti-dextran antibodies in sera from humans who have never received any clinical dextran, one can mention the presence of the antigen (dextran) itself originating from food, dental plaque or dextran-producing bacteria in the gastro-enteric and respiratory tracts. A cross-reactivity between dextran and polysaccharides from many organisms, e.g., pneumococci and streptococci may also be detected in some human sera [11,12,15]. Recently, some researchers have identified a ubiquitous antigen in low levels, as native dextran in human sera [11].

Our data, analyzed with ELISA, showed that the unpurified antibodies from human MPC serum reacted preferentially with the native dextran T 40, consisting mainly of linear α(1,6)-linked glycopyrannosyl residues. As control, a mouse IgM plasmocytoma, directed mainly against α(1,3) dextran (B 1355 S) only poorly reacted with dextran T 40 (data not shown). Moreover, the human MPC serum contained antibodies that recognized with a lower affinity some tested dextran derivatives such as carboxymethyl benzylamide dextrans (CMDB) and carboxymethyl benzylamide sulfonate dextrans (CMDBS). However, both carboxymethyl dextrans (CMD) and sulfated carboxymethyl dextran (CMDSu) did not expose the antigenic determinant which was normally recognized by the anti-dextran antibodies and, consequently, these derivatives were not recognized by the anti-dextran antibodies contained in the human MPC serum. Other tested sulfated polysaccharides, dextran sulfate (DxS), heparin and fucan appeared insensitive to antibody recognition. The two most likely explanations are as follows. On the one hand, the non-accessibility by steric effect of the antigenic determinant upon the CMD, CMDSu and DxS could be because of their peculiar distribution in substituted groups upon the glucosidic polymer structure. The distribution of the substituents along the macromolecular backbone may influence the flexibility of the macromolecule which is more or less recognized by the antibodies. On the other hand, the reason could be the absence of some epitopic groups susceptible to being recognized by the anti-dextran antibodies. This is evidenced by the sulfated polysaccharides, heparin and fucan, whose chemical structures are different to dextran. According to these results, it should be pointed out that an unknown polysaccharide devoid of sulfate or carboxylate groups, but probably presenting hydrophobic structures (benzylamine-like), is strongly suspected to be the origin of cross-reactivity with dextran derivatives in the human sera. Hydrophobic characteristics of some substituents on the polysaccharidic backbone may play an important role in the response for antibody recognition.

We have performed two affinity chromatography experiments, using the SiD and SiD CMDBS supports to study the cross-reactivity of these anti-dextran antibodies towards dextran and its derivatives. The specificity of antigen–antibody interactions is used by coupling one native dextran or CMDBS as antigens. Chemical strategies such as pH and ionic strength have been undertaken to determine the most efficient eluent to purify human anti-dextran antibodies. On the SiD support bearing native dextran as ligand, reducing the pH to 2.6 using the glycine–HCl buffer or raising the pH to 10.5 using the glycine–NaOH were not sufficient to desorb the anti-dextran antibodies. Variation of ionic strength was examined as another parameter. Elution at high ionic strength is often a good approach in those cases when electrostatic interactions play an important role, even if the kinetics of desorption appear to be slow. The results indicated that NaCl concentrations from 1 to 3 *M* failed to elute the antibodies from the SiD support. In contrast, the fractions eluted with dextran 4000 g/mol and subsequently analyzed by ELISA, recognized the native dextran but not its derivatives. This indicated that competitive desorp-

tion with the oligo-dextran was the most efficient eluent to isolate the dextran-reactive antibodies [29] and that these purified antibodies with high affinity for dextran only recognized native dextran as antigen. This oligo-dextran prepared from sucrose with a glycosyl transferase extracted from *Leuconostoc mesenteroides* is mainly constituted of α(1,6) glycosyl units with two α(1,3)-linked glucose at the end of the backbone. In fact, this dextran has a chemical structure which is more or less specific for these antibodies.

With the CMDBS grafted on the SiD support, the effects of pH and the ionic strength suggested that another population of human anti-dextran antibodies was isolated. The adsorbed antibodies on this SiD–CMDBS support could be eluted with the glycine–HCl buffer (pH 2.6); these eluted antibodies reacted with CMDB 1 and CMDBS 1 but not with native dextran. Other antibodies from the same serum were also eluted from the SiD–CMDBS column, with the oligo-dextran solution and were directed against native dextran. Concerning the influence of the ionic strength, the results have shown that anti-dextran antibodies could not be eluted with saline concentration. This is probably due to non-ionic strong interactions developed between the anti-dextran antibodies and the immobilized ligand as antigen [30].

5. Conclusion

In the current study, we have purified human anti-dextran antibodies using the SiD support and an oligo-dextran solution. The analysis of eluted fractions by ELISA indicated that these purified antibodies showed an exclusive recognition for the dextran and none for its derivatives. Moreover, on the SiD–CMDBS support, we have purified another population of dextran-reactive antibodies, eluted with the glycine–HCl (pH 2.6) buffer. These eluted antibodies recognized the derivatized dextrans (CMDB and CMDBS) but not the native dextran. Thus, the human MPC serum contained at least two populations of anti-dextran antibodies whose specificity has been studied. Further experiments would be performed to determine the affinity constants of these antibodies. These antibodies are excellent tools to study and to estimate the rate of dextran-reactive antibodies involved in some diseases such as nephropathy or anaphylactoid shocks in man [8–15]. Another interesting perspective concerns the biological aspects of the substituted dextran derivatives in relation with the chemical nature and the distribution of the substituents. Indeed, various studies have demonstrated the specific interactions of some CMDB and CMDBS with HIV-1 envelope glycoproteins [18,23,24], the inhibitory effects of CMDBS 1 on complement activation in vitro and in vivo [25] or the antiproliferative capacity on cell growth, such as human breast cancer cells [26] or smooth muscle cells [27–29]. Thus, the anti-dextran or anti-derivatized dextran antibodies should be useful in identifying dextran derivatives for further biological in vitro and in vivo experiments.

Finally, affinity chromatography appears to be a good approach to purify some subclasses of anti-dextran antibodies. Moreover, this methodology would be of use in studying the mechanisms which occur during interactions between the immobilized ligands and these antibodies.

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