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Peptide immobilization on calcium alginate beads: applications to antibody purification and assay

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Abstract

Two different procedures were developed for the non-covalent immobilization of peptide antigens on calcium alginate beads. The antigenic peptide is first synthesized in a multimeric form starting from a polydentate lysine core, and then immobilized on alginate beads (average volume 0.05 ml) by entrapment or simply by non-covalent adsorption. Coupling yields, as monitored by RP-HPLC analysis of the immobilization time course and/or by amino acid analysis of derivatized beads, were close to 1–2 mg of peptide per ml of gel. Immobilization yields were not dependent on the peptide net charge, hydrophobicity or length, but mainly on the extent of peptide multimerization. After immobilization on alginate gel, peptide antigenic properties were fully retained, as clearly demonstrated by the batchwise micropreparative purification of anti-peptide antibodies in good yields and with a high degree of purity, directly from crude sera in a single adsorption–elution step. Derivatized beads were sufficiently stable towards repeated washing–equilibration procedures, allowing very limited peptide leakage from the matrix. Peptide beads were also successfully used for the development of solid-phase immunoassays in test-tubes to characterize the corresponding antibodies, with the immobilization yield and signal-to-noise ratio being greatly enhanced in comparison with other types of conventional supports.

1. Introduction

Natural or synthetic ligands able to recognize target proteins or peptides with sufficient selectivity and specificity find wide applications in affinity chromatography, once their efficient immobilization on compatible solid supports is achieved. The number of biomolecular ligands is still increasing at a very great rate, owing to the success of recently introduced technologies for the production of molecule-specific recognition agents, such as monoclonal antibodies [1], or peptides derived from the screening of synthetic molecular libraries [2]. However, the availability of solid supports suitable for affinity applications is very limited. Agarose was the first affinity support to be widely used [3], but highly cross-linked agarose [4], methacrylate gel [5], silica [6] and glass beads [7] have also found applications in several systems. The standard approach in designing ideal affinity supports has been to combine, on the one hand, hydrophilicity and absence of charged groups on the matrix surface to avoid non-specific interactions and, on the other, a chemically and mechanically stable particle core that can withstand prolonged use in aqueous buffers. To provide maximum flexible use of a wide range of biomolecular separations, most often matrices are designed to contain reactive or active groups to which interactive

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ligands can be conveniently attached. Non-covalent immobilization of ligands on solid supports for the preparation of affinity media has been little explored, as the predictable ligand leakage from the support could constitute a source of contamination during the purification process.

In this study, we explored the possibility of using alginate gel for non-covalent immobilization of peptide ligands, and its applications as a biospecific support for antibody purification by affinity chromatography and for the development of analytical immunoassays. Physical entrapment in a polymeric gel matrix, such as alginate, is one of the simplest methods of immobilization. The alginic acid, a constituent of marine algae, is a copolymer of β-D-mannuronic and α-L-guluronic acid, of widely varying composition and sequence [8,9]. Alginate gel is prepared from sodium alginate in the presence of calcium ions, which leads to the immediate formation of gel spheres, sufficiently stable towards mechanical treatments.

The alginate gel entrapment technique can be carried out in a single-step process under very mild conditions and is therefore compatible with most living cells [9]. The open-lattice structure of the gel leads to leakage of molecules such as proteins of small molecular size. Thus alginate is generally used for the immobilization of whole cells or cell organelles [10]. Two different procedures have been developed in this study for the non-covalent immobilization of peptide antigens on calcium alginate beads: (i) by surface adsorption on gel beads and (ii) by internal entrapment, preparing gel beads in the presence of the peptide ligand. Both procedures require the synthesis of the antigenic peptide in a multimeric form starting from a polydentate lysine core, similarly to the preparation of multiple antigenic peptides for antiserum production [11]. In order to test the bioavailability of the peptides after immobilization on alginate beads, anti-peptide antibodies were raised in rabbits and tested for their ability to recognize the corresponding immobilized antigens. Binding characteristics were evaluated using different assay formats, by measuring the ability of immobilized peptides to detect and/or purify the corresponding antibodies from crude sera. The effect of several parameters on the peptide immobilization, such as peptide net charge, length, extent of multimerization, bead size, possibility of recycling and leakage, were also investigated to assess the usefulness of alginate as a bioaffinity matrix.

2. Experimental

2.1. Materials

9-Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids for peptide synthesis were purchased from Novabiochem (Laufelfingen, Switzerland), high-performance liquid chromatography (HPLC)-grade dichloromethane (DCM), N-methyl-2-pyrrolidone (NMP), methanol (MeOH), diethyl ether, water, acetonitrile, Tris [tris(hydroxymethyl)aminomethane)], acetic acid, citric acid and pentane from Merck (Darmstadt, Germany), trifluoroacetic acid (TFA) and hydrochloric acid (HCl) from Pierce (Rockford, IL, USA), solutions of dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt) used during peptide synthesis from Applied Biosystems (Foster City, CA, USA) and reagents used as scavengers during cleavage of peptides from resin, such as phenol, thioanisole and ethanedithiol, from Aldrich (Milan, Italy). Polystyrene microtitre plates were purchased from Becton Dickinson (Oxnard, CA, USA). Goat anti-rabbit IgG–horseradish peroxidase (HRP) conjugates, bovine serum albumin (BSA), *o*-phenylenediamine, alginic acid from *Macrocystis pyrifera*, low viscosity, and calcium chloride were from Sigma (St. Louis, MO, USA).

2.2. Solid-phase peptide synthesis

Peptides were synthesised by solid-phase peptide synthesis following the Fmoc methodology on an Applied Biosystems fully automated Model 431A peptide synthesizer, software version 1.2. For multimeric peptide synthesis, two

subsequent couplings of Fmoc–Lys(Fmoc) were performed on a gly–4-hydroxymethylphenoxyacetic (HMP) resin, dividing the amount of resin in half after each step. After completion, on a 0.1-mmol scale, the corresponding peptide antigen was assembled. Finally, the peptide resins were dried overnight under vacuum. Peptides were cleaved from the resin (100 mg) using 5 ml of TFA–phenol–thioanisole–water–ethanedithiol (83:6:5:4:2, v/v) and by incubating at room temperature for 3.5 h. The mixtures were then filtered and vacuum concentrated to 0.5 ml and the peptidic material was precipitated by adding 10 ml of cold diethyl ether. The crude material was then dissolved in 20 ml of 0.1 *M* acetic acid (HOAc). Multimeric peptides were extensively dialysed against 0.1 *M* HOAc and lyophilized. Linear peptides were dissolved in water–acetonitrile (50:50, v/v) and purified by reversed-phase HPLC (RP-HPLC).

2.3. Peptide immobilization by non-covalent adsorption on calcium alginate beads

A 3% (w/v) aqueous solution of sodium alginate (alginic acid from *Macrocystis pyrifera*, low viscosity) was prepared by suspending the polymer in distilled water. The suspension was stirred for 2 h at room temperature. The homogeneous mixture was centrifuged at 1300 *g* for 5 min, to remove air bubbles, and then extruded drop by drop using a syringe needle into a 0.2 *M* $CaCl_2$ solution. The resulting gel beads were left to harden in $CaCl_2$ for 1 h at room temperature and then washed exhaustively with distilled water. The washed gel was stable at 4°C for several weeks. For peptide non-covalent adsorption, ten alginate beads (1 mm diameter) were incubated with 0.2 ml of different peptide solutions [0.1–2 mg/ml in water–acetonitrile (9:1, v/v)] for 3 h at room temperature. After incubation, the gel beads were washed three times with 0.2 ml of 25 m*M* Tris–5 m*M* $CaCl_2$ (pH 7.5) and then incubated for 15 min in 0.2 ml of 0.1 *M* HOAc. The immobilization yields were evaluated by RP-HPLC determination of the peptide content in the incubation mixture at different times by gradient elution on an ABI-Aquapore RP 300 (30 × 2.1 mm I.D.) column equilibrated at a flow-rate of 0.5 ml/min with water–acetonitrile–TFA (97:3:0.1, v/v/v).

2.4. Antibody purification

Purification of polyclonal antibodies on multimeric antigenic peptides (MAP) beads was carried out by incubating 50 derivatized beads (20 μg of peptide per bead, prepared as described above) in 3 ml of crude antiserum diluted in 50 m*M* Tris–10 m*M* $CaCl_2$ (pH 7.5). After incubation, the alginate beads were washed four times with 3 ml of Tris buffer, to remove non-specific adsorbed protein, and then bound antibodies were eluted with 1 ml of 0.1 *M* HOAc. The total amount of bound and unbound antibody was determined by enzyme-linked immunosorbent assay (ELISA). The antibodies were checked for purity by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Control experiments with underivatized beads were also performed in order to evaluate the level of non-specific interactions.

2.5. Peptide immobilization by non-covalent entrapment in calcium alginate beads

Aliquots of 1 ml of solutions containing different amounts of purified multimeric peptides (from 1 to 5 mg) were mixed with 1 ml of 6% sodium alginate solution (alginic acid from *Macrocystis pyrifera*, low viscosity) and the mixtures were stirred for 1 h at room temperature. The gel beads were formed by dripping the alginate–peptide solutions into 0.2 *M* $CaCl_2$ solution using different syringe needles. The resulting gel beads were left to harden in $CaCl_2$ for 1 h at room temperature and then washed exhaustively with distilled water. The diameter of the beads ranged from 1 to 5 mm.

The entrapment yields were determined by RP-HPLC analysis of the aqueous immobilization medium (0.2 *M* $CaCl_2$) after dialysis against water and lyophilization. After immobilization, the alginate beads were incubated for 30 min in 0.1 *M* Tris (pH 7.5) and in 0.1 *M* HOAc and peptide leakage was monitored by RP-HPLC

HPLC analysis. Peptide elution on an ABI-Aquapore RP 300 column, was carried out as described above.

2.6. Amino acid analysis

The amino acid composition of synthetic peptides and of peptides immobilized in calcium alginate beads was evaluated by RP-HPLC analysis of Fmoc-derivatized acid hydrolysates.

2.7. Sera preparation

New Zealand rabbits were immunized with multimeric peptides (50–200 μg) dissolved in 0.15 *M* sodium chloride–0.05 *M* sodium phosphate buffer (pH 7.3) and emulsified with complete Freund's adjuvant containing H37RV (2 mg/ml) by injections in the rear foot pads. Two weeks later the same immunogen was administered subcutaneously. After two subsequent boosters, blood samples were collected from each animal for monitoring antibody titres. One week after the last boost the animals were bled and sera stored at −80°C.

2.8. ELISA assays

Binding assays with peptide immobilized on alginate beads

The multimeric peptide antigens were immobilized by entrapment or non-covalent adsorption in alginate gel as described above, and the resulting beads were equilibrated in 0.1 *M* Tris–10 m*M* $CaCl_2$ buffer (pH 7.5). The beads were then placed in vials (three beads per vial) and incubated in 0.2 ml of different antisera at various concentrations [diluent: 0.1 *M* Tris–10 m*M* $CaCl_2$ (pH 7.5)] for 1 h at room temperature and then washed with diluent buffer. Subsequently, 0.2 ml of horseadish peroxidase-labelled goat anti-rabbit (IgG) immunoglobulin solution, diluted 1:1000 with Tris buffer, was added to each vial and incubated for 1 h at room temperature. After washing with diluent buffer, each vial was treated with a chromogenic substrate solution consisting of a 1 mg/ml solution of *o*-phenylendiamine in 0.1 *M* sodium citrate (pH 5.0) containing 5.0 m*M* hydrogen peroxide for 1 min, and the absorbance at 492 nm was determined with a multi-scan bichromatic reader (Labsystem). Alginate beads with no peptide immobilized were used as controls.

Binding assay with multimeric peptides immobilized on microtitre plate

Polystyrene microtitre plates were incubated with 15 μg/ml multimeric peptide solution (100 μl per well) in 0.1 *M* sodium carbonate buffer (pH 8.5) overnight at 4°C. After peptide coating, the plates were washed with phosphate-buffered saline (PBS) [150 m*M* sodium phosphate–150 m*M* NaCl [pH 7.5)] and 200 μl of PBS containing 3% BSA were added to each well to block the uncoated plastic surface (incubation for 1 h at room temperature). The plates were then washed again with PBS and filled with the corresponding antisera at varying dilutions in PBS containing 0.5% BSA (pH 7.5). After 1 h, each well was incubated with 100 μl of anti-rabbit IgG–peroxidase diluted 1:1000 with PBS containing 0.5% BSA (pH 7.5). The plates, washed again with PBS buffer, were treated with chromogenic substrate solution composed of a 1 mg/ml solution of *o*-phenylendiamine in 0.1 *M* sodium citrate (pH 5.0) containing 5.0 m*M* hydrogen peroxide. The absorbance at 492 nm was determined with a multi-scan bichromatic reader (Labsystem).

2.9. Electrophoresis

Samples of affinity-purified anti-peptide antibody were analysed by SDS-PAGE (9% polyacrylamide, 0.1% SDS) using a Phast system apparatus (Pharmacia) according to the manufacturer's instructions. Proteins were stained using the silver-staining method [12].

3. Results

3.1. Multimeric peptides

The general structure of the tetrameric peptides used in this study is shown in Fig. 1. The

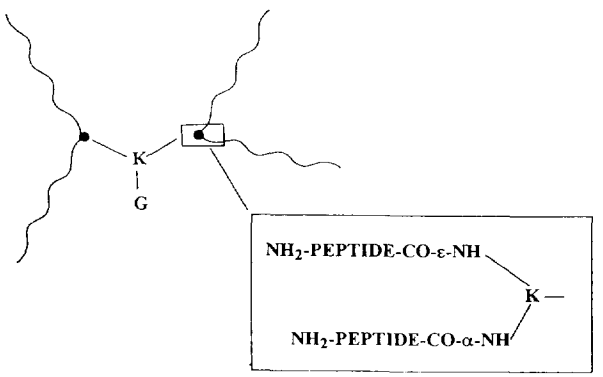

Fig. 1. General structure of a tetrameric peptide. The central Gly–Lys core (G–K) is linked via the α- and ε-lysine amino groups to two lysine residues, from which depart the four peptide antigen chains linked to the α- and ε-lysine amino groups.

central lysine residue is linked via the α- and ε-amino groups to two lysine residues, from which the peptide antigen chains depart. For immobilization studies on alginate gel, five different tetrameric peptides available in our laboratory were selected, denoted MAP1–5, each with a different sequence, with monomeric units ranging in length from 12 to 17 residues (M_r 5000–10 000) and total net charge from 0 to +24 at pH 7. In control experiments, several monomeric peptides of the same length and charge were also tested. All the peptides used in this study were purified to homogeneity and characterized by the amino acid composition of acid hydrolysates and by laser desorption mass spectrometry in order to confirm the intended chemical structure.

3.2. Multimeric peptide immobilization by gel entrapment

Entrapment of multimeric peptides on alginate beads was achieved by preparing sodium alginate aqueous solutions in the presence of the multimeric peptides at concentrations ranging from 1 to 5 mg/ml. Gel beads were then formed by dropping this solution into a calcium chloride solution using a syringe equipped with a narrow-bore needle (see Experimental). The bead diameter was determined by the needle size, and ranged from 1 to 5 mm. The immobilization yield was close to 1–2 mg of peptide per ml of sodium alginate, as monitored by amino acid analysis of exhaustively washed beads after vacuum drying and acid hydrolysis and/or by RP-HPLC analysis of the aqueous immobilization medium (see Experimental). Peptide leakage from the gel matrix was evaluated in different buffers [0.1 *M* Tris (pH 7.5) and 0.1 *M* HOAc]. Under all the experimental conditions tested, the entrapped peptide was not removed during a 30-min wash, as determined by RP-HPLC analysis of the washing solutions. The antigenicity of encapsulated peptides in alginate beads was then tested by measuring their reactivity with the corresponding anti-peptide antibodies prepared by rabbit immunization with the multimeric peptides. Beads were equilibrated in binding buffer [0.1 *M* Tris–10 m*M* $CaCl_2$ (pH 7.5)] and treated with different amounts of crude serum. As shown in Fig. 2 for MAP4, the absorbance was dependent on the amount of anti-peptide antiserum added, as detected by subsequent incubation with anti-IgG antibodies conjugated to peroxidase. In different experiments, the same amount of antiserum was added to beads prepared by entrapping different amounts of the corresponding multimeric peptide. As shown in Fig. 3, also in this case the signal was found to be proportional to the amount of peptide used in

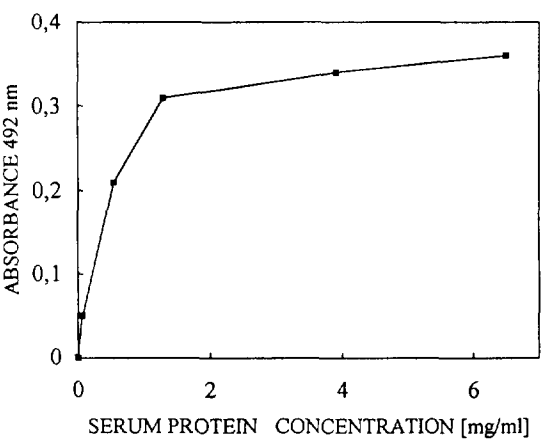

Fig. 2. Binding of anti-MAP4 antiserum at different concentrations, to MAP4, immobilized by entrapment in alginate beads at a 1 mg/ml concentration.

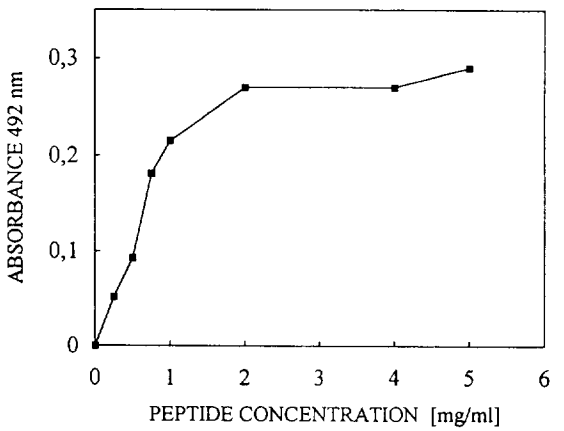

Fig. 3. Binding of anti-MAP4 antiserum, at a 1:1000 dilution, to different amounts of MAP4 immobilized by entrapment in alginate beads at different concentrations.

the immobilization procedure, as expected. The effect of bead size on the peptide immunoreactivity was also tested, by preparing a set of beads of different diameters but using the same peptide concentration during bead preparation. Using the same amount of serum in each experiment, the signal was found to be inversely proportional to the bead diameter in this case (Fig. 4), indicating that on smaller beads the peptide is more available for interaction. Similar results were obtained with all the remaining tetrameric peptides and their corresponding antisera. In control experiments, non-derivatized beads provided very low absorbance values (0.03–0.05 AUFS).

3.3. Multimeric peptide immobilization by adsorption

Peptide immobilization by non-covalent surface adsorption on calcium alginate beads was carried on by incubating multimeric peptides with alginate beads in water–acetonitrile mixtures. The extent of immobilization was determined by RP-HPLC determination of the peptide content in the incubation mixture after 1 h, for the five MAPs and for the monomeric peptide control. As shown in Fig. 5, the immobilization yield ranged from 70 to 100% of the added peptides (20 μg of peptide per 10 beads), and was not related to the presence of peptide charged residues. When the corresponding linear peptides were immobilized under identical conditions, the coupling yield was below 20%. As the immobilization yields were high and very similar for all multimeric peptides, but very low for monomeric peptides, peptide immobilization on alginate beads did not seem to depend on the peptide net charge, hydrophobicity or length,

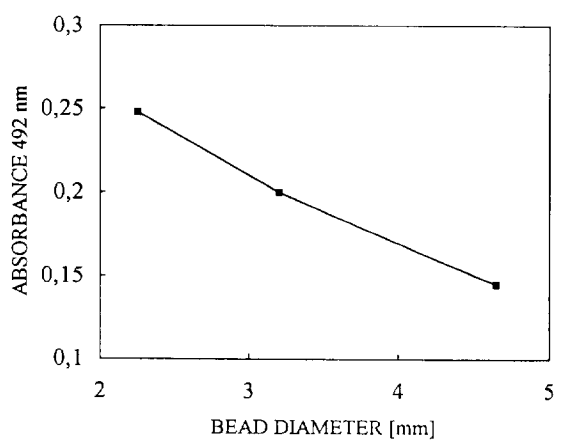

Fig. 4. Effect of bead size on immobilized peptide immunoreactivity. Beads were produced using three different needle gauges; each samples contained 0.2 g of bead alginate (wet mass) with MAP4 of 1 mg/ml of alginate, and treated with a fixed concentration of anti-MAP4 antiserum.

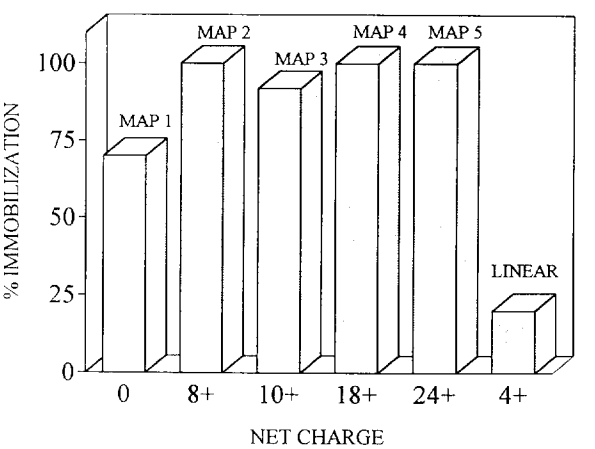

Fig. 5. Immobilization yields of different multimeric peptides by adsorption on alginate beads. In each case 20 μg of peptide in 0.2 ml of water–acetonitrile (9:1) was added to ten alginate gel beads. The amount of unbound peptide was determined by HPLC analysis.

but mainly on the extent of peptide multimerization. Only multimeric peptides were efficiently adsorbed on alginate beads, as the low percentage of monomeric peptide bound to the beads was removed completely after a subsequent washing step. MAP beads were stable towards repeated washings with 0.1 *M* Tris (pH 7.5) and 0.1 *M* HOAc and no peptide leakage from the gel matrix was observed under these conditions. The bead capacity for peptide loading was investigated in detail with MAP4, by immobilizing different amounts of the peptide, from 1 to 60 μg, on a single bead. The peptide coupling yield was close to 100% in the concentration range 1–40 μg and, consequently, up to 40 μg of multimeric peptide could be immobilized on a single bead, with a yield close to 100%.

Peptides adsorbed on beads were then used for the development of a solid-phase immunoassay in test-tubes to characterize the corresponding antibodies. A fixed amount of peptide (MAP4) was adsorbed on alginate beads as described before, and then the derivatized beads were incubated with the corresponding antiserum at different dilutions. The presence of antibody bound to the beads was detected by a subsequent treatment with anti-IgG conjugated to peroxidase as before. As shown in Fig. 6A, binding was again linearly dependent on the amount of antiserum added, and saturable. In this case, the absorbance was far superior to that obtained with the other method of immobilization, suggesting that a higher proportion of multimeric peptide was available on the bead surface for antibody binding. In control experiments, binding of antibodies to underivatized beads was negligible. The use of alginate beads for immunoassay development was then compared with conventional plastic microtitre plates, by immobilizing the same multimeric peptide at the same concentration. As shown in Fig. 6B, dose–response curves indicated a lower signal-to-noise ratio, as the level of a specific signal detected at high antiserum concentration was much higher than that obtained with underivatized alginate beads. Peptide beads were then tested for their stability towards repeated equilibration–desorption steps. Alginate beads derivatized with MAP4 were incubated with 0.1 *M* Tris for 50 min and then with 0.1 *M* HOAc for 15 min, and these cycles were repeated several times. The residual immunoreactivity on the beads after each cycle was determined by incubating the beads with the corresponding anti-peptide antiserum and by measuring the amount of antibody adsorbed on the beads. The beads retained almost 80% of the initial immunoreactivity even after nine repeated cycles, which decreased to 30% after seventeen cycles (Fig. 7).

Multimeric peptides adsorbed on alginate

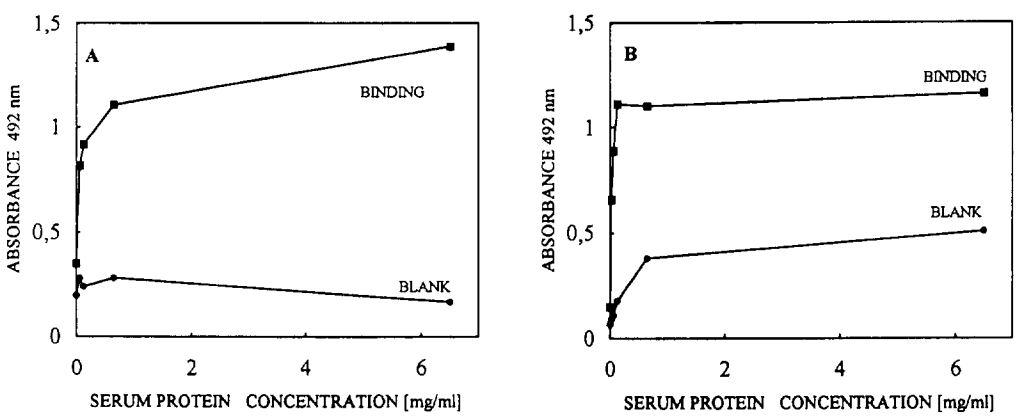

Fig. 6. (A) Binding of anti-MAP4 antiserum, at different dilutions, to MAP4 immobilized by adsorption on alginate beads (40 μg per bead). All experiments were performed by using one bead per test-tube. (B) Binding of anti-MAP4 antiserum, at different dilutions, to microtitre plates derivatized with 100 μl of MAP4 at a concentration of 20 μg/ml.

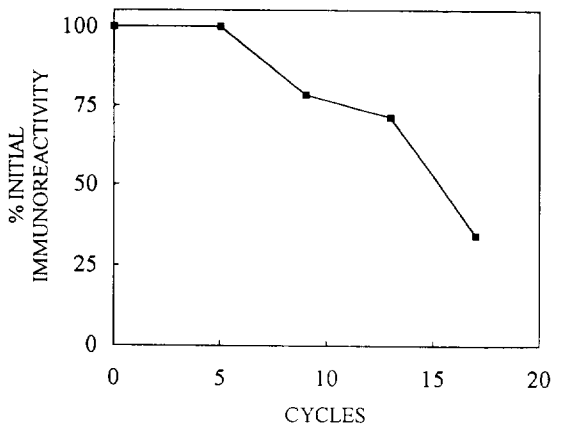

Fig. 7. Recovery of immunoreactivity on MAP4-derivatized beads, when repetitive experiments of buffer incubations were performed. Each cycle consisted of a 50-min incubation in 0.1 *M* Tris–10 m*M* $CaCl_2$ (pH 7.5) and a 15-min incubation in 0.1 *M* HOAc.

beads were also tested for their ability to purify the corresponding antibodies from crude sera. Vials were prepared with MAP-derivatized beads and the corresponding crude sera were added directly and incubated for 1 h at room temperature. Finally the beads were washed several times with the Tris buffer solution and treated with dilute HOAc to remove the adsorbed antibody. Serum incubation with peptide beads resulted in an efficient removal of immunoreactivity, which was recovered after treatment of the beads with dilute HOAc (Fig. 8). Underivatized beads were uneffective in removing immunoreactivity, which was fully recovered in the washings. In order to evaluate the purification process, antibodies released by acid treatment were analysed by SDS-PAGE under non-reducing conditions, and protein bands were detected using the silver-staining method. Fractions corresponding to the acid treatment of the beads after serum incubation contained mainly a band migrating at the expected molecular mass of 150 000, while other proteins present in the crude serum were efficiently removed (data not shown).

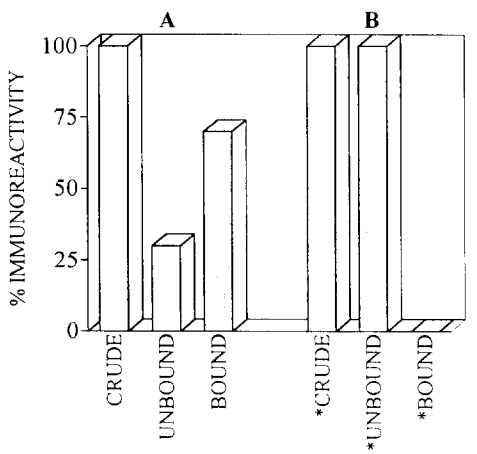

Fig. 8. Immunoreactivity recovered from the purification of anti-MAP4 antibodies (A) on beads derivatized by surface adsorption with MAP4 and (B) on underivatized beads, as measured by ELISA.

4. Discussion

Alginate gel has been used mainly for the non-covalent entrapment of high-molecular-mass proteins, mainly enzymes, cell organelles or whole cells [9,10], as the gel open-lattice structure does not allow entrapment of low-molecular-mass molecules, thus preventing its use for peptide immobilization. Multimeric peptides differ considerably from their linear counterparts not only in the higher molecular mass but also, more importantly, in the three-dimensional arrangement of the peptide arms, which leads to the filling of a larger molecular volume. After gel entrapment, MAPs were efficiently retained by the lattice structure of alginate, even if only a minor fraction of the total immunogenicity was recovered in the bead surface, as determined by bead ELISA. The high molecular mass of antibodies prevents access to the bead interior, so only the outer bead surface remains fully accessible. This was demonstrated by the effect of bead size on immunoreactivity. Reduction in the bead diameter was followed by an increase in bead immunoreactivity, since at the same peptide-to-alginate mass ratio smaller beads display a larger surface area than larger beads of the same mass, and consequently the proportion of peptide displayed on the bead surface is also higher.

While poorly efficient for use in affinity chromatography, this method of immobilization

could find wider applications when applied to small molecules able to diffuse into the bead interior. For example, it may prove useful for the screening of synthetic peptide libraries in a search for ligands for target peptides, synthesized in a multimeric way, and immobilized by entrapment in alginate. Surface adsorption proved more efficient for the retention of peptide immunogenicity, thus indicating that a larger fraction of peptide molecules was distributed on the outer bead surface. Absorbance values obtained with beads derivatized with the same amount of peptide according to the two different procedures were much higher with the surface adsorption method, and were found to be very similar to those obtained in a classical plastic microtitre plate ELISA. On the other hand, it should be noted also that non-specific interactions with this type of matrix were extremely limited, and in any case lower than those observed with conventional plastic microtitre plates.

The high immobilization yields obtained with the multimeric peptide in comparison with the linear counterparts indicate that multivalency is an essential prerequisite to achieve strong adsorption. Even if linear peptides can possibly acquire an expanded form and penetrate through the pores of the alginate beads, only 25% of the initial amount of peptide added to the beads is immobilized. The weak non-covalent interactions occurring between MAPs and the alginate matrix are greatly enhanced in comparison with linear peptides, since in the first instance many chains simultaneously can take part in stabilizing contacts, leading to stronger interactions. On the other hand, several pieces of evidence indicate that not all the peptide chains are directly involved in the interaction with the matrix, as a considerable amount of immunoreactivity is recovered after immobilization. Peptide immobilization by surface adsorption proved useful for micro-preparative purification of anti-peptide antibodies directly from crude sera, in a single binding–elution step. Apparently no traces of residual multimeric peptide were found in the purified fraction. The purity and recovery were sufficiently high to suggest the wide use of this method of peptide immobilization for antibody purification. As alginate beads can be conveniently prepared and stored, and remain for several months without degradation, they can be used to immobilize different multimeric peptides by surface adsorption in a very short time, to test their immunogenicity towards sera in a test-tube or to purify a small amount of the corresponding antibody for further studies.

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