ELSEVIER

Journal of Chromatography B, 715 (1998) 137–145

JOURNAL OF CHROMATOGRAPHY B

Affinity purification of immunoglobulin M using a novel synthetic ligand

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Abstract

While monoclonal antibodies of the G class can be conveniently purified by affinity chromatography using immobilized protein A or G, even on a large scale, scaling up IgM purification still presents several problems, since specific and cost-effective ligands for IgM are not available. A synthetic peptide (TG19318), deduced from the screening of a combinatorial peptide library, was characterized previously by our group for its binding properties for immunoglobulins of the G class and its applicability as a synthetic ligand for polyclonal and monoclonal IgG purification, from sera or cell culture supernatants. In this study, we have examined the ligand recognition properties for IgM, immobilizing the synthetic peptide on different affinity supports and examining its ability to purify IgMs from serum, ascitic fluid and cell culture supernatants. TG19318 affinity columns proved useful for a very convenient one-step purification of monoclonal IgMs directly from crude sources, loading the samples on the columns equilibrated with saline buffers at pH values ranging from 5 to 7, and eluting adsorbed IgM by a buffer change to 0.1 *M* acetic acid or 0.05–0.1 *M* sodium bicarbonate, pH 9.0. Antibody purity after affinity purification was very high, close to 85–95%, as determined by densitometric scanning of sodium dodecyl sulfate–polyacrylamide gels of purified fractions, and by gel permeation analysis. Antibody activity was fully recovered after purification, as determined by immunoassays. Column capacity was related to the type of support used for ligand immobilization, and ranged from 2 to 8 mg of IgM/ml of support.

Keywords: Immunoglobulins

1. Introduction

Conventional approaches for the purification of IgM have been based on the combination of different fractionation techniques, such as precipitation, [1,2], gel permeation chromatography and ion-exchange chromatography [3,4] or electrophoresis [5,6]. Protein A [7], protein G [8], or protein A/G [9], widely used for the affinity purification of antibodies from sera or cell culture supernatants, even on an industrial scale, do not recognize immunoglobulins of the M class well and are not used to capture and purify IgM from crude sources. Recent works pointed out the possibility of using alternative ligands for the affinity purification of IgM. Immobilization of complement protein C1q on solid supports led to affinity media that were useful for the isolation of IgM, based on a temperature-dependent interaction of the ligand with the immunoglobulins [10]. Even if the reported binding constant of C1q to IgG is much weaker than the binding constant for IgM, traces of contaminating IgG are found on IgM preparations obtained by affinity chromatography on C1q columns. Another ligand that is useful for the affinity purification of IgM is the mannose binding protein (MBP), which resembles the C1q polypeptide in

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0378-4347/98/$19.00
PII: S0378-4347(98)00104-2

structure [11]. The use of immobilized MBP for the purification of IgM is based on adsorption in the presence of calcium at a temperature of 4°C, and the room temperature-dependent elution of adsorbed immunoglobulins in the presence of ethylenediaminetetraacetic acid (EDTA). This ligand shows low binding affinity for IgG, but binds to bovine and human IgM with reduced affinity than that for murine IgM [12]. However, in addition to the complexity of MBP isolation, functional binding capacities of MBP columns are limited to 1 or 2 mg of IgM per ml of support. Other MBPs, such as snowdrop bulb lectin (GNA), have been used specifically for murine IgM isolation, but with the drawback of requiring elution of adsorbed immunoglobulins by the addition of 0.1 *M* methyl-α-D-mannoside [13]. Immobilized protamine has also been employed for IgM fractionation, but the low purity of adsorbed fractions had to be increased to acceptable levels by two additional gel permeation steps [14]. All of these procedures are time-consuming, labor intensive, may alter IgM functionality and are not compatible with industrial scaling up.

In a previous work, our laboratory identified a multimeric peptide that was able to bind specifically and selectively to the constant portion of immunoglobulins, and that could be used in affinity chromatography. This synthetic ligand, denoted PAM (protein A mimetic, TG19318) was selected through the synthesis and screening of a multimeric peptide library [15]. Initial characterization indicated a broader selectivity of this molecule than protein A, allowing the isolation of mono- and polyclonal IgG from different sources [15,16], as well as IgA [17] and IgE [18]. In this study, we have extended the characterization of TG19318, examining its selectivity for immunoglobulins of the M class, evaluating its ability to purify IgM directly from sera, ascitic fluid and from cell culture supernatants, and assessing the influence of different supports and elution buffers for affinity chromatography on the purification capacity.

2. Experimental

2.1. Samples for affinity chromatography

Serum derived from mouse blood was purchased from Sigma (St. Louis, MO, USA), as was purified polyclonal IgM. Ascitic fluid containing IgM and cell culture supernatant, obtained from the cultivation of a stable hybridoma cell line (B18μ) producing murine IgM against nitrophenol, were provided by Dr. R. Sitia (DIBIT, Milan, Italy).

2.2. Ligand preparation

TG19318 was prepared by solid-phase peptide synthesis on an automated peptide synthesizer 431A (Perkin-Elmer), as previously reported [15], and it was purified to homogeneity by reversed-phase high-performance liquid chromatography (RP-HPLC). Amino acid analysis of acid hydrolyzates of TG19318 confirmed the intended chemical nature, as well as the molecular mass, by time of flight matrix assisted laser desorption ionization (TOF–MALDI) mass spectrometry, which provided a value identical to the expected value (2141 a.m.u.). The chemical structure of TG19318 is shown in Fig. 1.

2.3. Affinity supports

Preactivated affinity supports for the direct im-

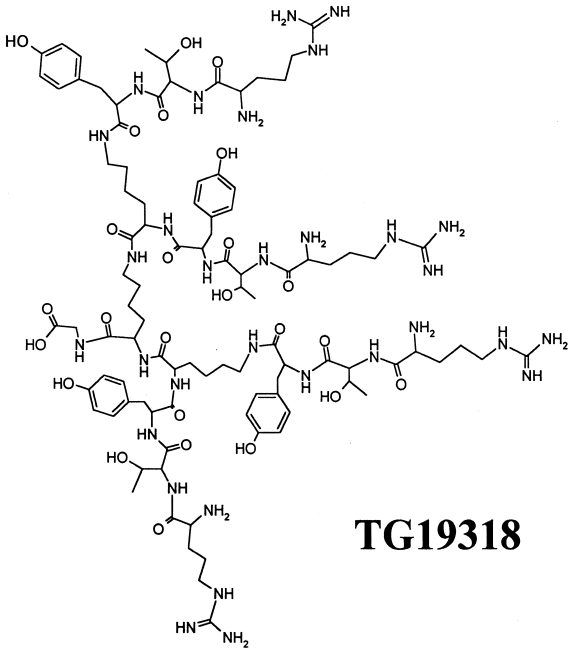

Fig. 1. Chemical structure of TG19318. The ligand is a tetrameric tripeptide of the sequence Arg–Thr–Tyr that was synthesized starting from a central tetra-branched polylysine core.

mobilization of peptides and proteins included CH-Sepharose 4B (Pharmacia, Uppsala, Sweden), Emphaze (Pierce, Rockford, IL, USA), Eupergit 30N (Sigma), Protein-Pak (Waters, Milford, MA, USA) and Epoxy-HyperD (Biosepra, Malborough, MA, USA).

2.4. Columns preparation

For ligand immobilization on solid supports, the peptide ligand TG19318 (10 mg) was dissolved at a concentration of 2 mg/ml in a 0.1 *M* sodium bicarbonate solution, pH 8.5, and this was added to 1 ml of buffer-conditioned solid support. The suspension was incubated for several hours at room temperature under gentle agitation, and the extent of peptide incorporation was monitored by RP-HPLC analysis of the reaction mixture at different times. For all of the supports investigated, coupling yields were close to 80–90%. Resins were then washed with 0.1 *M* Tris, pH 8.5, to deactivate residual active groups, and packed into a 100×10 mm I.D. glass column.

2.5. IgM affinity purification on TG19318 columns

In all cases, antisera, ascitic fluids or cell culture supernatants were diluted 1:1 (v/v) with the column equilibration buffer, filtered through a 0.45-μm filter (Nalgene) and loaded onto the column, which had been equilibrated at a flow-rate of 1.0 ml/min with the selected buffer. After elution of unbound material, the eluent was changed to 0.1 *M* acetic acid or to 0.1 *M* sodium bicarbonate, to elute bound antibodies. Desorbed material was immediately neutralized with 1 *M* NaOH or 0.1 *M* HCl and characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), gel permeation chromatography and enzyme-linked immunosorbent assay (ELISA) in order to determine the recovery of immunoreactivity after purification.

2.6. SDS–PAGE analysis

The purity of the bound materials from the different affinity columns was checked by SDS–PAGE analysis, performed under reducing or non-reducing conditions, using a 0.75-mm separating gel in a final concentration of 12% acrylamide. The electrophoresis was carried out using the Mini-PROTEAN II apparatus (Bio-Rad), following the manufacturer's instructions. Samples contained 1 μg of protein. Gels were run at room temperature at 200 V, at a constant voltage setting, with a current of approximately 60 mA. Molecular mass markers included myosin (200 kDa), *E. coli* β-galactosidase (116.25 kDa), rabbit muscle phosphorylase *b* (97.4 kDa), bovine serum albumin (BSA; 66.2 kDa), hen egg white ovalbumin (45 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa) and hen egg white lysozyme (14.4 kDa). Protein was detected using the silver staining method.

2.7. ELISA

The recovery of IgM's immunoreactivity from mouse serum and ascitic fluid affinity purification was checked using aliquots of crude material, unbound and bound fractions, directly coated on microtiter plates at the same concentration (10 μg/ml) in 0.1 *M* sodium carbonate buffer, pH 8.5, overnight at 4°C. After washing the plates five times with phosphate-buffered saline (PBS; 50 m*M* phosphate, 150 m*M* sodium chloride, pH 7.5), the wells were blocked with 100 μl of PBS containing 3% BSA for 2 h at room temperature, to prevent the adsorption of nonspecific proteins. Plates were then washed several times with PBS. To detect IgM, each well was filled with 100 μl of a goat anti-mouse IgM peroxidase conjugate solution, diluted 1:1000 with PBS containing 0.5% BSA, and incubated for 1 h at 37°C. Plates were then washed with PBS five times, and developed with a chromogenic substrate solution consisting of 0.2 mg/ml 2,2′-azino-bis(3-ethylbenzthiazoline 6-sulfonic acid) diammonium (ABTS) in 0.1 *M* sodium citrate buffer, pH 5.0, containing 5 m*M* hydrogen peroxide. The absorbance of each sample was measured at 405 nm with a Model 2250 EIA Reader (Biorad). The standard IgM concentrations used for the assay ranged from 0.1 to 10 μg/ml, with a detection limit of 0.5 μg/ml and linearity between 0.5 and 8 μg/ml.

The recovery of IgM's immunoreactivity from cell culture supernatant (B18μ) was tested by immobilizing the original antigen NP–BSA (nitrophenol–BSA) on microtiter plates, dissolved in 0.1 *M* sodium

carbonate buffer, pH 8.5, overnight at 4°C. The plates were washed and saturated as described before, and filled with crude, unbound and bound materials at the same concentration (10 μg/ml), diluted with PBS–0.5% BSA. Detection of antibody and the development of the chromogenic reaction were carried out as described above. The standard IgM concentrations used for this assay ranged from 0.05 to 10 μg/ml, with a detection limit of 0.1 μg/ml and linearity between 0.1 and 5 μg/ml.

2.8. Gel permeation analysis

Gel permeation analysis was performed using a Pharmacia Sephacryl HR-300 column (500×16 mm I.D.), equilibrated at a flow-rate of 0.5 ml/min with PBS, and the effluent was monitored at 280 nm. Crude material and IgM fractions, purified on the affinity columns, were first concentrated to 0.5–1.0 mg/ml, filtered using a 0.22-μm membrane and then applied to the column.

3. Results

3.1. Binding of murine IgM to immobilized TG19318

Preliminary experiments were carried out with purified murine IgM in order to evaluate the recognition properties of immobilized TG19318 on various supports. Affinity columns (1 ml bed volume), prepared by immobilizing the ligand at a fixed density (10 mg/ml) on CH-Sepharose 4B, HyperD, Eupergit C30 N, Emphaze and Protein-Pak, were equilibrated with 50 m*M* sodium phosphate buffer, pH 7.0, and loaded with 1 mg of purified IgM. All of the columns tested efficiently retained the sample applied, and adsorbed IgM was fully recovered by a buffer change to 0.1 *M* acetic acid or to 0.1 *M* sodium bicarbonate, pH 9.0, as detected by ELISA with anti-IgM antibodies. Capacity determinations were carried out by overloading the columns with samples containing 10 mg of IgM and measuring the amount of adsorbed immunoglobulin after elution with acetic acid. The supports with the highest capacity proved to be HyperD (8 mg of IgM/ml of support), Emphaze (7 mg of IgM/ml of support) and Sepharose (5 mg of IgM/ml of support), while Protein-Pak (3 mg of IgM/ml of support) and Eupergit (2 mg of IgM/ml of support) showed reduced binding capacity.

3.2. Effect of buffer composition on antibody purification

The effect of buffer composition on TG19318 recognition for IgM was examined by affinity chromatography experiments on 1 ml bed volume columns that were prepared by immobilizing the ligand on CH-Sepharose 4B. The columns were equilibrated with different buffers and a sample containing 10 mg of purified murine IgM was loaded onto the column. After elution of unbound material, the eluent was changed to 0.1 *M* acetic acid, to elute the adsorbed antibody. The protein content in the column bound fractions was determined by the BCA method (Pierce) and by ELISA with anti-IgM antibodies. The buffer composition and pH affected the column's capacity significantly, with the binding capacity being higher at low ionic strength and at a pH around neutrality (Table 1). The effects of the buffer's ionic strength and pH were similar with the other supports tested. The addition of 0.5 *M* NaCl to the dissociation buffer led to very sharp elution peaks, allowing the recovery of purified antibody in a limited volume.

Table 1
Dependence of TG19318 antibody binding capacity for murine IgM on ionic strength and pH

pH	Molarity (m*M*)	Antibody recovered[a] (mg)
6.5	100	4.5
7.0	100	6
8.0	100	5
8.5	100	3.5
7.0	50	7
7.0	150	5
7.0	200	3

[a] Determined on the TG19318–Sepharose column (1.0 ml bed volume) that had been equilibrated at a flow-rate of 1.0 ml/min with sodium phosphate.

3.3. Purification of monoclonal IgM from ascitic fluid

The binding selectivity of TG19318 was first examined by evaluating the ability of the Sepharose-immobilized ligand to purify murine IgM from crude ascitic fluid. A 300-μl volume of crude ascitic fluid, containing 2 mg of IgM, as detected by the IgM-specific ELISA, was applied to the TG19318–Sepharose column that had been equilibrated at a flow-rate of 1.0 ml/min with 100 m*M* sodium phosphate, pH 7.0, and, after elution of a significant amount of material at the column's void volume, a second protein fraction was obtained by a buffer change to 0.1 *M* acetic acid (Fig. 2). The protein content of the fractions obtained after purification was assessed by SDS–PAGE analysis, first under non-reducing conditions. As shown in Fig. 3, the column-bound fraction contained only a protein band that migrated with the expected molecular mass of IgM (950 kDa), with no traces of albumin, the major protein contaminant in ascitic fluids. Analysis of the material that was not retained on the column indicated that the majority of albumin eluted at the void volume, and just traces of high molecular mass protein were not retained by the column. Under reducing conditions, SDS–PAGE analysis indicated that all of the high molecular mass material was transformed to fragments that migrated at the expected molecular mass for reduced IgM, a result that was consistent with the presence of only IgM in the column-bound fraction (Fig. 4). As shown in Fig. 5, gel permeation analysis confirmed the SDS–PAGE data, since no traces of albumin were detected in the purified IgM preparation. The two overlapping small peaks following the IgM fraction were formed by a large number of contaminating proteins, each present in very low amounts, which were undetectable as major components in the SDS–PAGE gel, but were coeluting on gel permeation. The majority (95%) of the IgM originally found in the crude sample was recovered by affinity chromatography on the TG19318–Sepharose column. Similar results were obtained using the same sample and under the same conditions with the TG19318–HyperD affinity column, where, as before, SDS–PAGE and gel permeation analysis of the purified fraction indicated the complete removal of contaminating albumin (data not shown).

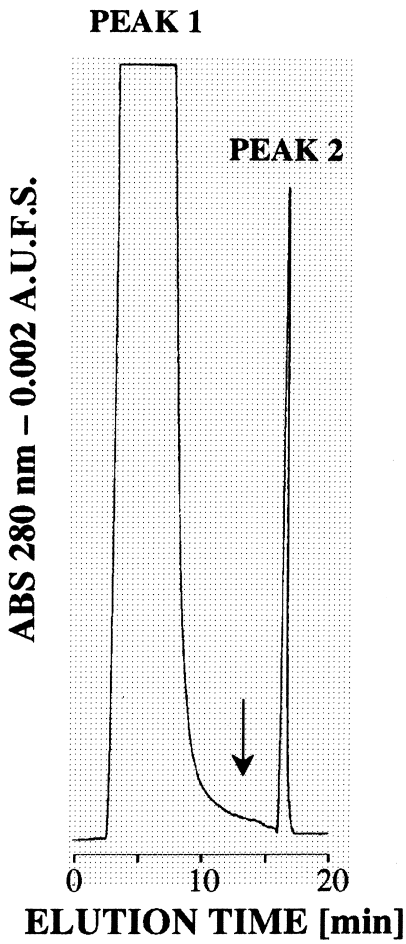

Fig. 2. Purification of mouse monoclonal IgM from ascitic fluid on a TG19318–Sepharose affinity column. Crude ascitic fluid (300 μl) was applied to the column, which had been equilibrated with 50 m*M* phosphate buffer, at a flow-rate of 1.0 ml/min. After elution of the unbound material (peak 1), the adsorbed material was eluted with 0.1 *M* acetic acid (peak 2). Each fraction was collected for SDS–PAGE analysis.

3.4. Purification of monoclonal IgM from cell culture supernatant

Immobilized TG19318 proved useful also for the capture of monoclonal IgM directly from crude cell supernatants. A 10-ml volume of crude cell culture supernatant, obtained from a stable hybridoma cell line secreting murine IgM against nitrophenol, containing a low concentration of IgM (100 μg/ml), was loaded on a TG19318–HyperD column (1 ml

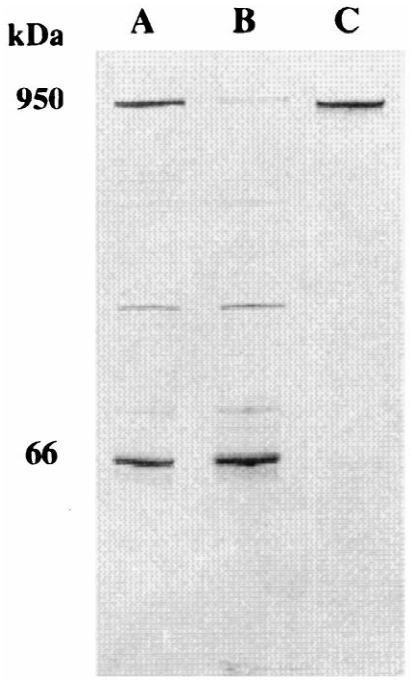

Fig. 3. SDS–PAGE analysis on a 12% polyacrylamide gel of fractions obtained from the purification of mouse IgM from ascitic fluid under nonreducing conditions. Lane A, crude ascitic fluid; lane B, unbound fraction (peak 1); lane C, bound fraction (peak 2).

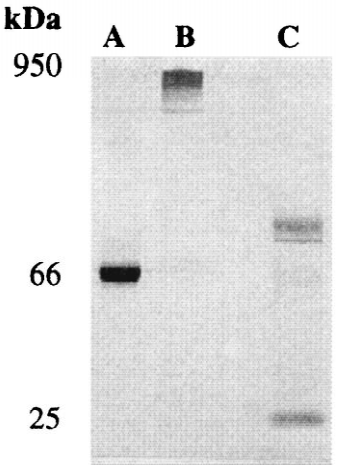

Fig. 4. SDS–PAGE analysis on a 12% polyacrylamide gel of reduced and non-reduced IgM purified from ascitic fluid. Lane A, crude ascitic fluid; lane B, bound fraction under non-reducing conditions; lane C, bound fraction, reduced.

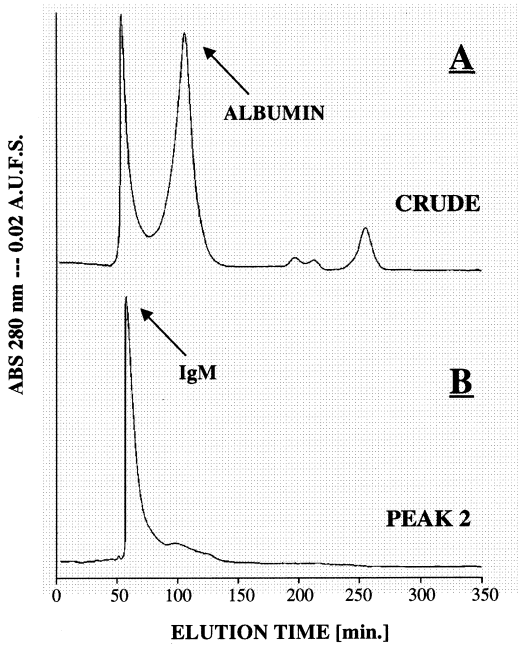

Fig. 5. Gel permeation profiles on Sephacryl HR-300 (500×16 mm I.D.) of crude ascitic fluid and affinity purified IgM from the TG19318–Sepharose column. Samples (50 μl) of ascitic fluid (top) and purified IgM (bottom) were applied on the gel permeation column, which had been equilibrated with PBS at a flow-rate of 0.5 ml/min, and the effluent was monitored at 280 nm. The peaks were identified by comparison with the retention times of molecular mass standards.

bed volume) that had been equilibrated at a flow-rate of 1 ml/min with 50 m*M* sodium phosphate buffer, pH 7.0. After loading the sample, the column was washed with the equilibration buffer until complete elution of the unretained material was achieved, and then the eluent was changed to 0.1 *M* acetic acid. Material desorbed by the acid treatment was collected and immediately neutralized. Determination of the protein content by the BCA method and of the IgM content by the IgM-specific ELISA indicated that 800 μg of antibody were recovered in the bound fraction (80% recovery yield). SDS–PAGE analysis of eluted fractions showed an excellent degree of purification, since no traces of albumin were detected in the column-bound fraction, and all of the material migrated at the expected molecular mass for IgM (Fig. 6). Densitometric scanning of the gel lane containing the purified fraction showed purity close to 95%. In contrast, the flow-through material from

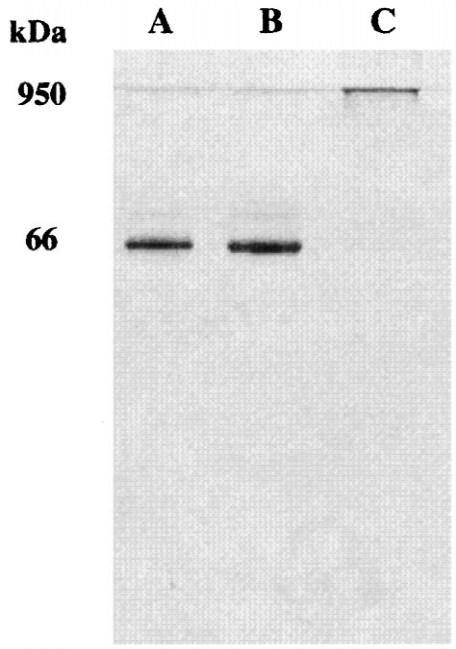

Fig. 6. SDS–PAGE analysis on a 12% polyacrylamide gel of fractions obtained from the affinity purification on a TG19318–HyperD column of mouse monoclonal IgM from cell culture supernatant (B18μ). Lane A, crude cell culture supernatant; lane B, fraction that did not bind to the column; lane C, fraction that did bind to the column.

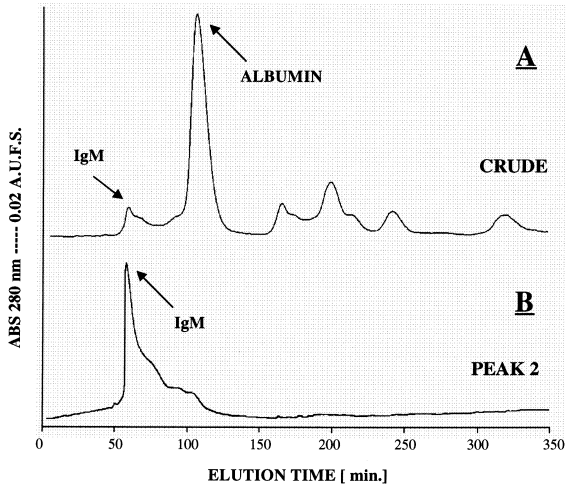

Fig. 7. Gel permeation profiles on Sephacryl HR-300 (500×16 mm I.D.) of crude cell culture supernatant (B18μ) and affinity purified IgM from the TG19318–HyperD column. Samples (100 μl) of cell culture supernatant (top) and purified IgM (bottom) were applied on the gel permeation column, which had been equilibrated with PBS at a flow-rate of 0.5 ml/min, and the effluent was monitored at 280 nm. The peaks were identified by comparison with the retention times of molecular mass standards.

the column contained the vast majority of the albumin and of the other contaminants. The extent of purification was also evaluated by gel permeation chromatography on calibrated columns. As shown in Fig. 7, the gel permeation profile of the affinity purified IgM validated the SDS–PAGE data, indicating that a single affinity step was sufficient to remove albumin and capture and concentrate the IgM fraction. The effect of the purification conditions on the maintenance of the antibody's antigen binding ability was evaluated by ELISA on microtiter plates coated with the NP–BSA antigen. Results indicated that the affinity fractionation step was mild and did not lead to loss of immunoreactivity, since the purified antibody was fully active.

3.5. Purification of IgM from mouse serum

IgM from mouse serum was purified by affinity chromatography on the TG19318–Sepharose column after a preliminary IgG adsorption step on protein A–Sepharose. A sample of mouse serum (300 μl) was first loaded on a protein A–Sepharose affinity column (2 ml bed volume) that had been equilibrated with 50 m*M* sodium phosphate buffer, pH 7.0, at a flow-rate of 1.0 ml/min. The material that was not retained by the column was collected and loaded on the TG19318–Sepharose column, which had been equilibrated at a flow-rate of 1.0 ml/min with 50 m*M* sodium phosphate buffer, pH 7.0. As before, fractions corresponding to the bound material were collected for gel permeation and SDS–PAGE analysis under reducing and nonreducing conditions (Fig. 8), and antibody recovery was determined using an ELISA with a goat anti-mouse IgM antibody conjugated to peroxidase being used for detection. The vast majority of immunoreactivity (close to 80%) was found in the bound fraction, while only residual activity was detected in the flow-through fraction. SDS–PAGE analysis indicated that the column-bound fraction contained mainly IgM without detectable amounts of albumin. However, it was found using gel permeation chromatography that the IgM

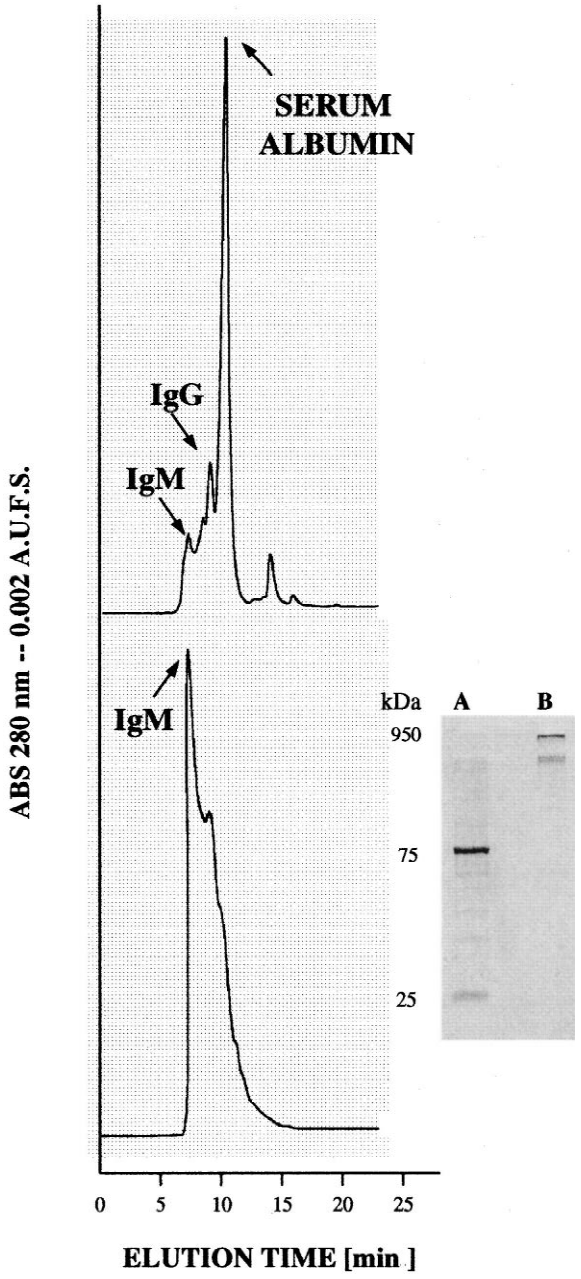

Fig. 8. Right: Gel permeation profiles on Sephacryl HR-300 (500×16 mm I.D.) of crude IgG-deprived mouse serum and affinity purified IgM from the TG19318–Sepharose column. Samples (50 μl) of IgG-deprived mouse serum (top) and purified IgM (bottom) were applied on the gel permeation column, which had been equilibrated with PBS at a flow-rate of 0.5 ml/min, and the effluent was monitored at 280 nm. The peaks were identified by comparison with the retention times of molecular mass standards. Left: SDS–PAGE analysis of reduced (A) and non-reduced (B) IgM purified on the TG19318–Sepharose column.

preparation was contaminated by other proteins, mainly IgA and IgG, which were not retained by the preliminary adsorption step on protein A.

4. Discussion

Monoclonal antibodies are becoming an important class of biomolecules for both the diagnosis and the treatment of a large variety of human diseases. Even if the large majority of monoclonal antibodies (mAbs) currently under development for clinical treatments are immunoglobulins of the G class, IgMs are also finding application for the diagnosis and cure of certain important diseases, such as cancer [19]. The medical and commercial relevance of antibodies has stimulated the development of innovating techniques to make mAb production and purification more cost effective. While the production of mAbs by cultivation of suitable hybridoma cell lines can be easily scaled up at the industrial level, purification may still constitute a problem when specific and selective ligands that are needed to capture and concentrate the mAb from dilute feedstock are not available. For immunoglobulins of the G class, this problem may not exist given the availability of protein A or G, but for IgM, selective ligands required for large-scale purification are not yet available. Calcium-dependent lectins, such as MBP or complement protein C1q, even if selective enough for laboratory studies, are not compatible with industrial applications, due to the high costs associated with their production, the low antibody binding capacity attainable, the complexity of the binding and elution conditions, and the limited stability and reusability. In addition, these ligands cannot be used for IgM purification from sera since also other glycoproteins that are normally found in serum may bind to the immobilized lectins, thus saturating the column, reducing the capacity and purity. Conventional purification methods based on the use of a combination of classical chromatographic techniques, such as gel filtration, ion-exchange or hydrophobic interaction, cannot be used for large-scale purification, since they are very time-consuming and inefficient, resulting in IgM preparations that are often contaminated by other proteins. The TG19318 ligand, a multimeric tetrameric tri-

peptide, can be obtained at a low cost by chemical solution phase or solid-phase synthesis on the multikilogram scale, does not contain biological contaminants such as viruses, pyrogens, DNA fragments as many recombinant or extractive biomolecules, and can withstand a large array of sanitizing agents without having denaturation problems. Immobilization on preactivated solid supports can be accomplished easily given the presence of four amino groups and one carboxyl group, and retention of IgM binding ability has been maintained with all four of the different supports tested in this study. Earlier studies on the immobilization of multimeric peptides on solid surfaces for the preparation of affinity media clearly indicate that only a limited number of peptide chains are linked to the solid phase, leaving the others fully available for interaction [20]. The column binding capacity for IgM ranged from 2 to 8 mg of IgM per ml of sorbent, depending on the characteristics of the support used. The differences in binding capacities observed may be ascribed to the different chemical nature, structure and activation chemistry of the supports tested, in a similar manner to that observed previously for natural ligands, such as protein A [21]. Immunoglobulin adsorption was achieved under mild conditions, at a physiological pH, at a moderate ionic strength and at room temperature; conditions that are fully compatible with the use of crude feedstock derived from cell culture supernatants. Elution of adsorbed antibody was also achieved under conditions that did not cause IgM denaturation or loss of antigen binding capacity, as detected by ELISA.

Interaction affinity is strong enough to allow purification of antibodies directly from diluted supernatants where the immunoglobulin concentration is very low, from 10 to 50 μg/ml. The main contaminant, albumin, is always efficiently removed in the purification step, in a manner that is independent of the type of support used for TG19318 immobilization. In addition, the ligand is also useful for IgM purification from IgG-deprived mouse serum, although immunoglobulins of this class represent only a minute amount (1.5%) of the total protein. The availability of TG19318 as an affinity ligand will make IgM purification much more convenient and affordable, opening up new avenues in the study and application of this important class of immunoglobulins.

References

[1] H.F. Deutsch, J.I. Morton, J. Biol. Chem. 231 (1958) 1107.
[2] H.E. Schultz, J.F. Heremans, Molecular Biology of Human Proteins, Vol. 1, Elsevier, Amsterdam, 1966, p. 224.
[3] H. Chaplin, S. Cohen, E.M. Press, Biochem. J. 95 (1965) 256.
[4] J.L. Fahey, E.W. Terry, in D.M. Weir (Editor), Handbook of Experimental Immunology, Blackwell, Oxford, 1967, Ch. 8, p. 1.
[5] H.J. Muller-Eberhard, Scand. J. Clin. Invest. 12 (1960) 33.
[6] K. Onoue, T. Kishimoto, Y. Yamamura, J. Immunol. 98 (1967) 303.
[7] H. Hjelm, K. Hyelm, J. Sjoquist, FEBS Lett. 28 (1972) 73.
[8] L. Bjork, G. Kronvall, J. Immunol. 133 (1984) 969.
[9] M. Eliasson, A. Olsson, E. Palmcrantz, K. Wiberg, M. Inganas, B. Guss, M. Lindberg, M. Uhlen, J. Biol. Chem. 231 (1958) 1107.
[10] A. Nethery, R.L. Raison, S.B. Easterbrook-Smith, J. Immunol. Methods 126 (1990) 57.
[11] M. Ohta, M. Okada, I. Yamashina, T. Kawasaki, J. Biol. Chem. 265 (1990) 1980.
[12] J.R. Nevens, A.K. Mallia, M.W. Wendt, P.K. Smith, J. Chromatogr. 597 (1992) 247.
[13] N. Shibuya, J.E. Berry, I.R. Goldstein, Arch. Biochem. Biophys. 267 (1988) 676.
[14] A. Wichmann, H. Borg, Biochim. Biophys. Acta 490 (1977) 363.
[15] G. Fassina, A. Verdoliva, M.R. Odierna, M. Ruvo, G. Cassani, J. Mol. Recogn. 9 (1996) 564.
[16] G. Fassina, A. Verdoliva, G. Palombo, M. Ruvo, G. Cassani, J. Mol. Recogn., in press.
[17] G. Palombo, S. De Falco, M. Tortora, G. Cassani, G. Fassina, J. Mol. Recogn., in press.
[18] G. Palombo, M. Rossi, G. Cassani, G. Fassina, J. Mol. Recogn., in press.
[19] S.M. Quadri, A.B. Malik, X.Z. Tang, R. Patenia, R.S. Freedman, H.M. Vriesendorp, Cancer Res. 55 (1995) 5736.
[20] G. Fassina, J. Chromatogr. 591 (1992) 99.
[21] P. Fuglistaller, J. Immunol. Methods 124 (1989) 171.