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PURIFICATION OF ASCITIC FLUID-DERIVED MURINE MONOCLONAL ANTIBODIES BY ANION-EXCHANGE AND SIZE-EXCLUSION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

HANK H. HWANG, MARK C. HEALEY* and ALICE V. JOHNSTON

Department of Animal, Dairy and Veterinary Sciences, College of Agriculture, Utah State University, Logan, UT 84322-5600 (U.S.A.)

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SUMMARY

Ascitic fluid-derived murine monoclonal antibodies (MoAbs) of immunoglobulin (Ig) M and IgG isotypes (IgG1 and IgG2a subisotypes) were previously prepared against an isolate of *Actinobacillus* sp (CAs8C) for the purpose of identifying and characterizing outer membrane antigens on this bacterium. An attempt was made to purify these MoAbs by anion-exchange and size exclusion high-performance liquid chromatography (HPLC). Hybridomas producing the IgG1 and IgG2a MoAbs posed unique difficulties in that they also secreted irrelevant IgG2b MoAbs that were present in the ascitic fluids. Anion-exchange chromatography (Protein-Pak DEAE-5PW column), with a simultaneous change in gradients of pH and ionic strength, was used to purify IgG and as a first step in the purification of IgM. There was good separation of IgG2b from IgG2a, but not from IgG1. Size-exclusion chromatography (Protein-Pak 300 SW column) was required to complete the purification of IgM. The presence of MoAbs in the HPLC fractions was confirmed by discontinuous gradient polyacrylamide gel electrophoresis (denatured and either reduced or non-reduced conditions) and the enzyme-linked immunosorbent assay. HPLC-purified MoAbs were free from transferrin and albumin and retained their specificity for As8C.

INTRODUCTION

Purification of immunoglobulins (Ig), particularly monoclonal antibodies (MoAbs), is required for many applications in numerous fields of science and technology. Several isolation and purification techniques have been used, but the procedures are frequently long and tedious, and often do not result in a high degree of antibody purity [1–3]. For example, the purification of antibodies by conventional anion-exchange chromatography requires several hours to complete and is often limited by poor recovery [2]. Precipitation with ammonium sulfate followed by DEAE-cellulose chromatography has been used to isolate

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mouse monoclonal antibodies (MoAbs), but in some cases the MoAb activity was reduced significantly by the precipitation process [4]. Affinity chromatography using protein A has been successfully employed for the purification of mouse IgG, but one subclass (IgG1) was bound only weakly and another (IgG3) did not bind [3]. Hydroxyapatite is very useful for simple and rapid fractionation of proteins, but has intrinsic limitations for routine use, and is difficult to scale-up for MoAb purification [5]. Clezardin et al. [6] demonstrated that IgM MoAbs derived from mouse ascitic fluid could be partially purified on an anion-exchange Mono Q column followed by a gel filtration Superose 6 column. Recently, a mixed-mode ion-exchange chromatographic matrix, utilizing silica gel as the support, was used for the rapid purification of immunoglobulins. This chromatographic matrix demonstrated little or no affinity for albumins, transferrins, proteases, or pH indicator dyes from tissue culture media [5].

Separation and recovery of proteins from ion-exchange chromatographic media are affected by factors such as buffer type and pH, length of the gradient, flow-rate of the mobile phase, ionic strength and nature of the counter ion, and characteristics of the proteins [7, 8]. The selection of ideal conditions for protein purification involves changing some or all of these parameters. We have previously reported on the production of three MoAbs (IgM, IgG1 and IgG2a) that were prepared against an isolate of *Actinobacillus* sp (As8C) for the purpose of identifying and characterizing outer membrane antigens on this bacterium. Hybridomas producing the IgG1 and IgG2a MoAbs posed unique difficulties in that they also secreted irrelevant IgG2b MoAbs that were present in the ascitic fluids. The object of the present study was (1) to use anion-exchange and size-exclusion high-performance liquid chromatography (HPLC) to purify these ascitic fluid-derived MoAbs, and (2) to demonstrate that these MoAbs retained their specificity for As8C following purification.

EXPERIMENTAL

MoAbs, equipment and reagents

Procedures for bacteriologic culture, production of hybridomas, and production of ascitic fluid containing MoAbs have been described [9, 10]. The panel of MoAbs used were mouse IgM, IgG1, and IgG2a. Hybridomas producing IgG1 and IgG2a MoAbs were previously demonstrated to also secrete irrelevant IgG2b MoAbs of unknown antigenic specificity (unpublished data). Target As8C antigens for the IgM and IgG2a MoAbs have been characterized [11]. Ascitic fluid-derived MoAbs were prepared by injecting hybridoma cells intraperitoneally into adult female BALB/c mice. The ascitic fluid was pooled for individual MoAbs before use. Negative control ascitic fluid was produced by injecting the non-secreting SP2/0 myeloma cells intraperitoneally into adult female BALB/c mice.

HPLC was done on a binary gradient system (Millipore, Waters Chromatography Division, Milford, MA, U.S.A.) equipped with a Model 680 automated gradient controller, a Model 481 Lambda-max variable-wavelength detector, a Model U6K universal injector, and two Model 510 EF HPLC pumps. A FRAC-100 fraction collector (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) and

a BD-40 chart recorder (Kipp & Zonen, Delft, The Netherlands) were used. All buffer solutions were filtered through a 0.45-μm Falcon 7104 bottle top filter with grid (Becton Dickinson, Oxnard, CA, U.S.A.) and degassed under vacuum before use.

Anion-exchange chromatography

Analytical (75 mm×7.5 mm) and preparative (150 mm×21.5 mm) HPLC Protein-Pak DEAE-5PW columns (Waters) were used. The analytical column was employed to establish the appropriate chromatographic conditions, while the preparative column was used to purify the ascitic fluid-derived MoAbs. Ascitic fluids were diluted 1:20 with buffer A (20 m*M* Tris–HCl, pH 8.5) and passed through a 0.45-μm Acrodisc membrane filter (Gelman, Ann Arbor, MI, U.S.A.) before loading the columns. Sample volumes applied to the analytical and preparative columns were 25 μl and 0.1–2.0 ml, respectively. The IgG1 and IgG2a MoAbs were purified using an 80-min linear stepwise gradient of 0–100% in buffer B (20 m*M* Tris–HCl, 0.3 *M* sodium chloride, pH 7.0). The IgM MoAb was purified by first using a 60-min non-linear stepwise gradient of 0–100% in buffer B. HPLC fractions containing IgM were pooled, concentrated 190 times with a type B-125 macrosolute concentrator (Amicon, Danvers, MA, U.S.A.), and dialyzed overnight at 4°C against 50 m*M* sodium sulfate and 20 m*M* sodium phosphate buffer (pH 6.5). The pooled sample was then subjected to size-exclusion chromatography for further purification. The column effluent was monitored at 280 nm and the attenuation of the detector was 0.02 absorbance units full scale (a.u.f.s.). The flow-rates for analytical and preparative chromatographics were 1.0 and 3.0 ml/min, respectively. After each run, the column was re-equilibrated to initial conditions before the next injection. During a typical analysis, fractions comprising individual peaks were pooled, dialyzed against 5 m*M* Tris–HCl buffer (pH 8.5) overnight at 4°C, lyophilized, and resuspended in distilled water. After preparative chromatography, the protein content of each peak was determined by the method of Lowry et al. [12]. Samples were stored at −20°C for later use.

Size-exclusion chromatography

Analytical (300 mm×7.8 mm) and preparative (600 mm×21.5 mm) HPLC Protein-Pak 300 SW columns were used. An isocratic mode was employed with a mobile phase of 50 m*M* sodium sulfate and 20 m*M* sodium phosphate buffer (pH 6.5). Sample volumes were 10 and 0.1–2.0 ml and flow-rates were 1.0 and 3.0 ml/min for analytical and preparative chromatography, respectively. Peaks were collected and treated as described for anion-exchange chromatography.

Polyacrylamide gel electrophoresis (PAGE)

HPLC fractions were examined by use of discontinuous gradient PAGE essentially as described by Laemmli [13]. Individual fractions were pretreated in 0.1% sodium dodecyl sulfate (SDS) and 0.5% 2-mercaptoethanol and boiled in water for 10 min. A 1% solution of bromphenol blue was added as a tracking dye. Separating gels, 1.5 mm thick, were prepared as 10–20% gradients with 5% stacking gels. Electrophoresis was carried out for approximately 4 h at 250 V and 60 mA

in 25 m*M* Tris–192 m*M* glycine buffer that contained 1% SDS (pH 8.6). An additional fraction containing non-reduced IgM was pretreated with 0.2% SDS and 5 m*M* iodoacetamide, boiled in water for 10 min, and analyzed on a 3–10% SDS polyacrylamide gradient gel. Electrophoresis was done for 10 h under the same conditions as used previously. Molecular masses were estimated from a calibration curve prepared with standard proteins purchased from Sigma (St. Louis, MO, U.S.A.). Gels were stained with Coomassie Blue R-250 (Sigma).

MoAb isotype, subisotype and ELISA value determinations

The isotype and subisotype of the MoAbs present in the HPLC fractions were determined using an enzyme immunoassay 55051-K mouse monoclonal subisotyping kit (HyClone Labs., Logan, UT, U.S.A.). The HPLC fractions were then diluted 1:200 and MoAb specificity for As8C was demonstrated by the enzyme-linked immunosorbent assay (ELISA), essentially as described by Healey and co-workers [9, 11]. The only significant change in the ELISA procedure was that horseradish peroxidase (HRPO)-conjugated rabbit anti-mouse IgG subisotype-specific antibody (HyClone) was used in addition to HRPO-conjugated goat antibody to mouse IgG (heavy and light chain-specific), 96-well Immulon microtiter plates (Dynatech Labs., Chantilly, VA, U.S.A.) were substituted for immunoassay cuvettes, and the results were analyzed using a microplate autoreader (Bio-Tek Instruments, Winooski, VT, U.S.A.) at 450 nm.

RESULTS AND DISCUSSION

Ascitic fluid-derived murine MoAbs (IgM, IgG1, and IgG2a) prepared against an isolate of *Actinobacillus* sp (As8C) were purified by anion-exchange and size-exclusion HPLC. Hybridomas producing the IgG1 and IgG2a MoAbs also secreted irrelevant IgG2b MoAbs of unknown antigenic specificity that were present in the ascitic fluids (unpublished data). Although uncommon, hybridomas that secrete MoAbs of more than one subisotype have been observed by other investigators [14]. Ascitic fluids containing the MoAbs were first applied to a high-performance DEAE-5PW anion-exchange column. Total protein recovery

TABLE I

FRACTIONATION OF ASCITIC FLUID CONTAINING MONOCLONAL ANTIBODIES (IgM, IgG1, IgG2a) BY ANION-EXCHANGE HPLC

Preparative (150 mm×21.5 mm) HPLC Protein-Pak DEAE-5PW columns were used.

Ascitic fluid	Total protein (mg)	Protein recovered* (mg)	Recovery (%)
IgM	3.90	3.48	89.2
IgG1	3.60	3.39	94.2
IgG2a	4.50	4.69	104.2

* Fractions comprising individual peaks were pooled, dialyzed against 5 m*M* Tris–HCl buffer (pH 8.5) overnight at 4°C, lyophilized, and resuspended in distilled water. The protein content of each peak was determined by the method of Lowry et al. [12].

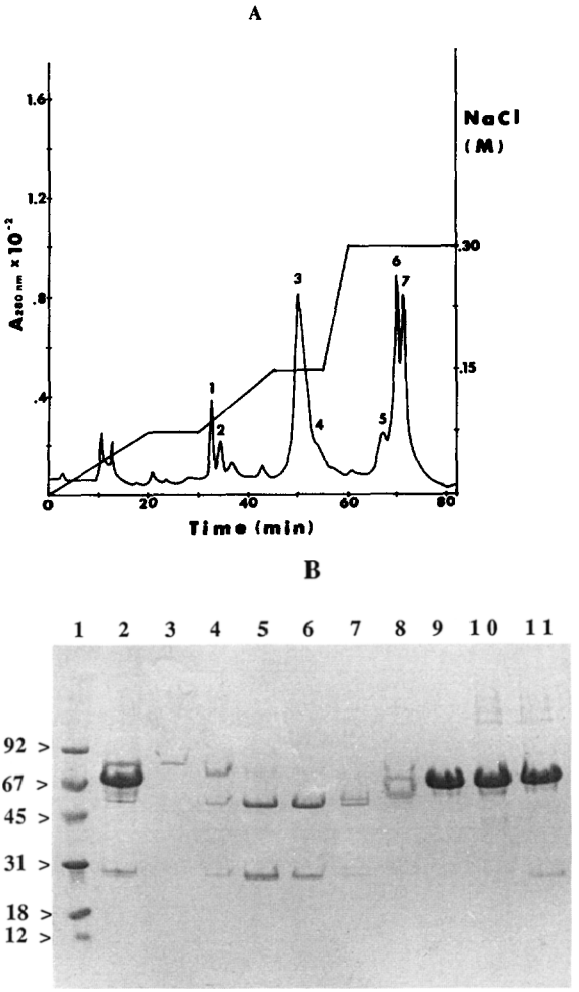


Fig. 1. (A) Fractionation of ascitic fluid containing the IgG1 MoAb by anion-exchange HPLC using a preparative (150 mm×21.5 mm) Protein-Pak DEAE-5PW column. The ascitic fluid was diluted 1:20 with buffer A (20 m*M* Tris–HCl, pH 8.5) and passed through a 0.45-μm membrane filter. A 0.1-ml volume of diluted sample (1.8 mg protein per ml) was loaded onto the column and eluted with an 80-min linear stepwise gradient of 0–100% in buffer B (20 m*M* Tris–HCl, 0.3 *M* sodium chloride, pH 7.0) at a flow-rate of 3.0 ml/min. The column effluent was monitored at 280 nm and the attenuation of the detector was 0.02 a.u.f.s. Protein recovery in peak 3 was 55.8 μg, i.e. a yield of 31.0%. Peak 1 = transferrin; peak 2 = unknown; peak 3 and shoulder 4 = immunoglobulins (IgG1 and IgG2b); peak 5 = albumin; peaks 6 and 7 = albumin and other proteins. (B) Peaks were analyzed by SDS-PAGE on a 10–20% gradient gel under reduced conditions. Electrophoresis was carried out for approximately 4 h at 250 V and 60 mA in 25 m*M* Tris–192 m*M* glycine buffer that contained 1% SDS (pH 8.6). Fractions comprising individual peaks in chromatogram A were pooled, dialyzed against 5 m*M* Tris–HCl buffer (pH 8.5) overnight at 4°C, lyophilized, and resuspended in distilled water. Lane 2 was loaded with 60 μg of ascitic fluid and lanes 3–11 were each loaded with 20–50 μg of eluted proteins. Lane 1, molecular masses of reference proteins; lane 2, ascitic fluid containing the IgG1 MoAb; lanes 3–11 correspond to peaks in chromatogram A as follows: lane 3, peak 1; lane 4, peak 2; lanes 5 and 6, peak 3; lanes 7 and 8, shoulder 4; lane 9, peak 5; lane 10, peak 6; lane 11, peak 7.

TABLE II

MONOCLONAL ANTIBODY ELISA VALUES AND SUBISOTYPES FOLLOWING ANION-EXCHANGE HPLC FRACTIONATION OF ASCITIC FLUIDS

Preparative (150 mm×21.5 mm) HPLC Protein-Pak DEAE-5PW columns were used.

Ascitic fluid	HPLC (profile)	ELISA* (value)	Subisotype (antigen-independent system)**		
			IgG1	IgG2a	IgG2b
IgG1	Peak 3	0.250±0.063	1.763±0.049	N.D.	1.236±0.223
	Shoulder 4	0.034±0.007	1.345±0.055	N.D.	1.999±0.069
IgG2a	Peak 5	0.146±0.019	N.D.	0.949±0.078	0.130±0.017
	Peak 6	0.038±0.006	N.D.	0.684±0.039	1.485±0.243

* Determined by the enzyme-linked immunosorbent assay using 1:200 dilutions of individual HPLC fractions and horseradish peroxidase-conjugated goat anti-mouse IgG (heavy and light chain-specific) antibody. Numbers are the mean ± the standard deviation of triplicate determinations.
** Determined using an antigen-independent enzyme immunoassay 55051-K mouse monoclonal subisotyping kit (HyClone). Numbers are the mean ± the standard deviation of triplicate determinations. N.D. = not determined.

TABLE III

MONOCLONAL ANTIBODY ELISA VALUES AND SUBISOTYPE SPECIFICITY FOR *ACTINOBACILLUS* SP (As8C) FOLLOWING ANION-EXCHANGE HPLC FRACTIONATION OF ASCITIC FLUIDS

Preparative (150 mm×21.5 mm) HPLC Protein-Pak DEAE-5PW columns were used.

Ascitic fluid	HPLC (profile)	ELISA* (value)	Subisotype (antigen-dependent system)**		
			IgG1	IgG2a	IgG2b
IgG1	Peak 3	0.250±0.063	0.577±0.049	0.034±0.002	0.062±0.002
	Shoulder 4	0.034±0.007	0.112±0.011	0.022±0.001	0.170±0.031
IgG2a	Peak 5	0.146±0.019	0.003±0.001	0.374±0.025	0.063±0.010
	Peak 6	0.038±0.006	0.005±0.002	0.137±0.012	0.179±0.028
SP2/0***			0.012±0.001	0.047±0.007	0.145±0.051

* Determined by the enzyme-linked immunosorbent assay using 1:200 dilutions of individual HPLC fractions and horseradish peroxidase-conjugated goat anti-mouse IgG (heavy and light chain-specific) antibody. Numbers are the mean ± the standard deviations of triplicate determinations.
** Determined using an antigen (As8C)-coated 96-well microtiter plate and horseradish peroxidase-conjugated rabbit anti-mouse IgG subisotype-specific antibody. Numbers are the mean ± the standard deviation of triplicate determinations.
*** Negative control ascitic fluid produced by injecting non-secreting SP2/0 myeloma cells intraperitoneally into adult female BALB/c mice.

for each ascitic fluid was quantitative (Table I). The recovery of total protein in the present study ranged from 89 to 104%. This is in agreement with Deschamps et al. [15] who reported recoveries from 60 to 100% when a TSK DEAE-5PW column was used for HPLC purification of mouse MoAbs in ascitic fluid.

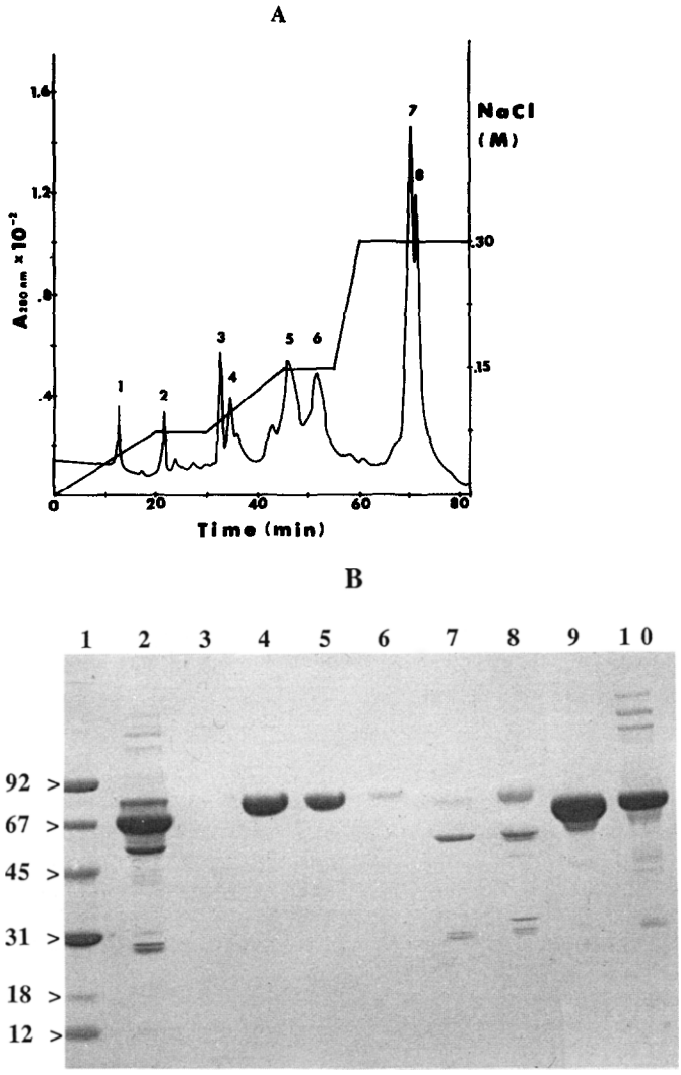
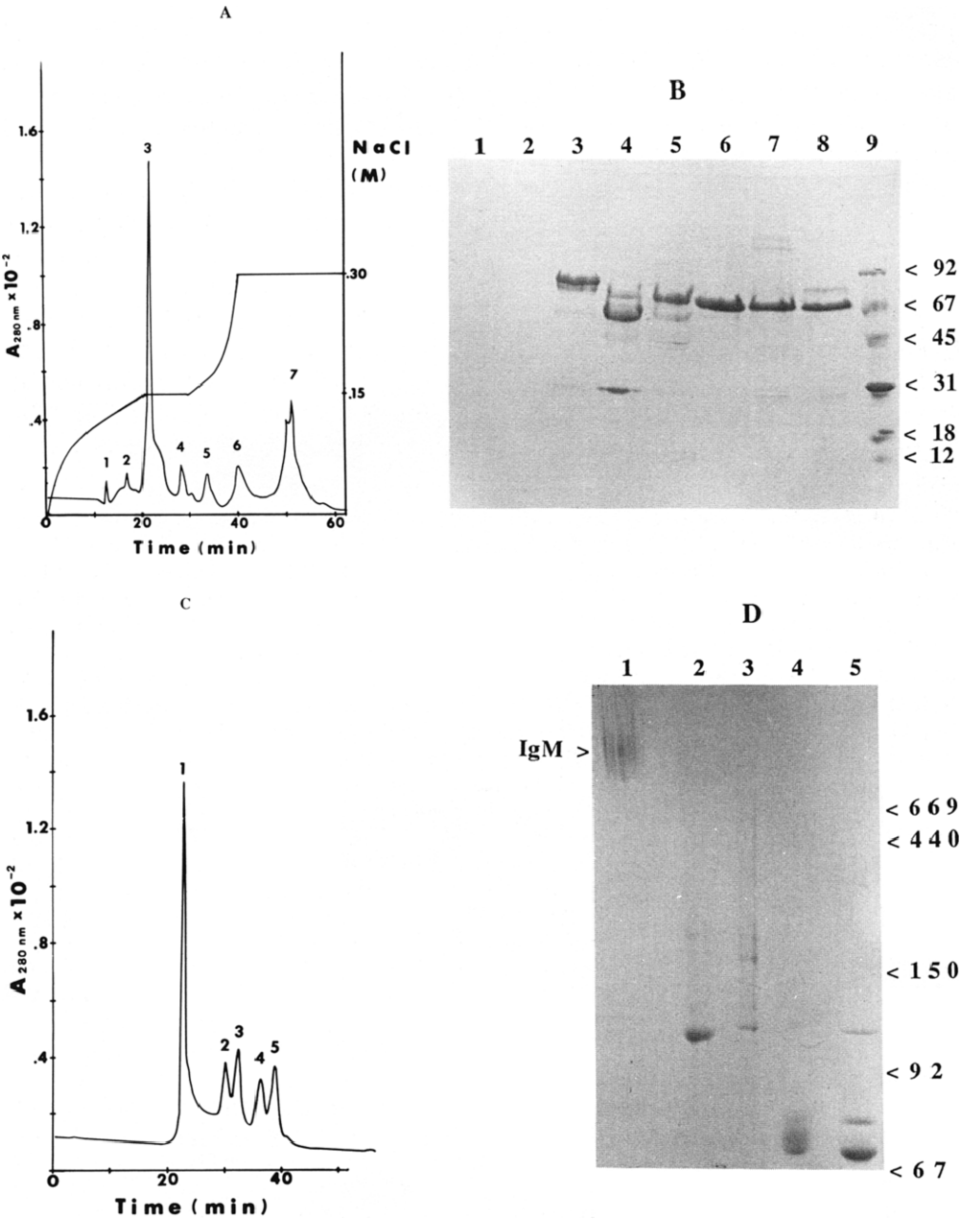


Fig. 2. (A) Fractionation of ascitic fluid containing the IgG2a MoAb by anion-exchange HPLC using a preparative (150 mm×21.5 mm) Protein-Pak DEAE-5PW column. Chromatographic conditions were identical to those described in Fig. 1A. A 0.1-ml volume of diluted sample (2.25 mg protein per ml) was loaded onto the column. Protein recovery in peak 5 was 59.4 μg, i.e. a yield of 26.4%. Peak 1=unknown; peaks 2–4=transferrin; peaks 5 and 6=immunoglobulins (IgG2a and IgG2b); peak 7=albumin; peak 8=albumin and other proteins. (B) Peaks were analyzed by SDS-PAGE on a 10–20% gradient gel under reduced conditions. Electrophoresis conditions were identical to those described in Fig. 1B. Lane 2 was loaded with 100 μg of ascitic fluid and lanes 3–10 were each loaded with 10–70 μg of eluted proteins. Lane 1, molecular masses of reference proteins; lane 2, ascitic fluid containing the IgG2a MoAb; lanes 3–10 correspond to peaks 1–8, respectively, in chromatogram A.

The HPLC profile for ascitic fluid containing the IgG1 MoAb, as well as the irrelevant IgG2b MoAb, revealed that the MoAbs (peak 3 and shoulder 4) were well separated from transferrin and albumin (Fig. 1A). While Deschamps et al. [15] and Clezardin et al. [16] were able to isolate ascitic fluid-derived mouse

MoAbs from transferrin and albumin, Burchiel et al. [1] reported that transferrin co-eluted with mouse IgG2b and IgG3 MoAbs from an anion-exchange HPLC column, and a further separation by size-exclusion chromatography was required. Subisotyping the MoAbs present in peak 3 and shoulder 4 revealed that the separation of IgG1 from IgG2b was incomplete (Table II). It is reasonable to assume that polyclonal IgG antibody is present in mouse ascitic fluid. The ELISA values for peak 3 and shoulder 4 are not in agreement with their respective IgG1 sub-



isotype values because the enzyme immunoassay 55051-K mouse monoclonal subisotyping kit is an antigen-independent system and does not discriminate between monoclonal and polyclonal IgG1. However, IgG1 specificity for As8C was demonstrated by using an antigen-dependent ELISA system for peak 3 and shoulder 4 (Table III). The ELISA values for this peak and shoulder were comparable with their respective IgG1 subisotype values using this system. We attribute the moderate ELISA value for IgG2b in shoulder 4 to non-specific binding (high background), as was also observed for the SP2/0 ascitic fluid. We have previously confirmed that BALB/c mice used for hybridoma production (normal mouse sera) and SP2/0 negative control ascitic fluid do not possess antibodies specific for As8C [9–11]. The MoAb heavy and light chains, with respective relative molecular masses of 50 000 and 25 000, were demonstrated in peak 3 and shoulder 4 following SDS-PAGE (Fig. 1B).

The HPLC profile for ascitic fluid containing the IgG2a MoAb, as well as the irrelevant IgG2b MoAb, revealed that the MoAbs (peaks 5 and 6) were again well separated from transferrin and albumin (Fig. 2A). There was good separation of IgG2a from IgG2b as evidenced by the comparability of ELISA and IgG2a subisotype values for peak 5 (Table II). The relatively large amount of IgG2a in peak 6 can again be explained by the inability of the subisotyping kit to discern monoclonal from polyclonal antibody. Specificity of IgG2a for As8C was confirmed by using an antigen-dependent ELISA system for peaks 5 and 6 (Table III). The ELISA values for these two peaks were comparable with their respective IgG2a

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Fig. 3. (A) Fractionation of ascitic fluid containing the IgM MoAb by anion-exchange HPLC using a preparative (150 mm×21.5 mm) Protein-Pak DEAE-5PW column. The ascitic fluid was diluted 1:20 with buffer A (20 m*M* Tris–HCl, pH 8.5) and passed through a 0.45-μm membrane filter. A 0.1-ml volume of diluted sample (1.95 mg protein per ml) was loaded onto the column and eluted with a 60-min non-linear stepwise gradient of 0–100% in buffer B (20 m*M* Tris–HCl, 0.3 *M* sodium chloride, pH 7.0) at a flow-rate of 3.0 ml/min. The column effluent was monitored at 280 nm and the attenuation of the detector was 0.02 a.u.f.s. Protein recovery in peak 7 was 67.3 μg, i.e. a yield of 34.5%. Peaks 1 and 2 = unknown; peak 3 = transferrin; peaks 4 and 5 = unknown; peak 6 = albumin; peak 7 = IgM and other proteins. (B) Peaks were analyzed by SDS-PAGE on a 10–20% gradient gel under reduced conditions. Electrophoresis conditions were identical to those described in Fig. 1B. Lanes 1–7 were each loaded with 20–60 μg of eluted proteins and lane 8 was loaded with 90 μg of ascitic fluid. Lanes 1–7 correspond to peaks 1–7, respectively, in chromatogram A; lane 8, ascitic fluid containing the IgM MoAb; lane 9, molecular masses of reference proteins. (C) Fractionation of peak 7 from chromatogram A by size-exclusion HPLC using a preparative (600 mm×21.5 mm) Protein-Pak 300 SW column. Prior to loading the column, peak 7 was concentrated 190 times and dialyzed overnight at 4 °C against buffer prepared with 50 m*M* sodium sulfate and 20 m*M* sodium phosphate (pH 6.5). A 0.1-ml volume of sample (6.3 mg protein per ml) was loaded onto the column and an isocratic mode was employed with the dialyzing buffer as a mobile phase at a flow-rate of 3.0 ml/min. The column effluent was monitored and collected as it was for anion-exchange HPLC. Protein recovery in peak 1 was 0.14 mg, i.e. a yield of 22.0%. The total yield of IgM from ascitic fluid was 7.6%. Peak 1 = IgM; peaks 2–5 = unknown. (D) Peaks in chromatogram C were analyzed by SDS-PAGE on a 3–10% gradient gel under non-reduced conditions. Fractions were dialyzed against 50 m*M* Tris and 0.2% SDS (pH 7.5) overnight before being lyophilized. They were then resuspended in distilled water, treated with 0.2% SDS and 5 m*M* iodoacetamide, boiled in water for 10 min, and loaded onto the gel. Electrophoresis was carried out for 10 h at 250 V and 60 mA in 25 m*M* Tris–192 m*M* glycine buffer that contained 1% SDS (pH 8.6). Lane 1 was loaded with 60 μg and lanes 2–5 were each loaded with 10–75 μg of eluted proteins. Lanes 1–5 correspond to peaks 1–5, respectively, in chromatogram C.

subisotype values using this system. Again, we attribute the moderate ELISA value for IgG2b in peak 6 to high background. MoAb heavy and light chains were demonstrated in peaks 5 and 6 following SDS-PAGE (Fig. 2B).

The HPLC profile for ascitic fluid containing the IgM MoAb is illustrated in Fig. 3A. In an anion-exchange HPLC preparative run, 24 fractions were collected and the presence of IgM in each fraction was monitored by ELISA (data not shown). Fractions 20–22 were ELISA-positive and correspond to peak 7 on the chromatogram. The remaining fractions were ELISA-negative. The retention time for IgM on the DEAE-5PW anion-exchange column was approximately 80 min as compared to 45–55 min for the immunoglobulins under the same conditions. Examination of peak 7 by SDS-PAGE revealed that the heavy chains (65 000) and light chains (25 000) of this MoAb co-eluted with other proteins (Fig. 3B). Consequently, a non-linear stepwise gradient was used to accelerate the elution process (Fig. 3A). A size-exclusion HPLC column was required to further purify the IgM in peak 7. Five fractions were collected and the presence of IgM in each fraction was verified by ELISA (data not shown). Only the first fraction (peak 1) contained IgM (Fig. 3C), which is consistent with the relatively high molecular mass (970 000) of this immunoglobulin, since a protein of this size should be eluted in the void volume of the size exclusion column. The identity of IgM was confirmed using SDS-PAGE under non-reduced conditions (Fig. 3D). Other techniques have been used to purify mouse IgM. Ehrnström and Gustavsson [17] used an automated two-column switching technique to purify ascitic fluid-derived IgM by cation-exchange HPLC followed by size-exclusion chromatography.

In conclusion, the method of HPLC described above allowed us to purify ascitic fluid-derived murine MoAbs of isotype IgM and IgG (IgG1, IgG2a, and IgG2b subisotypes). There was good separation of IgG2b from IgG2a, but not from IgG1. The desired purity of IgM could not be obtained in a single chromatographic step and thus required further purification by size-exclusion chromatography. HPLC-purified MoAbs were free from transferrin and albumin and retained their specificity for As8C.

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