Epitope Affinity Chromatography and Biophysical Studies of Monoclonal Antibodies and Recombinant Antibody Fragments

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Abstract

Peptide epitope affinity chromatography is a powerful technique for the purification of antibodies. This study aims to demonstrate the versatility of the technique and to show how biophysical techniques such as circular dichroism (CD) and fluorescence quenching (FQ) can aid the rational design of affinity ligands and characterization of antibody-based reagents. The performance of a number of peptide ligands for the purification of a range of different antibodies and recombinant fragments is investigated by automated fast-protein liquid chromatography. Purified products are analyzed for purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. They are then radiolabelled and the immunoreactivity is determined. Ligands are analyzed for secondary structural characteristics by CD and for binding affinity by FQ. Finally, a study is performed to investigate the thermal stability of a recombinant antibody fragment by CD analysis. It is found that simple ligand modifications such as the introduction of a C-terminal cysteine residue can improve purification performance. The FQ studies show that the modified peptide has a higher affinity for antibody. The CD analysis shows that it has a tendency to dimerize because of the formation of disulfide bonds. The versatility of epitope affinity is demonstrated through the purification of a recombinant diabody (dbFv) and by the use of a separate peptide matrix for the purification of an unrelated antibody. All studies result in antibody preparations of high purity and immunoreactivity. The CD analysis of the dbFv shows that it is denatured at 37°C and is therefore unsuitable as a targeting reagent for use in humans in its present form. It is concluded that epitope affinity chromatography coupled with biophysical analyses plays an important role in the production and characterization of antibody-based reagents for targeted diagnosis and therapy of human diseases.

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Introduction

Peptide epitope affinity chromatography was first described by Price et al. in 1991 (1) for the purification of antibodies reactive against the tumor-associated antigen, MUC1 mucin. The matrix was comprised of a short peptide ligand corresponding with the immunodominant region (2) of the MUC1 protein core conjugated to CNBr-activated Sepharose 4B and was reported to have a capacity in excess of 40 mg of antibody per 1 mL of gel for the monoclonal IgG3 antibody, C595. In addition, purification of anti-MUC1 antibodies of various classes and from diverse feedstocks was demonstrated.

During epitope affinity chromatography, an antibody is captured via the paratope through a specific interaction with the peptide epitope ligand. The eluted antibody is therefore only of the desired specificity and has an immunoreactivity of approximately 100%. Highly pure antibody preparations can be obtained in a one-step purification procedure that requires no feedstock pretreatment (apart from clarification) or polishing of the final product. Indeed, radiolabelled monoclonal antibodies that are purified in this way have been administered directly to humans for targeted radioimmunoscintigraphy and radioimmunotherapy of bladder (3,4) and ovarian cancer (5).

Traditional antibody purification methods (such as protein A) rely on the antibody being captured via a site on the constant region. However it is often the case that antibody fragments produced by genetic engineering or enzymatic methods do not have the constant region with the required capture site. Epitope affinity chromatography therefore provides a method of purifying antibody fragments without the need for fusion proteins or tags. In addition, only active and therefore correctly folded recombinant products will be present in the final purified preparation.

Recently, the fine specificity of antibody binding to MUC1-

related peptides has been studied in order to produce epitope affinity matrices with improved performance characteristics for the purification of a specific anti-MUC1 monoclonal antibody. In one study, limited chemical libraries were used to identify mimotopes with a higher binding affinity than the native epitope peptide (6). In another study, synthetic MUC1-related glycopeptides (7) provided ligands with higher affinity and capacity than the original epitope affinity matrix (1).

Spectroscopic techniques such as fluorescence quenching (FQ) and circular dichroism (CD) have been used for the evaluation of the interactions between antibodies and antibody fragments and their natural and synthetic antigens, as well as their structural content (8). Furthermore, these techniques have proven to be valuable tools in the rational design of ligands for affinity chromatography (6). A CD study of peptides used in affinity chromatography offered a significant contribution to the establishment of the conditions for performance maximization of antibody purification processes (6).

The aim of this study was to demonstrate the power and versatility of epitope affinity chromatography in the analysis and purification of monoclonal antibodies and recombinant antibody fragments. Furthermore, we intend to offer a study of both the peptide ligands used and the antibody fragments purified and, finally, to clearly demonstrate how simple peptide ligands can be manipulated to provide matrices with improved performance.

Experimental

Monoclonal antibodies and recombinant antibody fragment

The murine anti-MUC1 mucin antibody C595 (IgG3) (9) and anti-MUC2 mucin antibody 994 (IgG1) were prepared from exhausted media from static cultures of the respective hybridomas. Cell-free supernatants were clarified by ultracentrifugation (40 000 g, 30 min) and ultrafiltration (0.45 μm) then stored at 5°C with sodium azide (0.05%, w/v) as a preservative.

The recombinant antibody fragment C595dbFv (10) was expressed in E. coli strain HB2151 using the pCANTAB 5E vector. The C595dbFv is comprised of the DNA encoding C595 antibody heavy and light chain variable regions with a shortened linker sequence that will not allow intramolecular association of the chains. This results in intermolecular association to produce the bivalent diabody.

Induction of antibody fragment production in shake plask culture was achieved using the lacZ promoter of the pCANTAB 5E vector by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) substrate into the super broth medium supplemented with ampicillin (100 μg/mL) (11). Supernatants were clarified by ultracentrifugation (40,000 g, 30 min) and ultrafiltration (0.45 μm) then stored at 5°C with sodium azide (0.05%, w/v) as a preservative.

Preparation of immunoaffinity matrices

Synthetic peptide ligands were prepared on an Applied Biosystems 431A Peptide Synthesis (Biopolymer Synthesis and Analysis Unit, Queen's Medical Centre, Nottingham, U.K.). Peptide purity was analyzed by mass spectroscopy using a Bio-Ion 20 plasma desorption mass spectrometer (Bio-Ion, Uppsala, Sweden) and reverse-phase high-performance liquid chromatography (HPLC) on a C18 column. Peptides were found to be greater than 98% pure.

Synthetic peptides were conjugated to beaded agarose (Sepharose 4B, Amersham-Pharmacia, Uppsala, Sweden) via their *N*-termini using CNBr-activated matrix. A standard coupling ratio of 1 μmol peptide per 1 mL gel was used, and conjugation procedures were as recommended by the manufacturer.

Epitope affinity chromatography

Chromatography columns were packed with 3 mL of each affinity matrix and equilibrated with phosphate buffered saline (PBS) containing 0.05% (w/v) sodium azide (PBSA). Column loading, washing, and elution were performed using a fast protein liquid chromatography (FPLC) system. Clarified bacterial or hybridoma supernatants were applied at 1 mL/min. Unbound material was washed away with ten column volumes of PBSA. Antibody was eluted using a linear gradient of NaSCN (Sigma Chemical Co., Poole, U.K.) in PBSA from 0 (Buffer A) to 3M NaSCN (Buffer B) over 20 mL. At the end of the gradient, Buffer B was held for 5 mL before switching to Buffer A for a further 15 mL. Data acquisition and analysis were performed using FPLCDirector software (Amersham Pharmacia). Peak fractions were desalted by gel filtration using PD10 columns (Pharmacia Biotech), and the protein concentration was determined spectrophotometrically ($E^{1\%}_{1\ cm}$ = 14.3).

Enzyme-linked inmmunosorbent assay

The reactivity of purified antibody and column-pass samples was determined using a standard enzyme-linked immunosorbent assay (ELISA) procedure to measure antibody binding to MUC1 mucin adsorbed onto the wells of microtitre plates. After blocking nonspecific binding sites by incubation with PBS containing BSA 1% (w/v), diluted antibody preparations were added at 50 μL/well. After incubation for 1 h, the plates were washed four times in PBS + tween 20 (0.1%, v/v), and HRP-conjugated rabbit antimouse immunoglobulin (1/1000 dilution in blocking buffer) (Dako, High Wycombe, U.K.) was added at 50 μL/well. Plates were incubated for 1 h, washed four times, and then ABTS(2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) chromogenic substrate was added at 50 μL/well. The ABTS was prepared as a 0.033% (w/v) solution in 0.1M citrate phosphate buffer (pH 4) with 33% (v/v) hydrogen peroxide added at 1 μL/mL. The color development was measured using a Milenia Kinetic Analyser (Diagnostic Products Corporation, Llanberis, Wales, U.K.).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Affinity purified antibody samples were diluted 1:1 with sodium dodecyl sulfate (SDS) loading buffer [bromophenol blue (0.05% w/v), sucrose (40% w/v), ethylenediaminete tetraacetic acid disodium salt (0.1 M, pH 8.0), and SDS (0.5%

w/v)] and pretreated by boiling for 5 min prior to loading. SDS-polyacrylamide (PAGE) was performed using a PhastSystem Separation and Control Unit (Amersham Pharmacia) in conjunction with PhastGel precast gels (homogenous acrylamide, 12.5%, w/v). Silver staining was performed using the PhastGel silver-staining kit on the PhastSystem Development Unit.

Technetium-99m labeling of antibodies

Purified antibody preparations were labeled with technetium-99m (^{99m}Tc) using the partial reduction method (12). Briefly, the purified antibody solution (10 mg/mL) was pretreated by incubation with β-2-mercaptoethanol (2-ME, Sigma Chemical Co.) at a molar ratio of 1000:1 (2-ME to Ab) for 10 min. The partially reduced antibody was then separated from the 2-ME by gel-filtration chromatography using a disposable PD10 column (Pharmacia Biotech) that was equilibrated with PBS. The antibody was diluted to 1 mg/mL in PBS, aliquoted, and stored at –20°C.

A vial of Amerscan Medronate II Agent (Nycomed Amersham, Amersham, U.K.) was reconstituted with sterile saline for injection (5 mL). An aliquot (100 µL) was then added to the partially-reduced defrosted antibody solution. This was followed by the addition of the required quantity of ^{99m}Tc sodium pertechnetate, which was previously eluted from a ^{99}Molyldodeum/^{99m}Tc generator (CIS Bio International, Gif-Sur-Yvette Cedex, France). The reaction was allowed to proceed at room temperature for 10 min and was followed by the separation of the ^{99m}Tc-antibody conjugate from free ^{99m}Tc pertechnetate using a PD10 column.

Immunoreactivity testing

The immunoreactivity of radioimmunoconjugates was measured using a Sepharose-bead-based assay. A set of peptide-conjugated Sepharose (produced according to the "Preparation of immunoaffinity matrices" section) standards was prepared by diluting various quantities of the matrix with unconjugated beads in PBS containing BSA (1%, w/v) to a final volume of 500 µL. Radiolabelled antibody (100 ng/mL, 500 µL) was then added to each tube and allowed to react for 4 h at 5°C with agitation. The tubes were then centrifuged briefly in order to sediment the Sepharose, and an aliquot (200 µL) of the supernatant was removed and placed in a separate tube. All pellet and supernatant tubes were analyzed for ^{99m}Tc activity by gamma scintillation counting for 1 min each (1282 Compugamma, LKB Wallac, Turku, Finland). The immunoreactivity of the labelled antibody was calculated by linear regression analysis as the proportion of antibody bound to antigen at theoretical infinite antigen excess, thus plotting the reciprocal of the amount of antigen-coated Sepharose against the reciprocal of the fraction of bound radioimmunoconjugate (13).

FQ

The C595 antibody solution (2.5 mL in a 3-mL quartz cuvette, 1-cm path length), in PBS (pH 7.4) and previously filtered through a Minisart NML 0.2-µm pore membrane (Sartorius, Gottingen, Germany), was excited at 290 nm, and the emitted light was measured at 345 nm using a PerkinElmer (Beconsfield, U.K.) L-3000 luminescence spectrometer. The excitation-slit width was set at 5 nm and the emission slit width was set at 10 nm. The test peptides were titrated into the antibody from a concentrated stock solution (~200 mM) until maximum FQ of the antibody was observed. The dilution effects of titrating a peptide solution into the antibody solution were ascertained by titrating the PBS solution into 2.5 mL of the antibody solution (as a control), and they were used for the correction of the actual peptide titration data. The values of Fo (observed intensity of fluorescence in the absence of peptides) and F (observed intensity of fluorescence in the presence of varying amounts of peptides) were noted during all of the titrations. In order to obtain equilibrium constants from the results for a single mode of binding, the previously established data-fitting techniques and equations were used (8,14).

CD

Peptides with sequences APDTRPAPG (A-G) and APDTRPAPGC (A-GC) were dissolved in a PBSA buffer. The A-G peptide was dissolved at a concentration of 0.35 mg/mL for the secondary structure measurements at the far UV region (190–240 nm) and 0.07 mg/mL for the aromatic contributions at the near UV region (240–320 nm). The A-GC peptide was used at a concentration of 0.07 mg/mL for all measurements. For the far UV measurements, the spectra were acquired using a 0.2-mm optical path length cell for the A-G peptide and a 1.0-mm optical path length cell for the A-GC peptide. The longer cell was required in the latter to compensate for the difference in the concentration of the two peptides and give comparable spectra. The far UV CD spectra were measured both at room temperature (20°C) and at 4°C in order to observe a possible change in the structural content of the A-GC peptide, because it had been previously established to be the case with the A-G and other substituted peptides (6). In the near UV region, the spectra of both peptides were measured using a 5.0-cm optical path length cell.

For the temperature denaturation studies, the C595 antibody and C595dbFv antibody fragment were used at a concentration of 0.3 mg/mL in 50mM of phosphate buffer. A thermocouple was immersed in the sample throughout the duration of the temperature studies to give an accurate indication of the sample temperature. The temperature was kept constant during each spectral accumulation.

All CD spectra were recorded under nitrogen using a Jasco (Tokyo, Japan) J720 Spectrometer with a band width of 2 mm that was automatically slit controlled, and they had the sensitivity set at 50 mdegrees with a response time of 4 s, a step resolution of 0.2 nm, and a scan speed of 20 nm/min.

Analysis of CD data versus temperature and pH

The CD signal from the C595 antibody and C595dbFv antibody fragment was recorded at 220 nm. The signal was analyzed versus temperature to determine the melting temperature (Tm) of the antibody and antibody fragment. The Van't Hoff equation was used to fit the experimental results (14) and to determine the Tm of the antibody. The antibody was shown to have two melting transitions, as previously deter-

mined (8), in which case two Tm values were determined (Tm1, Tm2). Similarly, two melting transitions were obtained from the C595dbFv antibody fragment.

Results and Discussion

Manipulation of ligand affinity

Figure 1 shows the breakthrough curves that were derived when a frontal analysis was performed by loading 110 mL C595 hybridoma supernatant onto matrices comprised of Sepharose that was conjugated to peptide ligands with sequences APDTRPAPG (Sepharose–A-G) and APDTRPAPGC (Sepharose–A-GC). The antibody remaining in the unbound material was measured by ELISA and it can be seen that the Sepharose–A-G matrix showed an immediate and gradual breakthrough of C595 antibody. No breakthrough of the antibody from the Sepharose–A-GC matrix could be detected during the duration of the loading. When the two matrices were eluted under conditions of the NaSCN gradient, single peaks of antibody were obtained from both matrices with retention times of 13.0 and 17.5 min for Sepharose–A-G and Sepharose–A-GC, respectively. Therefore, 0.9M of NaSCN was required for the desorption of antibody from the former matrix though the latter required 1.7M, thus suggesting that the affinity of the interaction between the C595 antibody and Sepharose–A-GC was higher than that between the antibody and Sepharose–A-G. Sepharose–A-G yielded 472 µg of the C595 antibody, and 637 µg antibody was recovered from Sepharose–A-GC. Fluorimetric studies confirmed that the affinity of the antibody for A-GC peptide was higher than that for A-G. The interaction of the peptides with the C595 antibody was measured and expressed as an equilibrium association constant using the antibody FQ technique. Experimental binding curves were obtained by plotting the changes in the

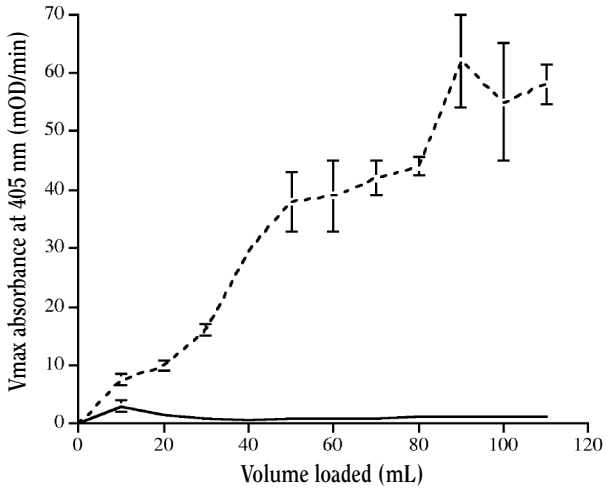

Figure 1. Investigation of the purification performance of affinity matrices Sepharose–A-G (----) and Sepharose–A-GC (—) by frontal analysis. The columns were loaded with C595 hybridoma supernatant (110 mL) and fractions were collected. These were then analyzed for antibody content by ELISA.

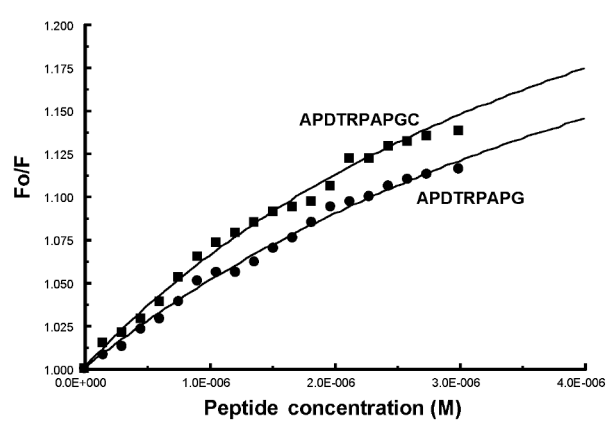

Figure 2. Binding of C595 antibody to A-G (●) and A-GC (■) peptides. Changes in the natural fluorescence of the antibody were plotted against the antigen concentration to calculate the equilibrium association constants for formation of the antibody–antigen complexes.

Table I. Immunoreactivity Antibody Purified by Epitope Affinity Chromatography

Matrix	Mean immunoreactivity of purified antibody	*n*
Sepharose–APDTRPAPG	88% ± 10%	10
Sepharose–APDTRPAPGC	90% ± 9%	5
Sepharose–KVTPTPTPTGTQTPT	86% ± 12%	3

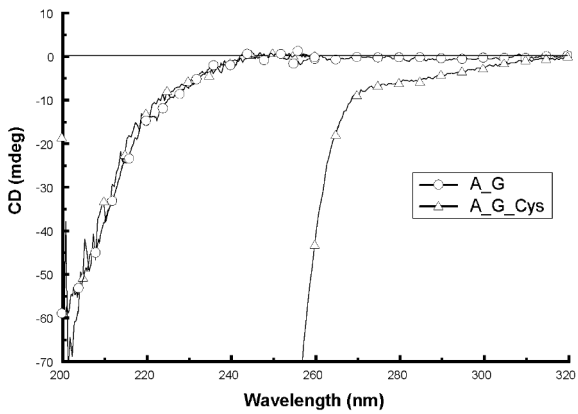

Figure 3. Circular Dichroism analysis of the peptides A-G and A-GC. The secondary structure analysis showed that the peptides in solution have very similar CD spectra, characteristic of low structural content peptides. At the tertiary structure contributions to the CD spectra, the A-G peptide presented no signal, characteristic of the lack of aromatic residues and disulfide bonds. However, the CD signal for the A-GC peptide confirms the presence of disulfide bonds in solution. The A-GC peptide also lacks any aromatic residues from its primary structure. The CD contribution in this region is therefore necessarily that of disulfide bonds formed between the cystine residues at the end of the A-GC peptides in a dimerization process.

fluorescence of the antibody at its emission maxima against the antigen concentration at each titration step (see Figure 2). The experimental fluorimetric data were fitted to a mathematical model to allow the theoretical calculation of Fo/F according to Missailidis et al. (14) (see also the "Experimental" section), which produced a good fit to the experimental data for the association constants K_A. The equilibrium association constants were found to be $K_A = 1.6 \times 10^5\ M^{-1}$ for the natural antigenic peptide A-G, which was in agreement with previously reported values by Spencer et al. in 1999 (8), and $K_A = 2.1 \times 10^5\ M^{-1}$ for the A-GC peptide.

The SDS-PAGE analysis of the purity of the antibody preparations that were derived from the columns showed no evidence of contaminating protein in either sample (data not shown). The immunoreactivity of each sample (Table I) was found to be comparable. It would therefore appear that, although the two matrices were equivalent in the purity and immunoreactivity of the product that was eluted from them, the Sepharose–A-GC matrix out-performed the Sepharose–A-G in terms of the affinity of the antibody for the ligand and capacity of the matrix.

In order to investigate whether the secondary structural characteristics of the A-G and A-GC peptides may contribute to the difference in their purification performance, CD analysis was performed on samples of the two peptides. The spectra are shown in Figure 3. The CD spectra in the far UV region, at room temperature, indicated that neither peptide was highly ordered in solution and in the absence of their ligand. There was an indication of a preferred left-handed extended polyproline II helical conformation, as observed previously for the A-G peptide (6). The two peptides examined showed no significant difference in their secondary structure contribution to the CD spectra at room temperature. However, when the spectra were measured in the near UV region, under identical conditions, a marked difference in the spectrum of the A-GC peptide was apparent. The A-G peptide presented no spectrum in this region, in accordance with the lack of aromatic residues in its amino acid sequence or formation of disulfide bonds. However, the A-GC peptide (which also lacked aromatic residues) demonstrated spectral characteristics indicating the presence of disulfide bonds between peptide molecules, with negative peaks in the 240–250-nm region. The negative CD spectrum of the A-GC peptide at this region therefore proves the presence of disulfide bonds between its cysteine residues. Furthermore, both peptides showed an increase of their secondary structural content when cooled to 4°C. Although this has been previously established for the A-G peptide (6), it has now been observed that this is the case with the A-GC peptide (data not shown) at a higher degree than the natural A-G peptide. It was considered that the increased capacity of the Sepahrose–A-GC matrix was caused by the dimerization of neighboring

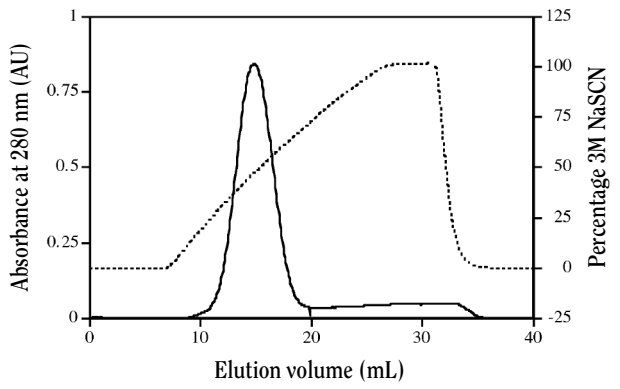

Figure 4. Purification of C595dbFv (——) from bacterial supernatant using a Sepharose-A-GC matrix. After loading, the columns were washed and eluted with a linear gradient from 0 (buffer A) to 3M NaSCN (-----).

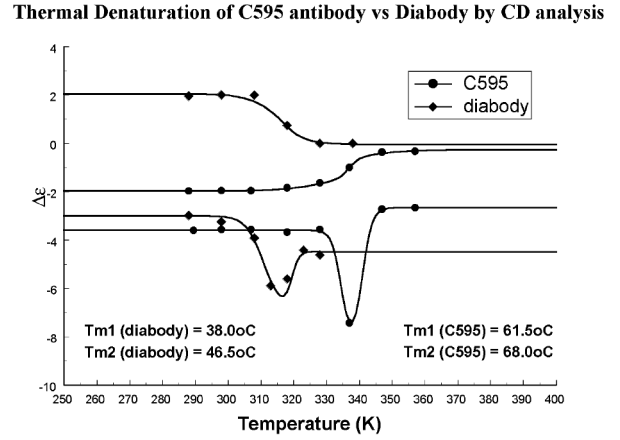

Figure 5. Thermal denaturation of C595dbFv diabody molecule in comparison with the melting of the C595 antibody. Fractional changes in the CD spectra at 215 and 280 nm were plotted against temperature to monitor the secondary and tertiary structure signals, respectively. Apparent Tm values were obtained by fitting the data to a two-transition equation. Both the diabody and antibody had similar melting profiles but with a significant difference in their melting temperatures. The C595 antibody presented Tm values of 61.5°C and 68°C for the secondary structure and 61°C and 64°C for the aromatic regions, respectively. Contrary to that, the diabody presented Tm values of 38°C and 46.5°C for both the secondary structure components and the tertiary structure aromatic determinants.

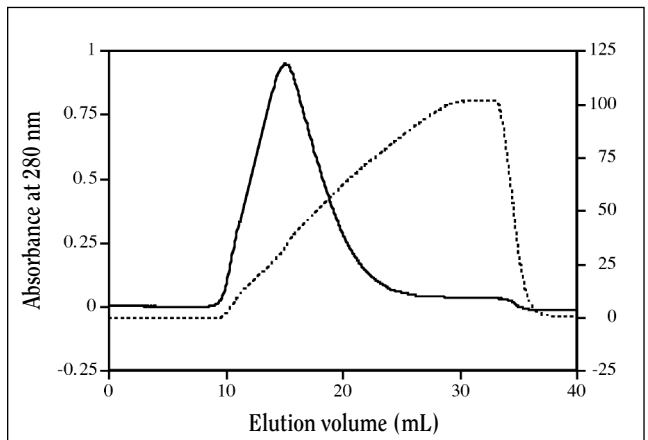

Figure 6. Purification of monoclonal antibody 994 (——) from hybridoma supernatant using a Sepharose–KVTPTPTPTGTQTPT matrix. After loading, the columns were washed and eluted with a linear gradient from 0 (buffer A) to 3M NaSCN (------).

peptide ligands on the surface of the matrix, which may lead to the reduced mobility of the peptide and secondary structural constraints that result in increased binding of the C595 antibody, in agreement with the increased association constant observed in the FQ studies.

Purification and characterization of recombinant anti-MUC1 antibody fragments

The periplasmic extract that was derived from the fermenter culture of recombinant C595dbFv (approximately 10mg/L) was applied to a column containing the Sepharose–A-GC matrix, and the captured antibody fragment was desorbed from the matrix using NaSCN gradient elution. The chromatogram depicting the elution profile can be seen in Figure 4. It can be seen that the recombinant product was eluted from the matrix as a single peak with a retention time of 14.8 min (1.4M NaSCN). Subsequent analysis of the activity and purity of the product by ELISA and SDS-PAGE, respectively, showed that it was fully immunoreactive, and there was no evidence of contaminating proteins.

The purified diabody has been characterized for its structural content by CD spectroscopy. The diabody presented a characteristic CD spectrum that was very similar to the one obtained from the whole antibody C595 (8) and typical of a molecule dominated by β-sheet structural conformation. Such a structure is expected from prior knowledge of the predicted structure of the diabody and the antibody components used in the engineering of this molecule (10). Furthermore, when the C595dbFv diabody and the C595 antibody were subjected to thermal denaturation (Figure 5), a clear melting profile emerged, with two transitions for both molecules. These transitions were confirmed to be at 61.5°C and 68°C for the secondary structure melting of the C595 antibody, in agreement with previously reported data (8). The C595dbFv diabody molecule, however, was shown to have a closely related melting profile (Figure 5) to that of the antibody, though the first melting transition was at 38°C and the second at 46.5°C. The diabody's melting profile is characterized by the adoption of a different and distinct partially-denatured conformation between 40°C and 45°C, which is similar to that adopted by the antibody but at a much lower temperature. This conformation disappears at the next temperature step (50°C), in which the diabody is in its completely denatured state. Cooling the diabody back to 25°C after temperature denaturation does not restore its structure or CD spectra in the near or far UV region, as is the case with the whole antibody molecule. This unexpected characteristic of the diabody has important implications for its use as a therapeutic molecule. It has been shown in this study that the diabody is partially denatured at 38°C, and this denaturation process starts before the human body temperature of 37°C. ELISA studies confirmed that the antibody quickly loses activity at 37°C (data not shown; G. Denton, personal communication), in agreement with the CD data, thus invalidating this agent as a molecule of potential clinical value. It is important to note that biophysical studies such as these, to fully characterize molecules of interest in terms of their physical properties, can be invaluable tools in assessing the clinical potential of new reagents. Furthermore, we would like to draw the attention of other investigators working with diabody molecules to their relative instability in comparison with whole antibodies. Work is underway within this group to apply site-directed mutagenesis to introduce interchain disulfide bonds into the diabody molecule in order to increase its stability.

Application of epitope affinity chromatography to other systems

In order to demonstrate the versatility of epitope affinity chromatography, a matrix was prepared comprised of a peptide related to the sequence of the protein core of the MUC2 mucin (KVTPTPTPTGTQTPT) conjugated to CNBr–Sepharose 4B. The ability of this matrix to purify an anti-MUC2 monoclonal antibody (994) was then tested. The chromatogram depicting elution of the antibody from the peptide affinity matrix is shown in Figure 6 and is seen to consist of a single peak of antibody eluting with approximately 1M NaSCN. The purified preparation was found to be 86% immunoreactive (Table I) and had no evidence of contaminating proteins by SDS-PAGE. Having proved that this technique applies successfully not only to the purification of the anti-MUC1 monoclonal antibody, C595, but also to another, unrelated system, we would like to claim the general applicability of the technique. We believe that epitope affinity chromatography is a powerful technique for the purification of monoclonal antibodies and recombinant antibody fragments. It provides a quick and highly specific one-step purification process that results in antibody preparations with high purity and immunoreactivity.

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