VERY RAPID MICROANALYSIS OF IgG IN ASCITES FLUIDS BY HPLC USING A NOVEL ANION-EXCHANGE COLUMN

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ABSTRACT

An HPLC system has been optimized for the rapid resolution and quantitation of IgG in mouse ascites fluids, making use of newly developed HPLC columns (MA7P). Chromatography using MA7P columns is characterized by remarkably narrow band widths and very short retention times. The HPLC system is capable of analyzing the IgG content of a typical ascites fluid with a cycle-to-cycle time of under 5 minutes, regardless of IgG subtype. Separation times under 1 minute are possible. Recoveries of 100 µg injections of IgG and other proteins are quantitative. Using a UV monitor, the system was optimized to generate a linear plot of peak area vs. amount of IgG injected from 100 ng to 3 µg per injection. These properties make the MA7P column very useful for both analytical and micropreparative applications. Ascites fluids from eleven different IgG1-producing hybridomas were analyzed. The IgG concentrations in those eleven ascites varied considerably. Retention times of the IgG varied slightly but significantly from hybridoma to hybridoma. Some monoclonals produce heterogeneous IgG. This heterogeneity can be detected by chromatography using MA7P columns.

INTRODUCTION

Monoclonal antibodies are being used in an increasingly large number of applications (1,2). Consequently, there is an increasing demand for methods which allow rapid analysis of ascites fluids and purified antibodies. High Performance Liquid Chromatography (HPLC) techniques allow rapid analysis of single samples or small numbers of samples (3-7). However, these procedures are time consuming when large numbers of samples must be analyzed using the same instrument. Therefore, an analytical HPLC technique with very short analysis times is highly desirable.

The recently introduced Microanalyzer™ MA7P cartridge column is a small bed volume column packed with a non-porous support which has a very high selectivity (3). This combination of properties allows for the very rapid and highly sensitive resolution of small aliquots of protein solutions. In this paper, data is presented on the use of the MA7P column for rapid analysis of IgG in ascites fluids.

The MA7P column is significantly faster than other HPLC columns available for this type of analysis. The HPLC system is useful both for quantitating the amount of IgG in the ascites fluid and for monitoring...
purification of the monoclonal antibody. Previous work with Bio-Gel HPHT columns has demonstrated that monoclonal antibodies are heterogeneous (8). Such heterogeneity is also observed in chromatograms using the MA7P column (3). Analysis of this heterogeneity may be helpful in selecting ascites with a high proportion of "active IgG". Since recovery of proteins from the MA7P column is excellent at very low protein inputs, the column can also be used for micropreparative applications.

MATERIALS AND METHODS

Materials

All buffer solutions were made with distilled, deionized water and reagent grade solutes. The following four ascites fluids (IgG subtype in parenthesis) were obtained from Sigma: MOPC 21 (IgG1); FLOPC 21 (IgG3); UPC 10 (IgG2a); and MOPC 141 (IgG2b). Ascites fluid 13H1 was obtained from Dr. Larry Stanker, Lawrence Livermore National Laboratory (Livermore, CA). Ascites fluids BB/A and BBLT-1 through BBLT-11 (Table 3) were obtained from Dr. Barry Bredt, Bio-Rad Clinical Division (Richmond, CA).

Methods

Hydroxylapatite (HPHT) fractionation of ascites BB/A was performed by Ms. Theresa Chow of Bio-Rad Laboratories, using the suggested procedure (8). The Protein-A MAPS kit with buffers was obtained from Bio-Rad, and was used according to the recommended protocol (9). Protein assays were performed as described by Bradford (10), using reagents from Bio-Rad.

Columns and Sample Preparation

Microanalyzer™ MA7P cartridge columns (4.6mm X 30mm), Bio-Gel HPHT hydroxylapatite columns (7.8mm X 100mm), and Bio-Gel TSK DEAE-5-PW columns (7.5mm X 75mm) were obtained from Bio-Rad Laboratories (Richmond, CA). Aquapore AX-300 cartridge columns (4.6mm X 30mm) were obtained from Rainin (Berkeley, CA). Bakerbond MAb material was obtained from J.T. Baker, Phillipsburg, NJ, and was packed into 4.6mm X 30mm cartridge columns. Mono Q columns (5.0mm X 50mm) and Polyanion SI columns (5.0mm X 50mm) were from Pharmacia (Uppsala, Sweden). Cartridge columns were housed in Bio-Rad cartridge holders. Samples were clarified by centrifugation (Eppendorf model 5414 for 2 min) and diluted with low salt buffer. Injections of 20 µl contained 10-50 µg of total protein.

HPLC System

The HPLC system used in these studies was a Bio-Rad Protein Microanalyzer System, consisting of two Model 1330 pumps, a gradient mixer (1.8 ml volume), and either a Model 7125 manual injector or a Model AS-48 Autosampler. Connections between the mixer and the injector, between the injector and the column, and between the column and the detector were kept to a minimum by using short lengths of 0.01 inch (i.d.) tubing. The tubing length from the injector to the detector was 10 cm. Since the detector cell had a volume of 8 µl, the extra-column volume (injector through detector) was about 20 µl. The system was operated by an Apple IIe computer with dual disk drive, Profiler hard disk option, and Bio-Rad Gradient Processor System (Version 3.7) software. Data from the Bio-Rad Model 1305A detector was integrated with a Model 3392A integrator, interfaced with the computer.

RESULTS

The chromatograms in Figure 1 demonstrate that HPLC using the MA7P column can resolve IgG from other components of various mouse ascites fluids in a short time. The four panels show chromatograms of ascites fluids from myeloma tumors producing immunoglobulins with four different subtypes. In order to identify the IgG peak, the IgG was purified from