Glycoconjugate J (1989) 6:511-524

Purification and Immunochemical Characterization of Ascitic Fluid Glycoproteins Containing Certain Tumor-associated and Blood Group Antigen Markers

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Received May 24/October 2, 1989.

Key words: glycoproteins, ascitic fluids, tumor antigens

Ascitic fluids from patients with various types of cancer were screened for the CA 19-9 and CA 125 tumor-associated antigenic activities. Two fluids exhibiting the highest activities were tested for their binding to various lectin-Sepharose columns resulting in both being bound best to wheat germ agglutinin (WGA) Sepharose. The WGA column eluate of one fluid was further chromatographed by HPLC and three peaks were obtained with approximate molecular weights of 3.65 MDa, 664 kDa and 330 kDa, of which only the largest fraction contained the CA 19-9 activity. The fluids were also fractionated on a Sephacryl S-400 column with most of the activity being present in or near the void volume.

Monoclonal antibodies were used to demonstrate that the purified glycoproteins also contained the blood group A determinant, the four Lewis determinants Le^a,Le^b,Le^x and Le^y, and the sialylated-Le^x determinant, while other antibody analyses failed to detect other blood group and/or carbohydrate sequence determinants. Some of the blood group expressions could be separated from the CA 19-9 and CA 125 active glycoproteins by adsorption with various lectins other than the WGA.

Since the introduction of the hybridoma techniques [1] many monoclonal antibodies have been described as reactive with carbohydrate sequence associated antigens of human tumors. These carbohydrate sequences have been found in both glycolipids and glycoproteins and have provided useful markers for monitoring a number of human cancers [2-5].

Abbreviations used: NeuAc, *N*-acetyl-D-neuraminic acid; Gal, galactose, D-galactopyranose; Fuc, fucose, L-fucopyranose; GlcNAc, *N*-acetyl-D-glucosamine; GalNAc, *N*-acetyl-D-galactosamine; WGA, wheat germ agglutinin; PBS, phosphate buffered saline.

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The CA 19-9 reactive determinant has been shown to be the sialylated-lacto-*N*-fucopentaose II (sialylated-Le^a) structure:

NeuAcα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-R.

This determinant has been detected in sera from patients with colorectal, gastric and pancreatic cancers [6-15] and it has also been found in a fucose-rich sialoglycoprotein of normal human seminal fluid [16,17].

The CA 125 reactive determinant is elevated in the sera of more than 80% of patients with ovarian cancer and has been used in diagnosing and monitoring these patients [18-26]. The CA 125 antigenic determinant has also been found in sera of patients with various gastrointestinal cancers [27], human milk [28], human seminal plasma [29], normal cervical mucus [30] and normal central airway and lung tissue [31]. In addition, this determinant has been reported in sera of patients with various benign diseases such as liver cirrhosis, liver granulomatosis, pancreatitis and peritonitis [32]. The structure of the CA 125 determinant has not yet been elucidated although it is known to be associated with a mucin-like high-molecular-weight glycoprotein complex, and is thought to be composed of, at least in part, of a conformationally dependent peptide [33].

In this study we show that CA 19-9 and CA 125 antigenic activities may be isolated in a large molecular weight glycoprotein fraction of ascitic fluids obtained from patients with various types of cancer. Using a number of monoclonal antibodies we demonstrated that these glycoprotein preparations also contain certain blood group determinants.

Materials and Methods

Ascitic Fluids

Ascitic fluids were obtained from various cancer patients being treated at Northwestern Memorial Hospital and at Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA. The fluids designated AI and MI were from two different patients with stage three adenocarcinoma of the ovary. Upon collection, the ascitic fluids were aliquoted in 50 ml fractions and immediately frozen. Prior to purification, the ascitic fluids were thawed, and centrifuged at 1,000 x *g* for 20 min to remove insoluble materials.

Activity Determinations of Ascitic Fluids and Purified Fractions

The ascitic fluids, and gel filtration fractions derived from them, were analyzed for CA 19-9 and CA 125 activity. CA 19-9 activity was quantified using a solid-phase "sandwich" radioimmunoassay (Centocor CA 19-9., Malvern, PA, USA) according to provided instructions. Briefly, polystyrene beads coated with anti-CA 19-9 murine monoclonal antibody were incubated with the 100 µl of sample and 100 µl of 50 mM sodium citrate buffer at 37°C for 3 h. The beads were washed with deionized water (3 times, 5 ml), after which 200 µl of anti-(CA 19-9) mouse monoclonal antibody labeled with ^{125}I were added. The wells were incubated for 3 h at room temperature, and washed as above. Radioactivity remaining bound to the beads was then determined by counting for 1 min in a gamma counter.

CA 125 antigenic activity was quantified using a commercial solid phase radio-immunoassay based on the sandwich principle (Centocor CA 125.). In this assay, the radiolabeled antibody was incubated simultaneously with polystyrene-immobilized antibody and 100 µl of sample for 20 h. The beads were washed and counted in a gamma counter.

Gel Filtration

AI and MI ascitic fluids, which exhibited the highest levels of CA 19-9 and CA 125 activities, respectively, were each chromatographed on a 5.0 x 75 cm Sephacryl S-400 column (Pharmacia Inc., Piscataway, NJ, USA) in phosphate buffered saline (PBS, 0.01 M sodium phosphate and 0.15 M NaCl), pH 7.4. The samples were eluted at 30-40 ml/h with continuous monitoring of absorbance at 280 nm (LKB 2138 Uvicord S monitor). Ten to twelve ml fractions were collected when the column was loaded with 300 ml of ascitic fluid. Every other fraction was tested for antigenic activity as described above and for carbohydrate (neutral hexoses) by the phenol-sulfuric method [34]. The active fractions were pooled and portions were further purified by affinity chromatography on wheat germ agglutinin (WGA)-Sepharose columns (EY Laboratories, San Mateo, CA, USA).

Lectin Gel Fractionation

A lectin gel kit (EY Labs.) consisting of nine lectins individually immobilized to agarose beads was used to fractionate the AI and MI ascitic fluids. Two ml of ascitic fluid were loaded on a 1 ml lectin-gel column packed into a disposable Pasteur pipet, and 2 ml of the unbound material were collected. The gel was then washed with 20 ml of PBS prior to the elution of the bound material with 2 ml of a sugar solution appropriate to the particular lectin to dissociate bound glycoproteins. The sugars utilized are listed in Table 2 and were obtained from Sigma Chemical Co., St. Louis, MO, USA.

The eluates were dialyzed extensively against the buffer indicated in the kit instructions and subsequently against deionized water. Absorbances at 280 nm were used to estimate protein and each sample was analyzed for particular antigenic activities using monoclonal antibodies.

In other experiments involving the AI and MI fluids, the combined active fractions of a large scale S-400 purified material (ca. 200 ml) were rotated for 12 h with 20 ml of WGA-Sepharose gel. After centrifugation (1,000 x *g* for 20 min) and removal of the supernatant, glycoproteins bound to the gel were eluted with 20 ml of 0.2 M *N*-acetylglucosamine. The eluate was then dialyzed against PBS and stored frozen.

HPLC Fractionation

The fraction of the AI crude fluid eluted from a WGA-Sepharose column was analyzed on HPLC (Model 344, Beckman Instruments, Berkeley, CA, USA) using a Bio-Sil SEC-400 column (300 x 7.5 mm, Bio-Rad Labs., Richmond, CA, USA). The mobile phase consisted of PBS, pH 7.4, with a flow rate of 0.3 ml/min. Fractions collected (1 ml) were monitored by a U.V. detector at 215 nm (Beckman Instruments, Model 163 U.V. variable wave length spectrophotometer). Each fraction was then assayed for CA 19-9 activity.

Enzyme-linked Fluorigenic Immunoassay (ELFA)

Duplicate wells of black 96 well polystyrene MicroFLUOR B plates (Dynatech Laboratories, Alexandria, VA, USA) were coated with 100 µl of 2.5-5.0 µg/ml (protein) of the purified ascitic fluid glycoproteins/mucins (Sephacryl S-400 void volume fractions) in 0.1 M $NaHCO_3$, pH 9.0, for 2 h at room temperature. Protein content was estimated spectrophotometrically using 215 and 225 nm wavelengths [35]. The wells were overcoated with a solution of 1% bovine serum albumin (BSA, fraction V, Sigma) in PBS, pH 7.3, containing 0.02% sodium azide, for 1.5 h at room temperature and then washed three times with PBS containing 0.1% (by vol) Tween (PBS-Tween). Various dilutions of monoclonal antibodies were made in PBS-Tween-1% BSA and 100 µl volumes were added to duplicate wells.

The plates were incubated at 4°C overnight and the wells then washed three times. Next, 100 µl of β-galactosidase-conjugated goat anti-mouse IgG or goat anti-mouse IgG, IgM and IgA mixture (Zymed Laboratories, San Francisco, CA, USA) were added. After the plates were incubated for 4 h at 4°C, the wells were washed three times with PBS-Tween and then 100 µl of 4-methylumbelliferyl-β-D-galactoside (0.1 mg/ml) in a 10 mM sodium phosphate buffer, pH 7.5, containing 0.1 M NaCl and 1 mM $MgCl_2$ were added to each well. The plates were read at various time intervals in a plate reader (MicroFLUOR System, Dynatech Laboratories) to quantify intensity of the 4-methylumbelliferone product and the results were expressed in fluorescent intensity units (FIU).

In all the above experiments, two controls were always included to assess background (wells lacking mucin glycoprotein); and non-specific binding (either primary antibody only, or secondary antibody only controls).

Antibodies Used

PM-81 (anti-Le^x) was obtained from Hybritech Labs., San Diego, CA, USA; anti-A, anti-B, anti-H and anti-T from Dako Corp., Santa Barbara, CA, USA; anti-Le^a and anti-Le^b from Chembiomed Ltd., Edmonton, Alberta, Canada; anti-Le^y-containing ascitic fluid was kindly donated by Dr. Kenneth Lloyd of the Memorial Sloan Kettering Cancer Center, New York, NY, USA; the CSLeXI antibody (anti-sialylated-Le^x) was supplied by Dr. P. Terasaki, UCLA, Los Angeles, CA, USA; the anti-I and anti-i antibody preparations were donated by Dr. J. Moulds of Gamma Labs., Houston, TX, USA; and the anti-H (reactive with H type 1 and 2 chains) was donated by Dr C. Doinel of the Centre National de Transfusion Sanguine, Paris, France. The anti-P_1, anti-P^k, H124 (anti-Le^x), H162 (anti-Le^x branched), and H112 and H131 (both anti-type 1 chain) antibodies were produced in our laboratories.

Results

Activities of Ascitic Fluids

Seventy three cancer patient ascitic fluids were collected and tested for CA 19-9 and CA 125 determinant activities. Sixteen percent (12 of 73 fluids) exhibited either elevated CA 19-9 or CA 125 activity (Table 1). It is interesting to note that only the ascitic fluid (No. 1),

Table 1. CA 19-9 and CA 125 activities of 12 of 73 ascitic fluids.

Fluid No.	Units/ml	
	CA 19-9	CA 125
1	800,000	1,700
2	92,000	47
3	37,500	0
4	23,250	407
5	24	1,750
6	0	1,830
7	185	1,950
8	0	2,050
9	0	2,200
10	150	2,450
11	87	3,825
12	0	7,900

designated AI, exhibited both elevated CA 19-9 and 125 activities, whereas the other fluids exhibited elevations of either one or the other activity. Ascitic fluid No 12, designated MI, showed the highest CA 125 activity. Using Centocor's conversion formula, 1 unit = 0.59 ng/ml, the AI fluid was calculated to contain 472 μg/ml of CA 19-9 antigen.

Binding to Lectin-Sepharose Columns

The AI and MI ascitic fluids were fractionated on a variety of lectin-Sepharose columns. Both CA 19-9 and CA 125 activities as quantified by the ELFA method (Table 2) were best adsorbed by WGA-Sepharose. The data are summarized in Table 3 using + and - (positive and negative activity) to facilitate comparison of the results. Although CA 19-9 and CA 125 activities were best bound by the WGA-columns, some activity also was bound to the *Arachis hypogaea* (PNA), *Glycine max* (SBA), and *Ricinus communis* (RCA-I) lectins. The WGA eluted fraction of AI fluid also contained A, Le^a, Le^b, Le^x, Le^y and sialylated-Le^x determinants whereas MI fluid showed the same determinants but much weaker activities than found in the AI fluid. The α-*N*-acetylgalactosamine-reactive lectins, *Dolichos biflorus* (DBA) and *Helix pomatia* (HPA), and the α-fucose reactive lectin *Ulex europaeus* (UEA), bound A and fucosylated determinants (blood group Lewis activities), but very little of the CA 19-9 and CA 125 activity. The sialylated-Le^x determinant was bound by DBA, PNA, HPA and UEA lectins to a much greater extent than CA 19-9 and CA 125. MI fluid exhibited H determinant activity, being bound by UEA, SBA and HPA whereas the AI fluid did not contain any detectable amount of this determinant.

HPLC Fractionation

The WGA eluate from AI fluid was fractionated by HPLC in a PBS buffer system yielding four peaks of 215 nm absorbing material eluting at 3.65 MDa, 664 kDa, 330 kDa and 44 kDa mean molecular weights as shown in Fig. 1. Only the 3.65 MDa fraction contained 19-9 activity. Using the same column but a different buffer (20 mM Tris-HCl, pH 7.4), very similar peaks were obtained but with poorer resolution.

Table 2. Reactivities of various monoclonal anti-carbohydrate sequence antibodies to the fractions of AI (CA 19-9) and MI (CA 125) ascitic fluids adsorbed and desorbed from the lectin-Sepharose columns indicated.

Antibody [b]	Fluid	Lectin-Sepharose columns [a]								
		DBA	PNA	HPA	UEA	SBA	WGA	Con A	LPA	RCA-1
Anti-A	AI	3583 [c]	988	>4000	2571	2844	2830	198	42	453
	MI	2811	498	>4000	3127	2703	1299	119	15	111
Anti-B	AI	15	181	74	14	49	39	8	0	2
	MI	0	39	63	8	39	46	8	0	0
Anti-H_1,H_2	AI	136	103	207	138	55	36	9	0	0
	MI	320	26	934	1600	1247	55	10	0	18
Anti-Le^a	AI	663	580	1933	1640	868	2080	67	29	409
	MI	461	253	365	354	640	797	103	130	59
Anti-Le^b	AI	889	209	2235	1742	499	1587	45	25	201
	MI	456	69	463	483	330	407	103	27	55
Anti-Le^x	AI	115	145	350	446	273	366	45	10	+
	MI	80	30	83	58	108	161	27	11	-
Anti-Le^y	AI	382	140	1605	1832	269	571	90	80	145
	MI	1285	105	1568	2018	1384	624	72	33	91
Anti-sialyl-ated-Le^x	AI	1447	520	3438	2035	240	3902	287	204	++
	MI	236	240	287	216	223	464	392	198	- -
Anti-CA 19-9	AI	300	3000	50	1600	900	154,500	400	150	1400
Anti-CA 125	MI	0	200	80	0	200	640	60	0	160

[a] The two ascitic fluids were processed through the lectin Sepharose columns and were assayed with the various anti-carbohydrate sequence antibodies listed as described in the text. The abbreviations listed for the lectins and the carbohydrate used for desorption of glycoprotein from the lectin-Sepharose were as follows: DBA, *Dolichos biflorus*, 0.1 M *N*-acetylgalactosamine; PNA, *Arachis hypogaea*, 0.1 M lactose; HPA, *Helix pomatia*, 0.1 M *N*-acetylgalactosamine; UEA, *Ulex europaeus*, 0.1 M fucose; SBA, *Glycine max*, 0.1 M lactose; WGA, *Triticum vulgaris*, 0.2 M *N*-acetylglucosamine; Con A, *Concanavalia ensiformis*, 0.01 M α-methyl-D-mannopyranoside; LPA, *Limulus polyphemus*, 0.01 M *N*-acetylneuraminic acid; RCA-I, *Ricinus communis*, 0.1 M lactose.

[b] Anti-T antibody was weakly reactive with some of the lectin fractions. Anti-I, -i, -P and -P^k antibodies were not reactive with any of the lectin-Sepharose processed ascitic fluid fractions.

[c] Fluorescence intensity units of the ELFA assay as described in the text.

Sephacryl S-400 Chromatographic Purification of Ascitic Fluid Glycoproteins

The HPLC fractionation (Fig. 1) suggested that the CA 19-9 or CA 125 activities may be separated from other glycoproteins of the ascitic fluids by molecular sieve chromatography. Ascitic fluids AI and MI (diluted 1:1 with PBS before loading due to their relatively high viscosities) were fractionated on Sephacryl S-400. Fractions were monitored at 280 nm as

Table 3. Reactivity of AI and MI ascitic fluid glycoproteins fractionated through various lectin-Sepharose columns[a].

	WGA	PNA	DBA	HPA	UEA	RCA-I	SBA	Con A	LPA
AI Fluid									
Anti-CA 19-9	>++++	+	-	-	+	+	+	-	-
Anti-sialylated-Le^x	++++	++	++	+++	++++	+	-	<+[b]	<+
Anti-A	++	++	++++	+++	>++++	+	++++	-	-
Anti-B	-	-	-	-	-	-	-	-	-
Anti-H	-	-	-	-	-	-	-	-	-
Anti-Le^a	++++	+	+	++	++	+	+	-	-
Anti-Le^b	++	-	+	+++	++++	-	<+	-	-
Anti-Le^x	+	-	<+	+	+	-	-	-	-
Anti-Le^y	+	-	+	+++	+++	-	-	-	-
MI Fluid									
Anti-CA 125	+++	+	-	-	-	<+	<+	-	-
Anti-sialylated-Le^x	++	<+	<+	<+	<+	+	<+	+	<+
Anti-A	+++	+	+++	>++++	+++	-	+++	-	-
Anti-B	-	-	-	-	-	-	-	-	-
Anti-H	-	-	<+	+	++	-	++	-	-
Anti-Le^a	++	<+	+	<+	<+	-	++	-	-
Anti-Le^b	+	-	+	+	+	-	<+	-	-
Anti-Le^x	+	-	<+	<+	-	-	<+	-	-
Anti-Le^y	++	-	+++	+++	++++	-	+++	-	-

[a] The results from Table 2 are summarized using + and - to indicate degree of positive activity (+ to >++++) and negative (-) activity with the various anti-carbohydrate sequence antibodies reactive with the glycoprotein fractions eluted from the lectin-Sepharose columns.
[b] <+, weak but positive reactivity.

shown in Figs. 2 and 3 for MI and AI fluids, respectively. For both fluids, the CA 19-9 or CA 125 activities were eluted in the fractions in or near the void volume. Only MI fluid exhibited a small amount of activity in smaller molecular weight fractions. Carbohydrate analysis (phenolsulfuric acid method) showed that with both AI and MI column fractions most of the carbohydrate eluted together with the protein (in the inclusion volumes of the column) and that only a small fraction of the total carbohydrate was eluted in the void volume with the CA 19-9 or CA 125 activities.

Fractions exhibiting reactivities with anti-CA 19-9 antibody in AI fluid or anti-CA 125 antibody in MI fluid were pooled, dialyzed and re-assayed for each activity, as well as for carbohydrate by the phenol-sulfuric method and for protein content by calculation from absorbances at 225 and 215 nm. Neutral hexose contents were 100 µg/ml and 50 µg/ml, and protein contents were 250 µg/ml and 200 µg/ml for AI and MI, respectively.

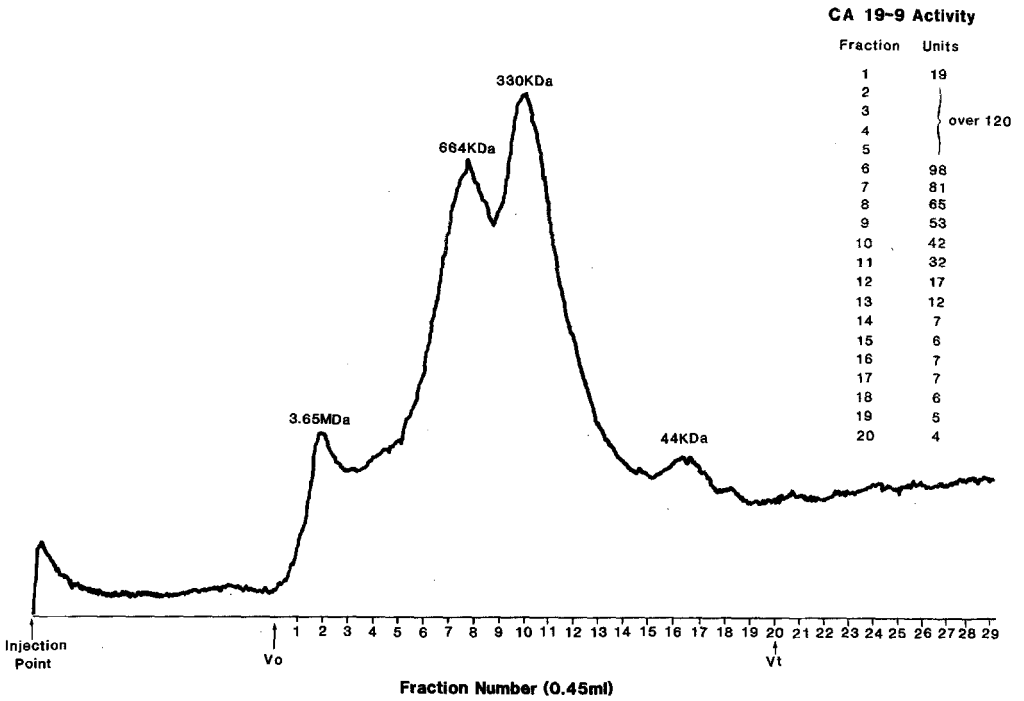

Figure 1. HPLC Fractionation of the WGA eluate from AI fluid as described in the Materials and Methods section. The eluate was monitored by 215 nm absorbance. On the upper right corner is shown the activity of the various fractions tested against anti-CA 19-9 antibody. Void volume (V_0) and total volume (V_t) of the column are indicated by the arrows.

Carbohydrate Sequence Determinant Activities of Purified CA 19-9 and CA 125 Ascitic Fluid Glycoproteins

The purified ascitic fluid glycoproteins (S-400 void volume fractions, and S-400 plus WGA eluted fractions) of AI and MI were separately adsorbed to the wells of polystyrene plates and incubated with antibodies specific for the determinants listed in Table 4. As before, the A and the Le^a, Le^b, Le^x and Le^y activities co-adsorbed with either the CA 19-9 or CA 125 activities. In addition the glycoprotein preparation exhibited a relatively strong reactivity to one of the anti-Le^x monoclonal antibodies (H162). Carbohydrate sequences detected by the anti-type 1 chain antibodies (H112 and H131), and the type 2 chain (I or i), or the H determinant reactive antibodies were not significantly expressed in the glycoprotein fractions. The sialylated-Le^x determinant was also expressed strongly in the AI glycoprotein fractions and was present, but with a weaker reactivity, in the MI glycoprotein fractions.

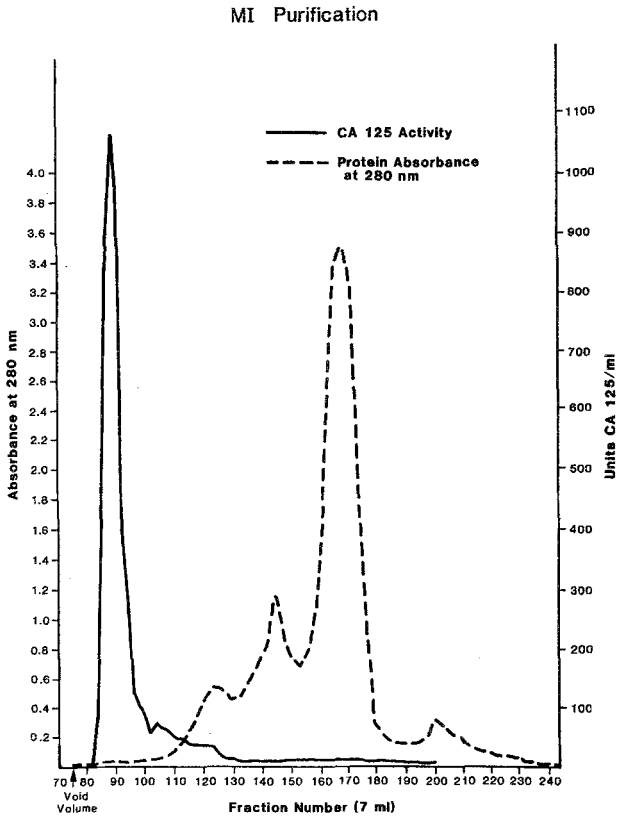

Figure 2. Gel chromatography of MI ascitic fluid using Sephacryl S-400. Fractions were monitored for protein by absorbance at 280 nm (- - -) and for CA 125 activity (—).

Discussion

Carcinoembryonic antigen (CEA) is among the most extensively studied tumor-associated glycoprotein antigens and assays for CEA and monoclonal antibodies to CEA are commonly used in the management of certain carcinoma patients [36, 37]. During the last several years a number of other tumor associated glycoproteins have been isolated and purified from serum and/or ascitic fluids obtained from patients with different types of cancer. In contrast to CEA, for which the antigenic determinants are known to consist of amino-acid sequences and conformations, the antigenic determinants of these other glycoproteins consist, in part, of carbohydrate sequences. Immunoassays for some of these glycoproteins indicate a possible role as diagnostic and/or prognostic cancer indicators [38]. Among the better characterized of these immunoassays are the CA 19-9 [6, 17], DU-PAN-2 [39, 40], Le^x and

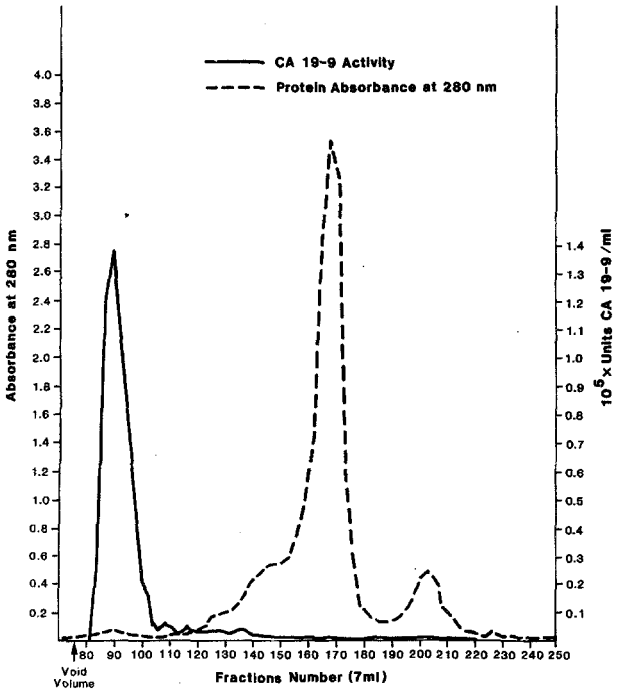

Figure 3. Gel chromatography of AI ascitic fluid using Sephacryl S-400. Fractions were monitored for protein by absorbance at 280 nm (- - -) and for CA 19-9 activity (—).

sialylated-Le^x [41-43] for gastrointestinal cancers; F36/22 [44, 45], B72.3 [36, 46] and HMFG1 and HMFG2 (human milk fat globule 1 and 2) [47] for breast cancer; and CA 125 [18-20] and MoV2 [48] for ovarian cancer. It is not yet clear whether the CA 125 epitope is protein or carbohydrate in nature, or a combination of both, although the results of Davis *et al.* [33] show that the epitope is, at least in part, conformationally peptide dependent in its reactivity with anti-CA 125 antibody.

We report here that high molecular weight glycoprotein fractions of ascitic fluids contain a number of tumor-associated antigenic determinants and that large amounts of the glycoproteins can be readily isolated. We initially screened ascitic fluids for the CA 19-9 and CA 125 determinants and found a number (16%) with high activities. Almost all of the CA 19-9 and CA 125 activities in the AI and MI ascitic fluids were found in the void volume from

Table 4. Carbohydrate sequence determinants expressed in AI and MI ascitic fluid fractions after Sephacryl (S-400) chromatography or S-400 followed by adsorption and elution from WGA-Sepharose (S-400, WGA).

	AI (CA 19-9)		MI (CA 125)	
Antibody	(S-400, WGA)	(S-400)	(S-400, WGA)	(S-400)
Anti-A	1568[a]	1210	952	380
Anti-B	0	0	3	0
Anti-H	20	20	18	50
Anti-Le^a	1530	1870	679	150
Anti-Le^b	1290	860	350	50
Anti-Le^x	325	500	127	45
Anti-Le^y	384	450	288	390
Anti-sialylated-Le^x	2102	1380	260	101
Anti-Le^x (H124)	132	20	29	7
Anti-Le^x (H162)	1358	2200	322	35
Anti-I	14	n.d.[b]	62	n.d.
Anti-i	0	n.d.	0	n.d.
Anti-Type 1 (H112)	11	10	27	14
Anti-Type 1 (H131)	6	30	16	5
Anti-P_1	0	n.d.	0	n.d.
Anti-P^k	0	n.d.	0	n.d.

[a] Numbers are fluorescent intensity units of the ELFA assay as described in the text.
[b] n.d. = not determined.

a Sephacryl S-400 column. Fractionation by HPLC of the WGA bound glycoproteins from ascitic fluids also showed that the active fractions were of high molecular weight. These results are similar to those found for the serum CA 19-9 antigen which elutes in the void volume of a Sephacryl S-400 column [10] and for DuPan-2 antigen of ascitic fluid which partially chromatographs in the void volume of a Sepharose CL-2B column [40].

The specific activity (activity units of either CA 19-9 or CA 125/protein) of the Sephacryl S-400 volume fractions was 4200 fold greater than the original ascitic fluids. Thus, this simple chromatography step yielded considerably purified fractions containing these two tumor-associated determinants.

When ascitic fluids were passed through lectin-Sepharose columns and the lectin-bound material eluted with particular sugars, only the WGA lectin effectively bound most of the CA 19-9 or CA 125 activities. The A, Le^a, and Le^b blood group activities, as well as the Le^x and Le^y activities, were bound by the *Dolichos biflorus*, *Helix pomatia* and *Ulex europaeus*

lectins, and to some extent by the *Glycine max* lectin in addition to the WGA lectin. Also, the sialylated-Le^x activity was bound to some extent by lectins other than WGA. Examining the data shown on Table 2 we can suggest that Con A, RCA-I, and LPA lectin bound little of the glycoprotein(s) for the activities tested and that therefore the particular determinants for which those lectins bind are not expressed or accessible on those glycoproteins. These lectins, therefore are not useful for purification and/ or characterization of such glycoproteins from ascitic fluid.

Because the Sephacryl S-400 void volume glycoprotein fraction contained all of these activities, the lectin binding results suggest that the fluids contain a mixture of glycoproteins with differing expressions of the various carbohydrate sequences. These results confirm the extensive heterogeneity of blood group glycoproteins described previously by other investigators [49] and that ascitic fluid glycoproteins are a good source for the carbohydrate epitopes expressed on these blood group glycoproteins as well as the other tumor-associated antigen epitopes CA 19-9, CA 125 and sialylated-Le^x. Conceivably the glycoproteins from Sephacryl S-400 could be further fractionated by lectin affinity chromatography but such experiments were not attempted, except with WGA. The results from Table 4 show that the Sephacryl S-400 and S-400 plus WGA fractionated glycoprotein preparation contained little or no activities for the type 1 or 2 chains. Thus, the carbohydrate sequences of these glycoproteins are "complete" in the sense that these precursors (Types 1 and 2 and H) are limited in expression and the oligosaccharide chains are highly fucosylated or sialylated.

Acknowledgements

This work was partially supported by the Donald P. Moore Postdoctoral Fellowship from the Cancer Research Institute to Lyman E. Davis.

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