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Isolation of CEA-like Material from Urinary Bladder Carcinoma

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Summary. Carcinoembryonic antigen (CEA)-like material from urinary bladder carcinoma was purified and compared with the immunological and physico-chemical properties of CEA isolated from colon cancer. After immunoadsorbent purification, the final step in a purification procedure similar to that adopted for colon cancer CEA, two main molecular species were identified: 1) Material identical with colon cancer CEA with respect to molecular size, PCA solubility, ability to bind to Con A, and most important the ability to bind to specific monkey anti-CEA serum. 2) Material with the same chromatographic and immunological properties as NCA.

Key words: Carcinoembryonic antigen (CEA) - Local urinary bladder carcinoma - Nonspecific cross-reacting antigen (NCA).

Patients with urothelial carcinoma show increased levels of Carcinoembryonic antigen (CEA) activity in serum and urine (1, 6). Determination of CEA-levels in patients with urinary bladder carcinoma is considered to be of clinical value in evaluating regression or progression of disease in response to treatment (7).

Urinary CEA-activity from bladder carcinoma patients and normals shows considerable size heterogeneity (5, 8). CEA-active material from bladder carcinomas has not yet been isolated and characterised.

The purpose of this investigation was to purify CEA-like material from urinary bladder carcinoma and to compare its immunological and physico-chemical properties with colon cancer CEA. Immunological differences between CEA molecules of different tumour origin may be used to improve the disease-related specificity of the clinical CEA-assays.

MATERIAL AND METHODS

Purification

Local recurrent transitional cell carcinoma of the urinary bladder from two individuals were obtained at autopsy and pooled. Tumour tissue was dissected free from normal tissue (460 g wet weight) and homogenised in water. The homogenate was precipitated with perchloric acid (PCA) (1 M, final concentration) and the PCA-soluble fraction was then neutralised, dialysed and lyophilised. The material was then fractionated on a Sepharose 4B column (90 x 5 cm) in 0.05 M NaH_2PO_4, pH 5.0 containing 0.15 M NaCl. CEA-activity was monitored by radioimmunoassay using the Hoffmann-La Roche Z-gel kit. Figure 1 shows the elution profile. Three CEA-active fractions (I, II and III) were collected. Each fraction was then passed over a Concanavalin A-Sepharose affinity chromatography column and eluted with methyl- α-D-mannoside (10 and 50 mg/ml). All bound material was eluted at the lower sugar concentration. The eluted fractions were finally fractionated on an anti-CEA-immunoabsorbent column prepared by conjugating the IgG-fraction of a spleen absorbed sheep-anti-CEA serum to cyanogen bromide activated Sepharose 4B (2). Bound material was eluted with 6 M guanidine HCl, dialysed and lyophilised. In order to analyse the small amounts of material eluted from the immunoadsorbent column the materials (fractions IB b, IIB b and IIIB b) were labelled with $Na^{125}I$ using the chloramine T-method (2). See Table 1 for fraction designation.

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Table 1. Purification of CEA from primary bladder cancer

		Dry weight mg	Specific activity µg CEA/mg	Total activity µg CEA
PCA-soluble		521	0.23	117.2
Sepharose 4B	I	60	1.54	92.4
	II	119	0.32	38.7
	III	73	0.65	47.5
Con A-Sepharose	IA	40	0.78	31.1
	IB	7.2	6.00	43.2
	IIA	79	0.12	9.5
	IIB	24	0.70	16.9
	IIIA	59	0.05	2.8
	IIIB	6.2	2.40	14.9
Anti-CEA-immuno-absorbent	IB a	5.1	<0.03	<0.15
	b	-	>75	7.5
	IIB a	17	<0.03	<0.5
	b	1.1	1.46	1.60
	IIIB a	4.8	<0.03	<0.15
	b	-	>55	5.5

Reference CEA, Anti-CEA and Anti-NCA Sera

Reference CEA was purified from liver metastases of colo-rectal cancer by a five step purification procedure described previously (3). Anti-CEA sera were raised in rabbits, sheep and monkeys (2). Rabbit and sheep anti-CEA sera were absorbed with A+B erythrocytes and PCA-extract of human spleen and were found not to precipitate with purified Nonspecific Crossreacting Antigen (NCA) or Biliary Glyco-Protein I (BGP I) (2) or with PCA extract of normal lung, spleen or colon. Immunosorbent purified monkey anti-CEA antibodies were prepared from a pool of unabsorbed anti-CEA serum from 4 monkeys (4). No cross reaction with NCA or BGP I, was seen at 10^3 times higher concentration than was needed for 50% inhibition with CEA using an enzyme-linked immunoadsorbent assay (4). Anti-NCA serum was obtained from rabbits injected with semi-

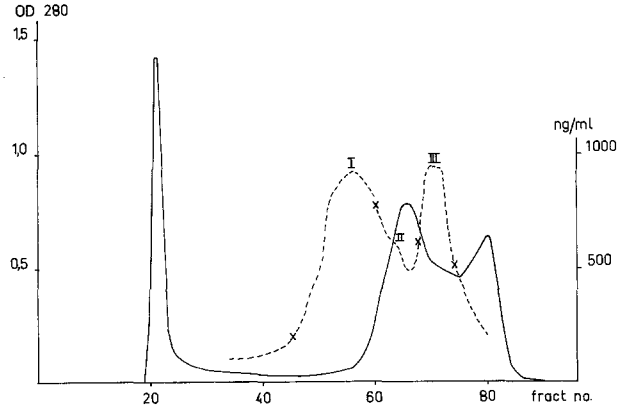

Fig. 1. Gelfiltration of the PCA-soluble fraction of bladder tumour homogenate on a Sepharose 4B column. Solid line: optical density at 280 nm. Broken line: CEA concentration in ng/ml

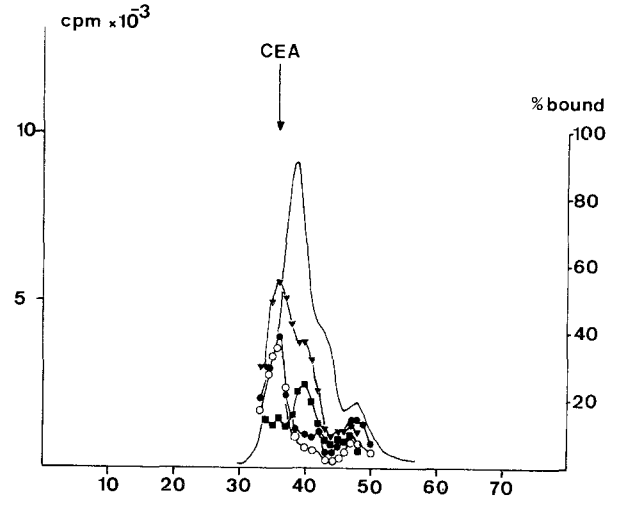

Fig. 2. Gelfiltration of ^{125}I-labelled fraction IB b (Table 1) on a Sephacryl S-200 column. Solid line: CPM/200 µl. (▼—▼) % radioactivity precipitated by unabsorbed rabbit anti-CEA serum. (●—●) % radioactivity precipitated by spleen and erythrocyte absorbed rabbit anti-CEA serum. (○—○ % radioactivity precipitated by monkey anti-CEA serum. (■—■) % radioactivity precipitated by specific rabbit anti-NCA serum

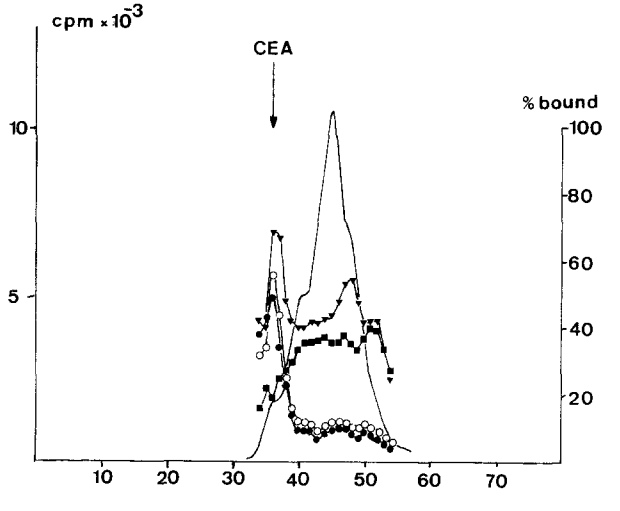

Fig. 3. Gelfiltration of ^{125}I-labelled fraction IIIB b (Table 1) on a Sephacryl S-200 column. Symbols as in Figure 2

purified spleen NCA and absorbed with PCA extract of one colon tumour with very low CEA and NCA content (3). The specificity of the antiserum for NCA has been documented (3).

Analytical Methods

The ability of anti-CEA and anti-NCA serum to precipitate ^{125}I-labelled purified CEA-like material from bladder carcinoma fractionated on a Sephacryl S-200 column (90 x 1 cm) was determined. Antibody bound material was separated from nonbound material either by the addition of sheep-anti-rabbit IgG covalently bound to cellulose particles or by the addition of carrier IgG and saturated $(NH_4)_2SO_4$ (monkey anti-CEA antibodies).

Size heterogeneity and molecular weights of purified CEA-like material from bladder carcinoma was determined by gradient polyacrylamide gel electrophoresis in sodium dodecylsulphate after complete reduction using a discontinuous buffer (2). The analyses were performed with ^{125}I-labelled material using autoradiography. Molecular weights were determined from the mobility of standard proteins (slope calculated by linear regression).

RESULTS

The recovery, specific activity (µg CEA-equivalents/mg material) and total CEA-equivalent activity obtained during purification of CEA-like material from bladder cancer are summarised in Table 1. The Hoffmann-La Roche Z-gel kit was used to determine CEA activity. Figure 1 shows the elution profile on Sepharose-4B gelfiltration of the PCA-soluble fraction of bladder tumour. Several points are of interest: i) the CEA-content of bladder tumours is very low. Only about 0.2 % of the dry weight of the PCA-soluble fraction appears to constitute CEA. CEA-measurement of 4 other individual bladder tumours gave values of 100-250 µg CEA-equivalents/kg tumour wet weight. The values should be compared with 25-100 mg CEA/kg tumour wet weight for liver metastases from colo-rectal cancer. ii) two peaks of CEA-activity were obtained when the PCA-extract of bladder tumour was fractionated on Sepharose-4B. iii) the specific activity increased during purification. Thus, the PCA-soluble fraction contained 0.23 µg CEA-equivalents/mg, Sepharose-4B fraction I 1.54 µg CEA-equivalents/mg, Con A-Sepharose eluted fraction (IB) 6.0 µg CEA-equivalents/mg and anti-CEA immunoadsorbent eluted fraction (IB b) >75 µg CEA-equivalents/mg. The dry weight of the latter fraction was too small to be determined accurately but was less than 100 µg. Increase in specific activity during purification was also observed for the other two Sepharose-4B fractions. iv) no CEA-active material (< 0.03 µg CEA-equivalents/mg) passed the anti-CEA immunoadsorbent column.

Figures 2 and 3 show the elution profiles on Sephacryl S-200 of immunoadsorbent purified bladder cancer CEA fractions (IB b and IIIB b, Table 1). The materials were labelled with ^{125}I in order to enable detection. As can be seen from the radioactivity profiles both fractions are heterogeneous with respect to molecular size indicating several populations of macromolecules. The S-200 fractions were analysed for material reacting with anti-CEA and anti-NCA sera using either anti-IgG antibodies or $(NH_4)_2SO_4$ to precipitate antibody bound material (Figures 2 and 3). The following results were obtained: i) both fractions contained macromolecules precipitating with specific anti-CEA sera (monkey anti-CEA and spleen absorbed rabbit anti-CEA serum) which eluted at the same position as reference CEA (arrow in Figures 2 and 3). However, only about 16 and 14% respectively of the total radioactivity of fractions IB b and IIIB b were precipitated by the specific CEA antisera. ii) specific anti-NCA serum precipitated about 15% of the radioactivity in fraction IB b and about 35% in fraction IIIB b. Anti-NCA reactive material showed considerable size heterogeneity. iii) unabsorbed anti-CEA serum precipitated a higher percentage of the labelled material in each fraction then either of the specific antisera alone. In fact the percentage of radioactivity precipitated by unabsorbed anti-CEA serum corresponded closely to the sum of the radioactivity precipitated by specific anti-CEA and anti-NCA serum. iv) 30% of the total radioactivity in fractions IB b plus IIIB b could be accounted for as anti-CEA precipitable material. Total precipitable material with anti-NCA antiserum was 50%.

SDS-PAGE analyses of ^{125}I-labelled immunoadsorbent purified bladder cancer CEA (fractions IB b and IIIB b) revealed the presence of several components in each fraction. Fraction IB b contained bands with the following apparent molecular weights: 175,000; 115-150,000 broad zone; 74,000 and 53,000. Fraction IIIB b contained the following bands: 175.000; 115-140.000 broad zone; 74,000; 53,000; 44,000 and 25-27,000. Colon cancer CEA and the CEA-related normal adult components, NCA and BGP I, gave apparent molecular weights of 175,000 ± 3,000; 115-125,000 and 83,000 ± 4,000 respectively when analysed by this method (2).

DISCUSSION

This study shows that small amounts of CEA-like material can be purified from bladder carcinoma using a purification procedure similar to that adopted for colon cancer CEA. The final product after immunoadsorbent purification appeared to be a mixture of several molecular species. Two species were tentatively identified: 1) material identical with colon cancer CEA with respect to molecular size (gel filtration and SDS-PAGE), PCA solubility and ability to bind to Concanavalin A and most importantly the ability to bind to specific monkey anti-CEA serum. This antiserum was shown not to react with CEA-related normal components even when assayed with a highly sensitive enzyme linked immunoadsorbent assay (4). 2) material with the same chromatographic and immunological properties as NCA.

It may seem surprising that NCA was isolated on the anti-CEA immunoadsorbent since the antiserum used was heavily absprbed with spleen tissue in order to remove NCA-crossreactive antibodies. The antiserum was furthermore shown to be CEA specific by immunodiffusion. The finding probably illustrates the difficulty in removing the last traces of crossreactive antibodies by absorption. It should be noted that only a few µg of NCA were isolated.

In addition to the two components tentatively identified the purified fractions contained four additional components with lower molecular weights. From the precipitation experiments (Figures 2 and 3) it seems likely that they are non-CEA related substances.

Further analyses are needed in order to establish whether the molecular species tentatively identified as CEA is in fact identical with colon cancer CEA. Such studies are in progress. Furthermore, considering the low amounts of CEA-active material isolated from bladder carcinoma, it seems important to establish the tumour origin of the isolated material. Studies on bladder carcinoma cell lines may resolve this question.

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