ISOLATION AND PURIFICATION OF CARCINOEMRYONIC ANTIGEN– LIKE MATERAL FROM BLADDER CARCINOMA[+]

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Abstract

This investigation is designed to purify carcinoemyonic Antigen(CEA)-like material from urinary bladder carcinom and to compare its immunological and physico – chemical properties with colon cancer CEA.

The results of this study shows that small amounts of CEA- like material can be purified from local bladder carcinomas using a purification procedure similar to that adopted for colon cancer (CEA).

المستخلص

تم في هذا البحث عزل وتنقية مادة شبيهة بالمستضد السرطان الجيني من اورام سرطانية للمثانة ومقارنة صفاته المناعية والفيزوكيمياوية بالمستضد السرطان الجنيني المعزول من اورام القولون.

اكدت النتائج المستحصلة ان كميات قليلة يمكن تنقيتها من الاورام السرطانية للمثانة باستخدام طريقة تنقية مشابهة لتلك المستخدمة في عزل وتنقية المستضد السرطاني الجنيني من القولون.

Introduction

Carcinoemyronic Antigen (CEA) is found on the cell surface membrane and is easily released into surrounding fluids. It is now apparent that CEA or CEA- like substances are also present in low levels in nonneoplastic, nonfetal tissue[1,2], including normal colonic mucosa, lung, and lactating breast tissue[3]. Since physiochemical of CEA has been observed, it appears that CEA represents a group of CEA substances that are cross- reactive[4,5] .

This heterogeneity has been cited as the cause of variation between the immunologic assays manufactured for detection of CEA, such that titers obtained by one assay method are not directly comparable to those obtained by another method[4,5,6].

Patients with urothelial carcinoma show increased leves of CEA activity in serum and urine[7,8,9,10,11].

Material and Methods

Purification: local recurrent transitional cell carcinoma of urinary bladder from two individuals were obtained at autopsy and pooled. Tumor tissue (460 g wet weight) was homogentzed in water and precipitated with prechloric acid (1M, final

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concentration). The PCA soluble 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 CEA kit provided by Byksangetec Diagostica GMbH & Co. KG/ France (Figure 1). Three CEA- activity pools. (1,11, and III) were collected. Each pool was then passed over a concanvalin in 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 immunoadsorbent column prepared by conjugating the IgG- fraction of a spleen absorbed sheep anti CEA serum to cyanogens bromide activated Sepharose $4B^{(2)}$. Bound material was eluted with 6M guaniding Hcl, dialysed and lyophilized. Material eluted from the immunoadsorbent column (fractions 1Bb, IIBb and IIIBb) was labeled with Na ^{125}I using the chloramines T- Method[12].

Reference CEA, anti – CEA and anti- NCA sera: Reference CEA was purified from liver metastase of colo-rectal cancer as earlier described [13]. Rabbit and sheep anti- CEA sera were absorbed with A+B erythrocytes and perchloric acid (PCA)-extract of human spleen and were found not to precipitate with purified Non specific cross reacting antigen (NCA) or Blood group patient (BGP) [14] or with PCA extract of normal lung, spleen or colon. Immunosorbent purified sheep- anti- CEA antibodies were prepared from a pool of four unabsorbed sheep anti CEA serum [3] . No crossreaction with NCA or BGPI was seen at 10^3 times higher concentration than was needed for 50 % inhibition with CEA using an enzyme linked immunoadsorbent assay [15]. Anti- NCA serum with PCA extract of one colon

Analytical Methods: The ability of anti –CEA and anti- NCA serum to precipitate ^{125}I- labeled purified CEA- like material from bladder carcinoma fractionated on a sephacryl S-200 column (90x1cm) was determined. Antibody bound material was separated from nonbound material either by the addition of sheep anti rabbit IgG covalently bound to cellulose particales or by the addition of carrier IgG and saturated $(NH4)_2SO4$.

Size heterogeneity and molecular weights were determined by gradient polyacrylamide gel electro-phoresis in sodium dodecylsulphate after complete reduction using a discontinuous buffer system[14]. The analyses were performed with$^{[125]}$I-labeled material using autoradiography. Molecular weight was determined from the mobility of standard proteins (slope calculated by linear regression).

Results

The recovery, specific activity (*mg* CEA- equivalent / mg material) and total CEA- equivalent activity obtained during purification of bladder cancer “CEA” are summarized in Table. 1. Figure 1 shows the elution profile on Sepharose 4B gelfiltration of the PCA- soluble fraction of bladder tumor. Several points are of interst: i) the CEA- content in bladder tumors 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 tumors gave values of 100 – 250 *mg* CEA- equivalent / Kg tumor wet weight. The values should be compared with those of 25-100 mg CEA / Kg tumor wet weight for liver metastases of colo-rectal cancer. ii) two peaks of CEA activity were obtained when the PCA- extract of bladder tumor was fractionated on

Sepharose 4B. iii) the specific activity increased during purification. Thus, the PCA-solube fraction contained 0.23 ng CEA- equivalents / mg. Sepharose 4B fraction 1 1.54ng CEA- equivalents/mg, con A-Sepharose eluted fraction (1 B) 6.0 ng CEA-equivalents / mg and anti-CEA immunoadsorbent eluted fraction (1 B b)> 75ng CEA – equivalents / mg. The dry weight of the latter fraction was too small to be determined accurately but was less than 100ng. Increase in specific activity during purification was also observed for the other two sepharose 4B fractions . iv) no CEA-active material (< 0.03 ng CEA equivalents / mg) passed the anti- CEA immunoadsorbent column.

Table (1) : Purification of CEA from primary bladder cancer

		Dry weight mg	**Specific activity** *mg* **CEA / mg**	**Total activity** *mg* **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	31.2
	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-adsorbent	IB a	5.1	< 0.03	< 0.15
	B	-	-	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	-	-	5.5

Figures 2 and 3 show the elution profiles on Sephacryl S-200 of immunoadsorbent purified bladder cancer “CEA” fractions (1Bb and III Bb, Table I). The materials were labeled with $^{[125]}$I in order to allow detection. As can be seen from the radioactivity profiles both fractions are heterogeneous with respect to molecular size indicating several populations of macromolecles. The S-200 fractions were analysed for material reacting with anti –CEA and anti- NCA sera using either anti

rabbit IgG antibodies or ($NH_4)_2$ SO_4 to precipitate antibody bund material (Figures 2 and 3). The following results were obtained : i) both fractions containing macromoleciles precipitating with specific anti CEA sera (sheep anti – CEA and spleen absorbed rabit anti- CEA serum) which eluted at the same position as reference CEA. However, only about 16 and 14% respectively of the total radioactivity of fractions IB b and IIIBb 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 IIIBb. Anti- NCA reactive material showed considerable size heterogeneity. iii) unabsorbed anti- CEA serum precipitated a higher percentage of the material in each fraction than 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.

SDS- PAGE analyses of $^{[125]}$I – labeled fractions IBb and IIIBb revealed the presence of several components in each fraction. Fraction IB b contatained 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 band: 175,000; 115 – 140,000 broad Zone; 74,000; 53,000; 44,000 and 25 – 27,000. Colon cancer CEA and CEA- related normal adult components, NCA and biliary glycoprotein I (BGPI) gave apparent molecular weight of 175,000 ± 8,000; 115 – 125,000 and 83,000 ± 4,000 respectivitely when analysed by this method [2].

Discussion

This study shows that the final product after immunoadsorbent purification appears to be mixture of several molecular species. Two species were tentatively identified: 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 sheep 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[15] material with the same chromato graphic and immunological properties as NCA.

It may seem suprising that NCA was isolated on the anti- CEA immunoadsrbent since the antiserum used was heavily absorbed 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 absorbtion. It should be noted that only a few ng of NCA was isolated.

In addition to the two components tentatively identified the purified fractions contained four additional components with lower mole cular weights. From the precipitation experiments (Figure 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. Considering the low amount of CEA – active material isolated from bladder

carcinoma it seems furthermore important to establish the tumor origin of the isolated material. Studies on bladder carcinoma cell line may resolve this question.

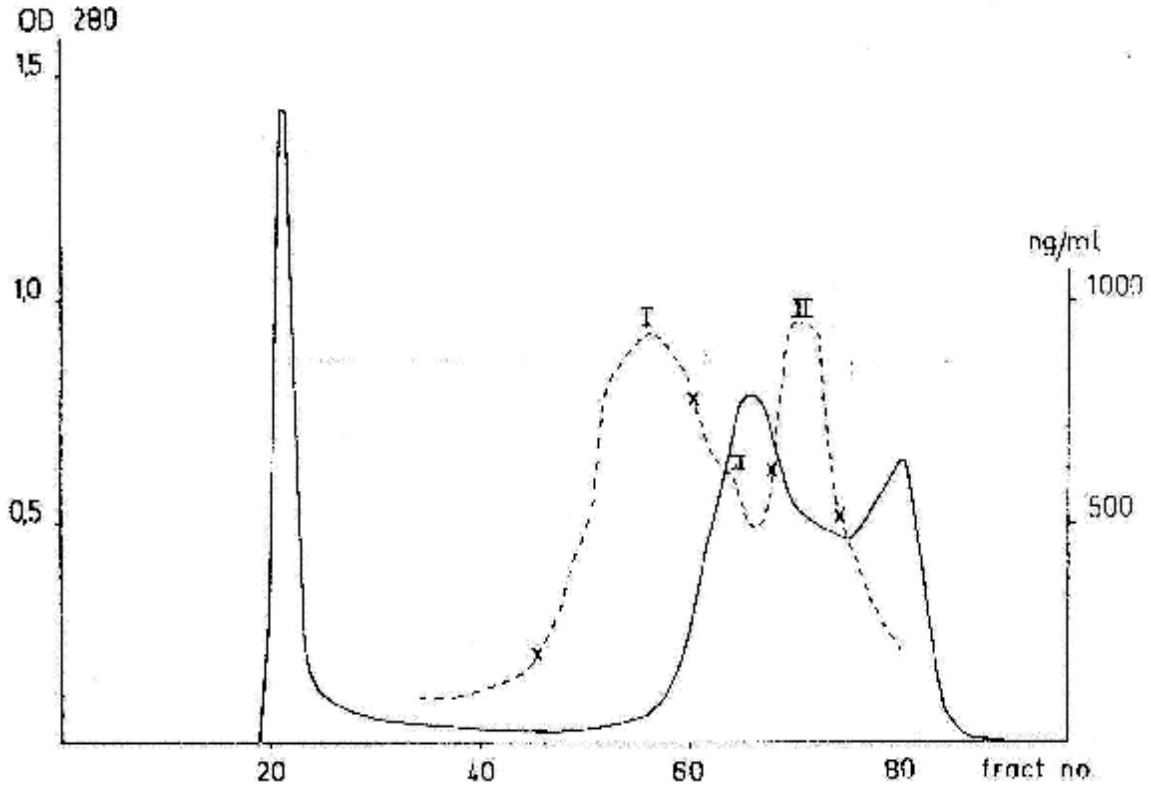


Fig. 1 Gelfiltration of the PCA-soluble fraction of bladder tumor ho-mogenate on a Sepharose 4B column.
Solid line: optical density at 280 nm. Broken line: CEA concentration in ng/ml.

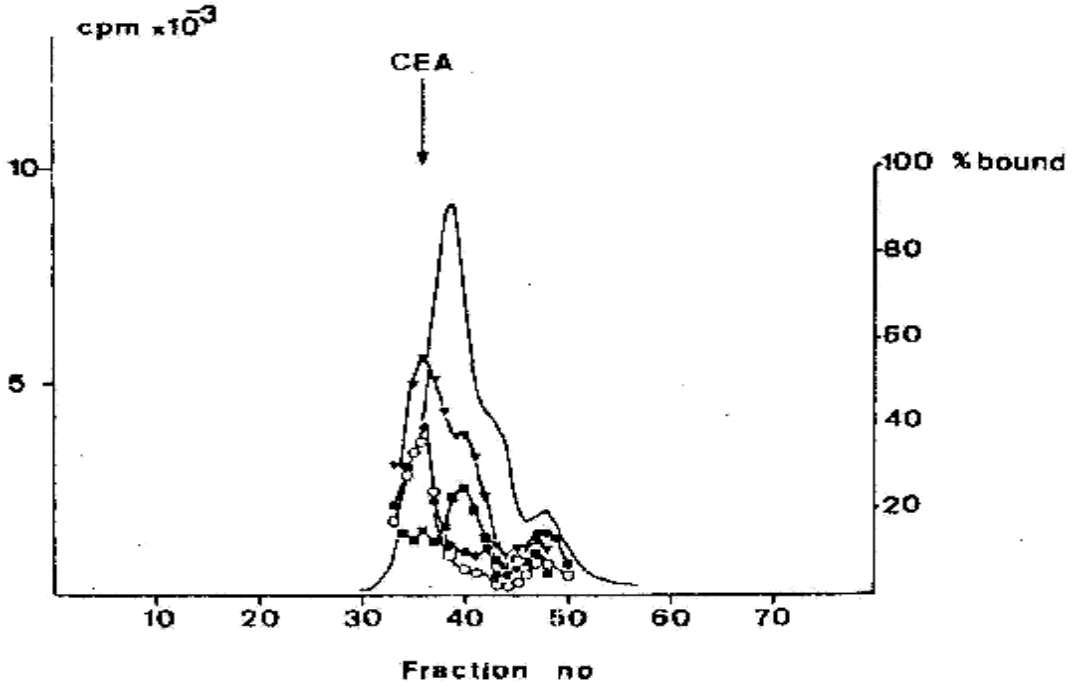
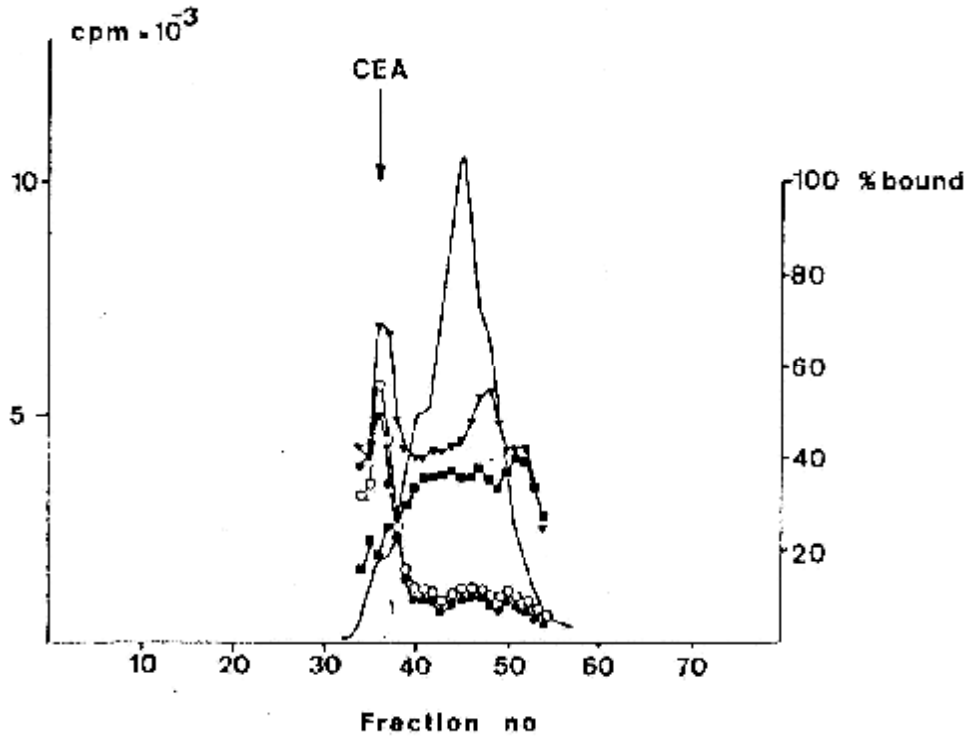


Fig. 2 Gelfiltration of 125I-labelled fraction IB b (Table I) on a Sephacryl S-200 column.
Solid line: CPM/200 ul. (▼ -▼) % radioactivity precipitated by unabsorbed rabbit

anti-CEA serum. (O--O) % radioactivity precipitated by spleen and erythrocyte absorbed rabbit anti-CEA serum. (0--0) % radioactivity precipitated by sheep anti-CEA serum. (■--■) %, radioactivity precipitated by specific rabbit anti-NCA serum.



Fi.g. 3 Gelfiltration of 125I-labelled fraction IIIB b (Table I) on a Sephacry 1 S-200 column. Symbols as in Fig. 2.

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