ISOLATION OF CA15-3 FROM BENIGN AND MALIGNANT BREAST TISSUES HOMOGENATES AND STUDY THE OPTIMUM [+]CONDITIONS OF THE BINDING WITH ^{125}I-ANTI-CA15-3 ANTIBODY

عزل CA15-3 من مجانسات انسجة الثدي الحميدة والخبيثة ودراسة الظروف المثلى لارتباطه مع الضاد المتخصص ^{125}I-anti CA15-3

Salwa H.N.Al-Rubae'I * **Sami A. AL-Mudhaffar** ** **Raad K. Muslih***

Abstract:

Gel filtration chromatography technique was used for partial purification of CA15-3 from benign and malignant breast tumors homogenates.

The results revealed the presence of one form of CA15-3 with a high molecular weight (440 KD). This type possesses a high affinity for the binding to its antibody ^{125}I-anti CA15-3. The experiments of optimum conditions of the binding between the partially purified CA15-3 and ^{125}I-anti CA15-3 antibody were determined, in benign breast tumor and malignant breast cancer homogenates.

Studies on the stability of both partial purified CA15-3 and crude CA15-3, show that the crude CA15-3 was more stable than the purified CA15-3.

المستخلص:

استخدمت تقنية الترشيح الهلامي للتنقية الجزئية للـ CA15-3 من مجانسات اورام الثدي الحميدة والخبيثة. اشارت النتائج الى وجود قمة واحدة ذات وزن جزيئي عالي(٤٤٠ كيلو دالتون) وتملك الفة ارتباط عالية تجاه الضاد المتخصص ^{125}I-anti CA15-3 antibody . تم تعيين الظروف المثلى لتجارب الارتباط بين الـ CA15-3 المنقى جزئيا من مجانسات اورام الثدي الخبيثة والحميدة مع الضاد المتخصص ^{125}I-anti CA15-3 antibody في مجانسات اورام الثدي الحميدة والخبيثة .اظهرت الدراسة ان استقرارية الـ CA15-3 غير المنقى اكثر من اسقرارية الـ CA15-3 المنقى جزئيا.

Introduction:

Serological tumor markers CA15-3 has been used for decades to monitor treatment response in patients with metastatic breast cancer [1]. Other studies isolated CA 15-3 by using

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[*]Assistant Professor in Chemistry Department, College of Science, Al-Mustansiriya University.
[**]Professor in Chemistry Department, College of Science, Baghdad University

Human carcinoma-associated and salivary mucins that detected by anti - bovine submaxillary mucin antibodies [2]. CA15-3 is high molecular weight glycoprotein (>400 KD) identified at the apical side of alevoli and duct of mammary glands [3]. Several authors have isolated, purified and characterized CA15-3 from different sources; either by the isolation of CA15-3 from a breast cancer patient's sera, using affinity chromatography, gel filtration, and then characterized by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) [4,5],or by purifying a high molecular weight glycoprotein from human milk and breast carcinoma by using gel filtration, affinity chromatography and then PAGE [6]. In the present study, benign breast tumors and premenopausal malignant breast cancer were used as a source for partial purification of CA15-3 and then determination its yield. The factors effects on the binding of partial purified CA15-3 to its antibody ^{125}I-anti CA15-3 antibody were also studied.

Materials and Methods:

Chemicals

All chemicals and reagents used in this study were of analytical grade, Immunoradiometric assay kit for CA 15-3 level from Diasorin Inc. (USA).Bovine serum albumin (BSA), ethylendiamine-tetraaceticdisodium salt (EDTA), NaN_3 from Fluka:(Switzerland). $CuSO_4.H_2O$, NaK-tartarateglycine, NaOH, HCl, $NaCO_3$, Na_2HPO_4, NaH_2PO_4 from BDH,limited,Poole (UK). Folin-Ciolteau from E. Merck AG. Dastmstapt, finally Blue dextran (2000), and sepharose CL-4B from Pharmacia fine chemicals (Sweden).

Instruments

Gamma counter type 1270-rack gamma II , Spectrophotometer ultra space type 4050 and Combicold rack were from LKB, UV-210 a double beam spectrophotometer from Shimadzu. pH-meter from Pye-Unicam. Cooling centrifuge; with a maximum speed 5000 r.p.m. from Hettich. Cooling centrifuge type 202-MK; with a maximum speed 13500 r.p.m. from Sigma. Memmert water bath, memmert incubator from West Germany. SM-shaker from England.

Patients

Three groups of breast tumors patients were included in this study.
Group I : Consisted of 40 patients with benign breast tumors
Group II : Consisted of 32 premenopausal patients with breast cancer.
Group III : Consisted of 15 postmenopausal patients with breast cancer.
Group IV : Consisted of 10 controls.

All patients were admitted for treatment to (Baghdad Teaching Hospital), (University Hospital, Al-Nahrain College of Medicine), (Nursing Home Private Hospital) and (Al-Arabi Private Hospital). Patients suffered from any disease that may interfere with this study were excluded. All surgical operation of breast tumors were carried out under the supervision of the following surgeons:Dr. Saab Sedeq, Dr.Munthir Al-Aubaidi, Dr.Azam Qanbar Agha, Dr. Abd Al-Salam Al-Tai, Dr. Zuhair Abid Al-Hadi. The host information of all patients and normal healthy subjects is summarized in table (1).

Table (1): The host information of breast tumors patients and healthy subjects studied.

Group	Patients	No.	Age	Type of tumor	Metastases
I	Benign breast tumor	40	18-42	23 fibroepithelial tumor (fibroadenoma) 17 fibrocystic changes (adenosis)	– –
II	Premenopausal malignant breast tumor	32	34-52	22 Infiltrative Ductal carcinoma 10 Ductal carcinoma	2 lymph nodes
III	Postmenopausal malignant breast tumor	15	55-73	Infiltrative Ductal carcinoma	4 lumph nodes
IV	Control	10	25-40		

Collections of Specimens

The tumors tissues were surgically removed from breast tumor patients by either mastectomy (cancer patients) or lumpectomy (benign tumor patients). The specimens were cut off and immediately rinsed with ice-cold isotonic saline solution. They were collected individually in plastic receptacle and stored at –20 ^oC until homogenization.

PBS–Buffer

Phosphate –buffered saline (PBS) 0.05 M, PBS buffer pH 7 containing 0.02% sodium azide was prepared as following:

A: Disodium basic phosphate (0.5M); 7.0980g Na_2HPO_4 and 9.0g of NaCl were dissolved in a final volume 1L deionized distilled water.

B:.Monobasic sodium phosphate (0.15M) 5.9990g of NaH_2PO_4 and 9.0g NaCl were dissolved in a final volume 1L deionized distilled water.

Phosphate buffer saline pH 7 was prepared by mixing a volume of solution A with appropriate amounts of solution B to obtain the required pH.

Preparation of Breast Tumors Tissue Homogenates

The frozen tissue were weighed, sliced finely and scalped in Petri dish standing on ice bath, and then homogenized with fivefold volumes of PBS buffer pH7.2, using manual homogenizer [7]. The homogenate was filtered through four layers of nylon gauze in order to eliminate fibers connective tissues, and then centrifuged at 4000 r.p.m for 45 min. at 4 ^{o}C in order to precipitate the remaining intact cells and the intact nucleus. The supernatant fraction at this speed was separated, divided in aliquots and freeze -20 ^{o}C until use.

Methods

A- Isolation of CA15-3 by Sepharose CL-4B Column

Preparation of the Column

The dimensions of the column were chosen according to the following equation [8].

Diameter= $\sqrt{\frac{m}{10}}$ Where: m = amount of protein in mg.

L = 30 x diameter **Where: L : length of the column**

Preparation of the Gel

The gel was prepared by allowing the pre-swollen gel to swell again in PBS buffer (0.05 M) pH 7.0, then left to settle and the excess of buffer was decanted. The step was repeated several times. Suction was then used to degas the gel and slurry was left for 24 hrs to equilibrate with buffer. The swollen gel was suspended and carefully poured into a vertical glass-column (0.7 x 30 cm) down the wall using a glass rod. After the gel has settled, the column was equilibrated with PBS for 24 hrs [8].

Void Volume Determination

The void volume of the column was determined by using blue dextran 2000 at concentration of 2 mg.mL^{-1} dissolved in PBS buffer pH 7.0 , then the elution was carried out with the same buffer at a flow rate of 20 mL.hrs^{-1}. Fractions of 2 mL were collected and their absorbance was measured at 600 nm. [8]. Figure (1) shows the elution profile of blue dextran 2000. The volume of the buffer required to elute the blue dextran which represents the void volume was (6 mL).

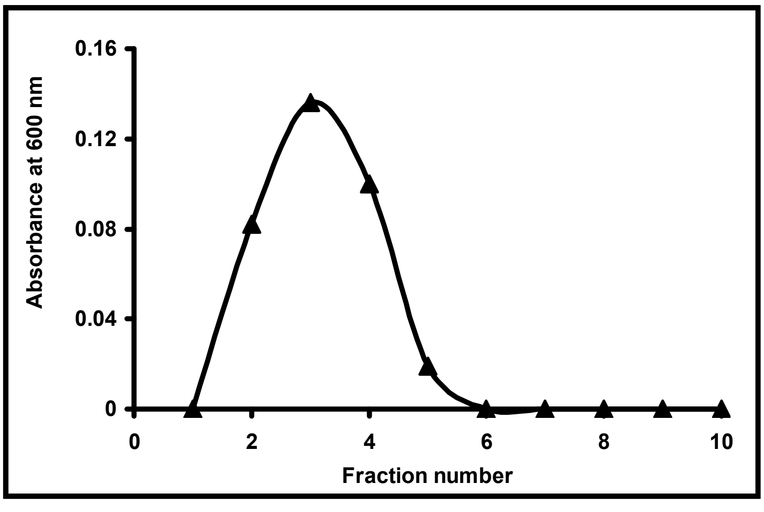


Figure (1): The elution profile of blue dextran 2000.

Column-Calibration

The column was calibrated by gel filtration kit, purchased from pharmacia fine chemicals which contained standard proteins. Standard protein solutions were prepared according to the manufacturers instructions, then applied through two 0.5 mL portions, proteins 1 and 3 in the first portion, protien 2 and 4 in the second portion. Elution was carried out with PBS buffer at a flow rate of 20 $ml.hrs^{-1}$. the absorbance of the fractions collected was measured at 280 nm to evaluated the elution volume (Ve) of the standard protein.

Standard Proteins

Pharmacia calibration kit for determination of M.wt by gel filtration was used. The kit comprises the highly purified proteins and their high M.wt is detailed in table (2).

Table (2): Standard proteins and their molecular weights.

Protein	M.wt (KD)	Conc. $Mg.mL^{-1}$
Thyroglobulin	**669**	**4.0**
Ferritin	**440**	**1.0**
Catalase	**322**	**6.0**
Aldolase	**158**	**6.0**

Calculations

The K_{av} values of the proteins eluted were determined using the following equation:

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

Where:

V_o= Void volume
V_e=Elution volume of each protein
V_t=Total gel - bed volume.

The calibration curve of K_{av} values vs. log M.wt. of the proteins were plotted.

Separation Procedure

Reagents

PBS buffer pH (7.0, 7.2 and 7.6) containing 0.02% sodium azide was prepared as described previously.

Procedure

The sample of tissue homogenate (0.5 mL) containing approximately 3.43 mg protein was applied to the surface of gel , equilibrated with 0.15 M PBS buffer pH 7.2 for benign and premenopausal malignant breast tumor respectively. The sample was eluted by using the same buffer pH (7.0 and 7.6) for (benign and premenopausal malignant breast tumors respectively) with a flow rate of 20 $mL.hrs^{-1}$ and fractions volume 2 mL were collected, gel filtration was carried out at 10 ^{o}C. The protein content of each fraction was determined using Lowry.et.al method [9]. The fractions contained CA15-3 were identified by the assay method. The binding of each fraction was calculated and plotted against the elution volume. The degree of purification (folds) of CA15-3 was calculated from the following formula.

$$\text{Purification fold of CA15-3} = \frac{\text{Specific binding of purified CA15-3}}{\text{Specific binding of crude CA15-3}}$$

Then yield % was determined as follows:

$$\text{Yield \%} = \frac{\text{Total protein content of purified CA15-3}}{\text{Total protein content of crude CA15-3}} \text{x100}$$

Dialysis for Concentration

After preparing dialysis tube, the fractions that contained high levels of the binding activity were pooled and concentrated by dialyzing against sucrose at 4 ^{o}C for 2hrs to get the required concentration to be used in the next experiments.

The Choice of the Optimum Conditions for the Binding of the Partially Purified CA15-3 to ^{125}I-Anti CA15-3 Antibody

A- Optimum Protein Concentration

Reagents

PBS buffer pH 7.0 and 7.6 was prepared as described previously.

Procedure

1-One hundred microliters of increasing amount (50,100,150,200 and 250) $\mu g.mL^{-1}$ protein of the dialyzable fractions of the partially purified CA15-3 from benign breast tumor was incubated with 50 µL of ^{125}I-anti CA15-3 antibody (0.35 $mg.mL^{-1}$) and completed to a final volume of 500 µL with 0.15 M PBS pH 7.0. The assay tubes were incubated for 90 min. at 45 ^{o}C. Two additional tubes, containing 50 µL (0.35 $mg.mL^{-1}$) of ^{125}I-anti CA15-3 antibody only, for total radioactivity computation, were set a side until counting.
2-At the end of incubation, the assay tubes were centrifuged at 4000 r.p.m for 45 min at 4^{o}C.
3-The supernatant were decanted, the rims at the tube were swabbed with cotton piece.
4-The radioactivity of the complex were counted using gamma counter.
5-The same experiment was repeated on premenopausal malignant breast tissues homogenates (100 $\mu g.mL^{-1}$ protein) with PBS buffer pH 7.6 and incubation time for 90 min at 15 ^{o}C.

Calculations

1. The counted radioactivity in each tube (expressed in c.p.m.) represents the bound fraction (B), (i.e., ^{125}I antiCA15-3 antibody/CA 15-3 complex).
2. The counted radioactivity in the tubes containing ^{125}I-anti CA15-3 antibody only represents the total count (T).
3. The (B/T) ratio for each tube counted as follows:

$$(B/T)\% = \frac{\text{Sample Counts (B)}}{\text{Total Counts (T)}} \times 100$$

4. The (B/T) ratio plotted against increasing amounts of protein concentration.

B- Influence of ^{125}I-Anti CA15-3 Antibody on the Binding

Reagents

PBS buffer pH 7.0 and 7.6 was prepared as described previously.

Procedure

Fifty microliters of increasing concentration (0.070, 0.140, 0.175, 0.210, 0.245, 0.280 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody were added to 100 µL (150 µg.mL^{-1} protein) of partially purified CA15-3 from benign breast tumors. The reaction was completed to 500 µL with PBS pH 7.0. The assay tubes were incubated for 90 min at 45 ^{o}C. Two additional tubes containing increased concentration of ^{125}I-anti CA15-3 antibody only, for total counts were counted. Steps 2,3 and 4 of the experiment (A) were repeated. The same experiment was repeated on premenopausal malignant breast tissues homogenate (100 µg.mL^{-1} protein) with PBS pH 7.6 and incubation time for 90 min at 15 ^{o}C.

Calculations

The (B/T) % was calculated as mentioned in experiment (A) and plotted against increasing concentration of ^{125}I-anti CA15-3 antibody.

C- Optimum pH

Reagents

PBS buffer pH (6.8, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.0) was prepared as described previously.

Procedure

To determine the optimum pH, 100 µL of a dialyzable fractions of partially purified CA15-3 from benign breast tumors (150 µg.mL^{-1} protein) were added to 20 µL of ^{125}I-anti CA15-3 antibody (0.140 mg.mL^{-1}). The volume of each fraction was completed to 500 µL with 0.15 M PBS of different pH (6.8 , 7.0 ,7.2 , 7.4 , 7.6 , 7.8 , 8.0). The assay tubes were incubated for 90 min at 45 ^{o}C. Two additional tubes, containing 20 µL (0.140 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody only , for total count , were set aside until counting. Steps 2,3 and 4 of experiment (A) were repeated. The same experiment was repeated on premenopausal malignant breast tissues homogenates (100 μg,mL^{-1} protein) and 25 μL (0.175 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody was incubated for 90 min at 15 ^{o}C.

Calculations

The (B/T) % was calculated as mentioned in experiment (A) and plotted against their corresponding pH values.

D- Optimum Temperature

Reagents

PBS buffer pH 7.0 was prepared as described previously.

Procedure

Twenty microliters (0.140 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody was added to 100 μL dialyzable fractions of partially purified CA15-3 from benign breast tumors (150 μg.mL^{-1} protein). The volume of reaction was completed to 500 μL with 0.15 M PBS buffer pH 7.0. The assay tubes were incubated for 90 min at 45 ^{o}C. The same steps were repeated at (37, 25, 15, 5^{o}C). Two additional tubes containing 20 μL (0.140 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody only, for total count, were set aside until counting. Steps 2,3 and 4 of experiment (A) were repeated. The same experiment was repeated on the premenopausal malignant breast tissues homogenates (100 μg.mL^{-1} protein) and 25 μL (0.175 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody in PBS buffer pH 7.0, with incubation time 90 min at 15 ^{o}C. The experiment was repeated at different temperatures (45,37,25 and 5 ^{o}C).

Calculations

The (B/T) % was calculated as mentioned in experiment (A) and plotted versus temperatures of incubation.

E- The Effect of Incubation Time

Reagents

PBS buffer pH 7.0 was prepared as described previously.

Procedure

Twenty microliters (0.140 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody were added to 100 μL of dialyzable fractions of partially purified CA15-3 from benign breast tumors containing (150 μg.mL^{-1} protein). The reaction volume was completed to 500 μL with 0.15 M PBS buffer pH 7.0, then incubated at 37 ^{o}C for (30, 60, 90, 120, 150, 180 min). Two additional tubes counting 20 μL (0.140 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody for total counts , were set aside until counting. Steps 2,3 and 4 of the experiment (A) were repeated. The same experiment was repeated on the premenopausal malignant breast tissues homogenates (100 μg.mL^{-1} protein) and 25 μL (0.175 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody with 0.15 M PBS buffer pH 7.0 and incubated at 15 ^{o}C for (30, 60, 90, 120, 150 and 180 min).

Calculations

The (B/T) % was calculated as metioned in experiment (A) and plotted versus the time of incubation for each group.

F- Stability of CA15-3 at –20 ^{o}C

Reagents

PBS buffer pH 7.0 was prepared as described previously.

Procedure

Crude and purified CA15-3 was stored at –20 ^{o}C for several time intervals. The frozen specimen was thawed at the end of each interval and the binding activity was measured at optimum conditions before and after partial purification. The remaining activity was calculated and plotted against storage periods.

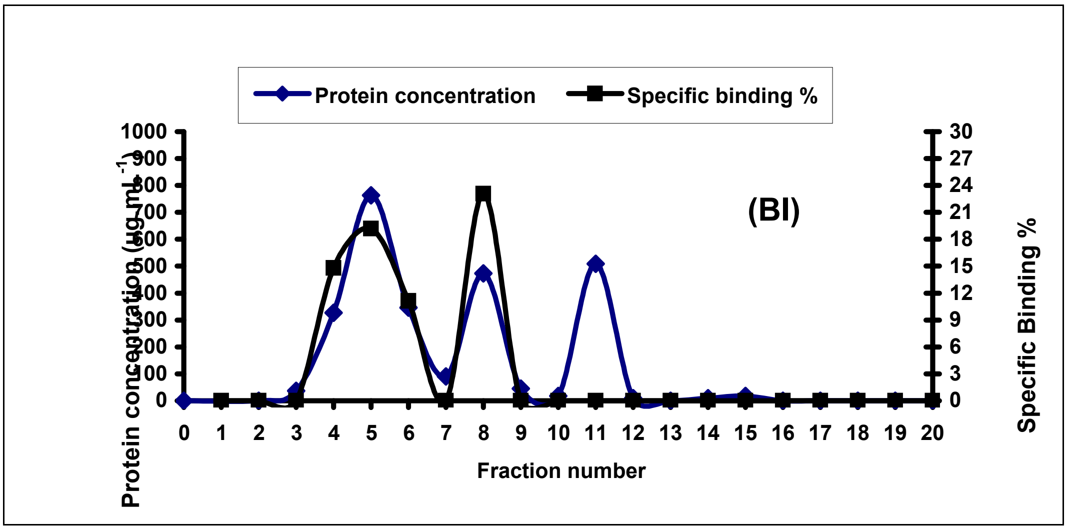
Calculations

The (B/T) % was calculated as mentioned in experiment (A) and plotted versus time storage for each group.

Results and Discussion

Partial Purification of CA15-3

Isolation of CA15-3 was performed by gel exclusion chromatography technique. CA15-3 was found to be separated from aggregates and other protein having smaller molecular weight by sepharose CL-4B. Figure (3&2) shows the elution profile of CA15-3 from benign breast tumors and premenopausal malignant breast cancer homogenates. The homogenate was loaded on the column as described in experimental part. The void volume (V_o) of column was (6 mL) as predicted from the elution profile of the blue dextran. The elution was performed with PBS buffer. The resultant fractions containing the binding activity of CA15-3 were collected, pooled and concentrated, then subjected to protein determination as in Lowry method [9].



Figure(2) : The elution profile of human CA15-3 from benign breast tumors (BI).

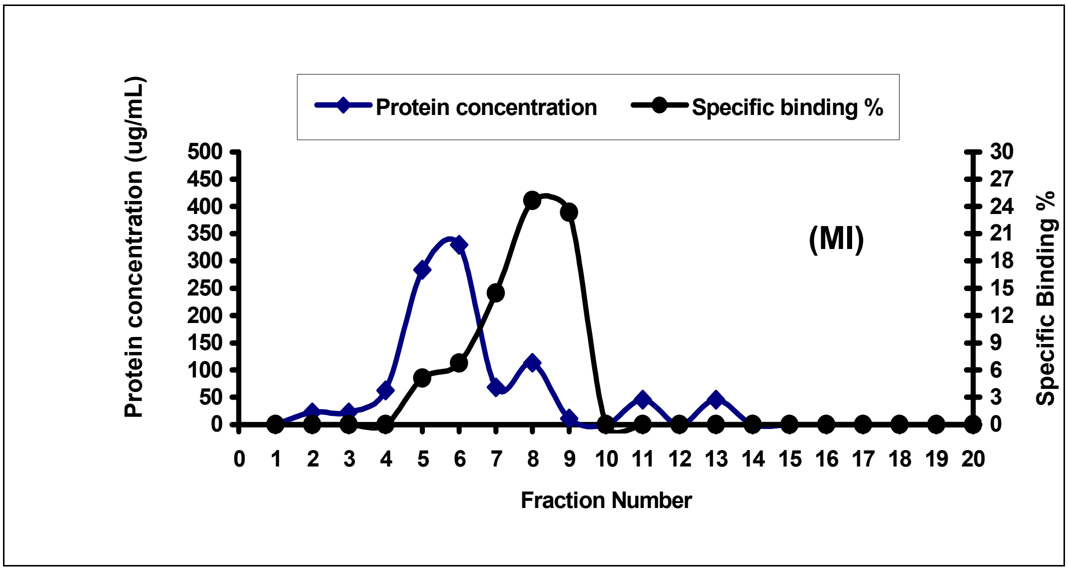


Figure (3): The elution profile of human CA15-3 from premenopausal malignant breast cancer (MI).

The elution volume V_e and then K_{av} values for the two peaks of CA15-3 (BI & MI) from benign breast tumors and malignant breast cancer respectively were calculated. The molecular weight of the partially purified CA15-3 obtained from figure (4) was 440 KD for peak (BI) and peak (MI) in two cases.

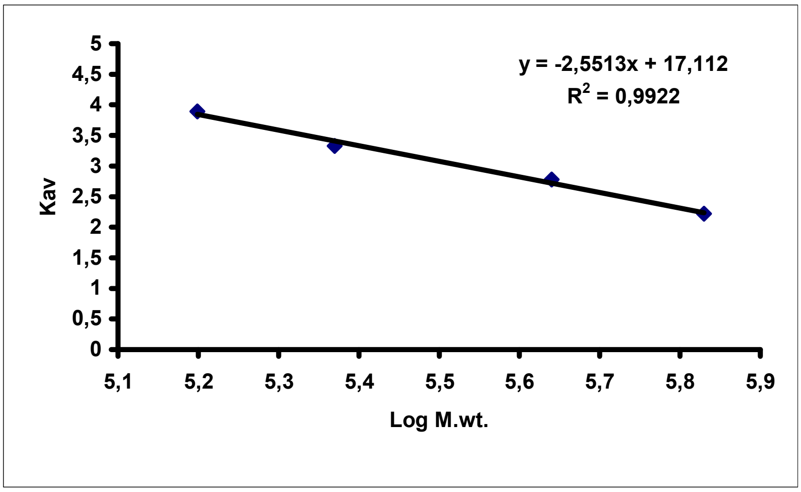


Figure (4): Calibration curve for determination of M.wt by gel filtration chromatography.

The values ranged from 300-450 KD [10,11,12]. Peaks of partially purified CA15-3 may be heavily aggregated; CA15-3 was obtained near the void volume of the column under separation conditions. From these results it was concluded that these components are capable of binding to the ^{125}I-anti CA15-3 antibody with different affinities and in general CA15-3 type (BI) have lower binding affinities than CA15-3 type (MI), the isolation of CA15-3 from benign breast tumors on gel filtration column showed 3.02 folds of purification for peak (BI), while the isolation of CA15-3 from premenopausal malignant breast cancer showed 5.0 folds of purification. Table (3) illustrates the purification parameters for the different purified CA15-3 forms isolated by gel exclusion chromatography technique. The glycosylation of the protein backbone may differ in carcinoma cells from normal epithelial cells causing a wide range of molecular weight for this mucin [13].

Table (3): Partial purification of CA15-3 by gel filtration.

CA15-3 Source		Total protein mg.mL^{-1}	Specifically bound ^{125}I-anti CA15-3	Specifically binding ^{125}I-anti CA15-3/mg protein	Yield%	Purification fold
Benign	Crude extract	3.43	10.17	2.97	100	1.00
	Gel filtration on sepharose CL-4B	2.91	30.70	10.55	84.84	3.02
Malignant	Crude extract	3.43	8.18	2.39	100	1.00
	Gel filtration on sepharose CL-4B	2.21	40.93	18.52	64.43	5.00

The Choice of Optimum Conditions for the Binding of Partially Purified CA15-3 with ^{125}I-Anti CA15-3 Antibody

Optimum Protein Concentration

Figure (5) shows the effect of increasing amounts of partially purified CA15-3 to a fixed amount of ^{125}I-anti CA15-3 antibody to produce (^{125}I-anti CA15-3 antibody/CA15-3) complex, that grow in size until they formed a precipitate. Above this zone an equivalence between CA15-3 and its antibody concentration is obtained, and amount of complex shows no further increases. A further addition of CA15-3 give rise to a solubilization of complex. The results revealed that 150 µg protein was the most appropriate concentration for the binding of (BI) and 100 µg protein for (MI). From these results, it could be concluded that the binding of ^{125}I-anti CA15-3 antibody with its partially purified CA15-3 (BI) needed a higher amount of protein concentration than partially purified CA15-3 (MI). This is may be due to lower concentration of CA15-3 in benign breast tumor as compared with malignant breast tumors. According to these results, in all subsequent experiments, (150 µg.mL^{-1} protein) in benign breast tumors and (100 µg.mL^{-1} protein) in malignant breast tumors were used, since they give the highest binding.

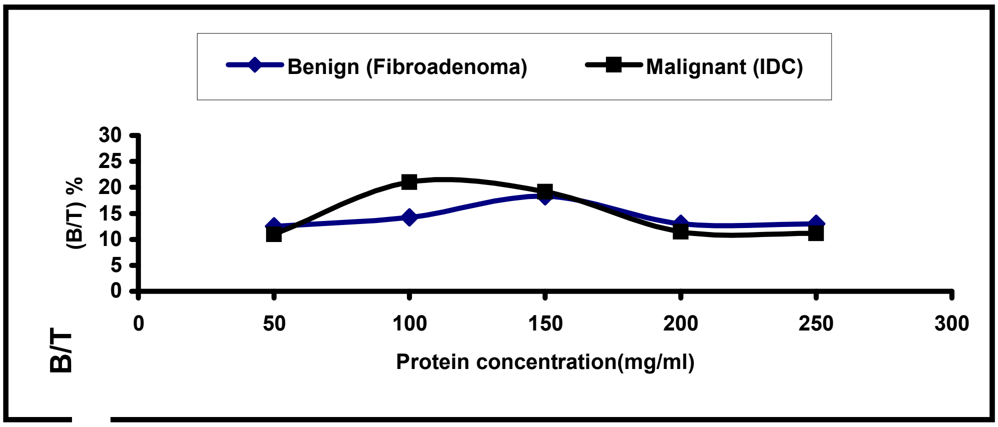


Figure (5): Influence of protein concentration on the binding of ^{125}I-anti CA15-3 antibody with partially purified CA15-3 from breast tumors.

Influence of ^{125}I-anti CA15-3 Antibody on the Binding

Figure (6) illustrate the effect of ^{125}I-anti CA15-3 antibody concentration on the binding with partial purified CA15-3 from benign breast tumors and premenopausal malignant breast cancer.

The maximum binding obtained when 0.140 mg.mL^{-1} of antibody in benign breast tumors and 0.175 mg.mL^{-1} of antibody in malignant breast tumors were used. From these results, it was found that (BI) purified fraction was saturated with small concentration of ^{125}I-anti CA15-3 antibody than those required for (MI). This is may be due to the increasement of the epitope (is the part of an antigen molecule that binds to any single antigen-combining site) [14] in partially purified CA15-3 in malignant breast tumors as compared to benign breast tumors.

According to these results, in all subsequent experiments (0.140 mg.mL^{-1}) and (0.175 mg.mL^{-1}) of ^{125}I-anti CA15-3 antibody in benign and malignant breast tumors were used, since they give the highest binding.

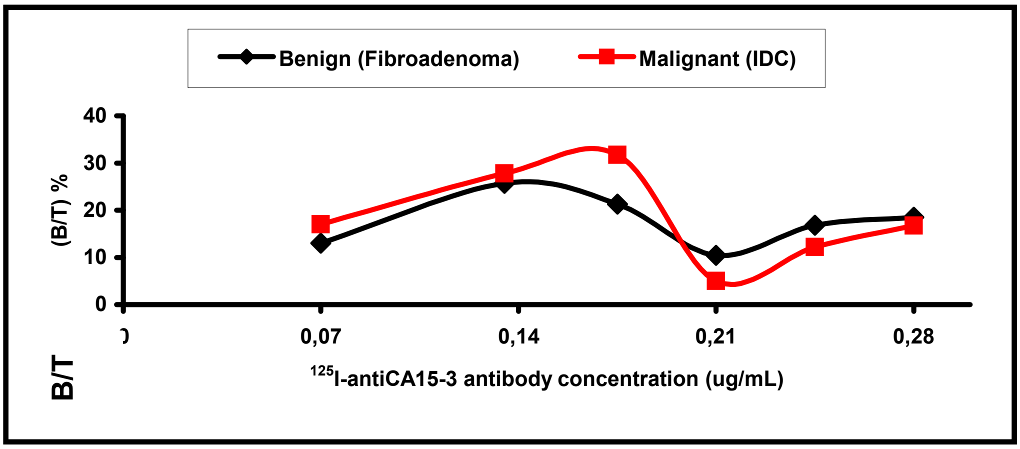


Figure (6): Effect of ^{125}I-anti CA15-3 antibody concentration on its binding with partially purified CA15-3 from breast tumors.

Optimum pH

Figure (7) shows the effect of increasing pH on the binding of ^{125}I-anti CA15-3 antibody to its purified antigen. The results revealed that the optimum pH for (BI) and (MI) purified fractions for the binding with its antibody was 7.0. These results indicate that the binding was pH dependent.

The similarity in pH (7.0) suggests that the CA15-3 isolated from different sources of tissues either benign or malignant breast tissues homogenates possesses the same epitopes in both cases. That means the induction of protonation-deprotonation process [15] occurs within the same changed polar groups on the amino acid residues present in the binding domain. According to the results obtained, the pH of the buffer used in all subsequent experiments was adjusted to pH 7.0.

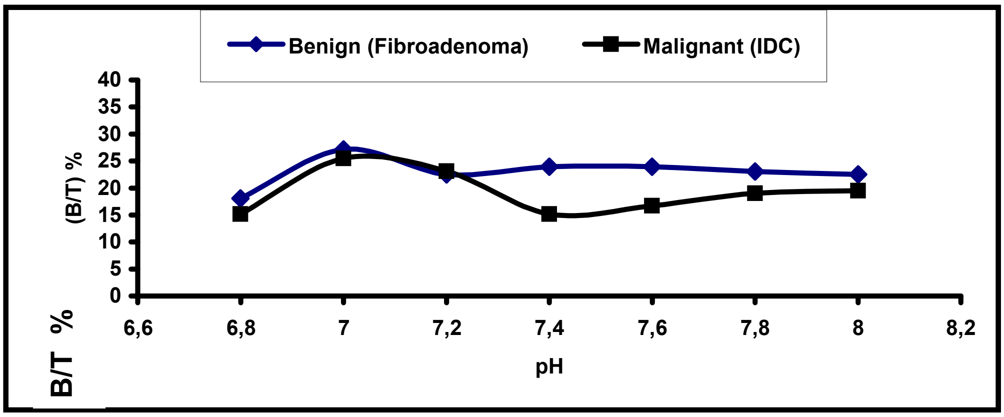


Figure (7): pH effect on the binding of ^{125}I-anti CA15-3 antibody with partially purified CA15-3 from breast tumors.

Optimum Temperature

The temperature dependency of the isolated CA15-3 binding to its antibody ^{125}I-anti CA15-3 was investigated. Figure (8) show the optimum temperatures on the binding of ^{125}I-anti CA15-3 antibody was 37^{o}C with partially purified CA15-3 (BI) and 15^{o}C with partially purified CA15-3 (MI). The difference of the temperature between crude and purified CA15-3 occurs in benign breast tumors, i.e. the optimum temperature was 45^{o}C of the binding of ^{125}I-anti CA15-3 antibody to crude CA15-3 while in the purified CA15-3 (BI) was 37^{o}C. On the other hand, the optimum temperature in both crude and partially purified CA15-3 from premenopausal malignant breast tumors was 15^{o}C. The temperature dependency of the binding suggests that the whole process is controlled by diffusion of the interacting of ^{125}I-anti CA15-3 antibody to CA15-3 in benign and malignant breast tumors. In view of these results, the temperatures (37 & 15 ^{o}C) for both benign and malignant breast tumors were used in all subsequent experiments.

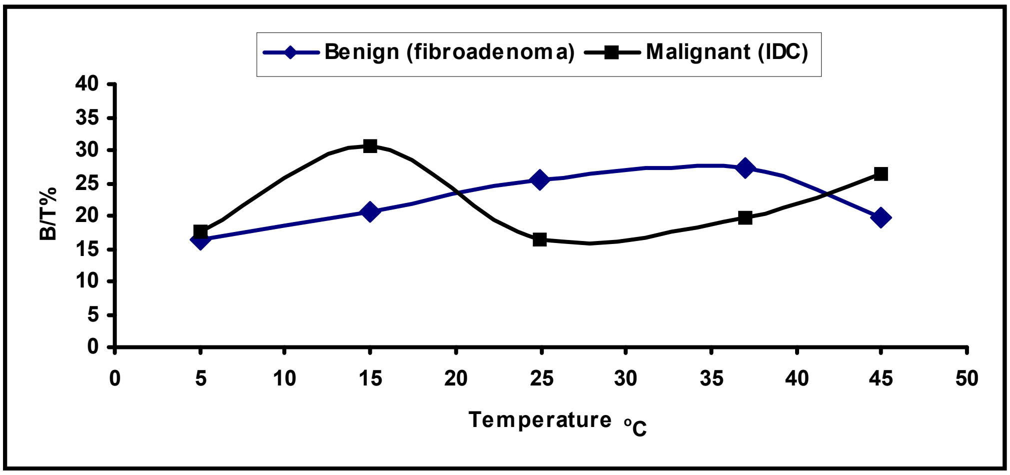


Figure (8): Effect of the temperature on the binding of ^{125}I-anti CA15-3 antibody with partially purified CA15-3 from breast tumors.

The Effect of Incubation Time

Figure (9) shows the time required for the highest binding of ^{125}I-anti CA15-3 antibody to partially purified CA15-3 in (BI) and (MI) was 90 min at 37 and 15 ^{o}C respectively. In view of these results, the incubation time used in all subsequent experiments was 90 min.

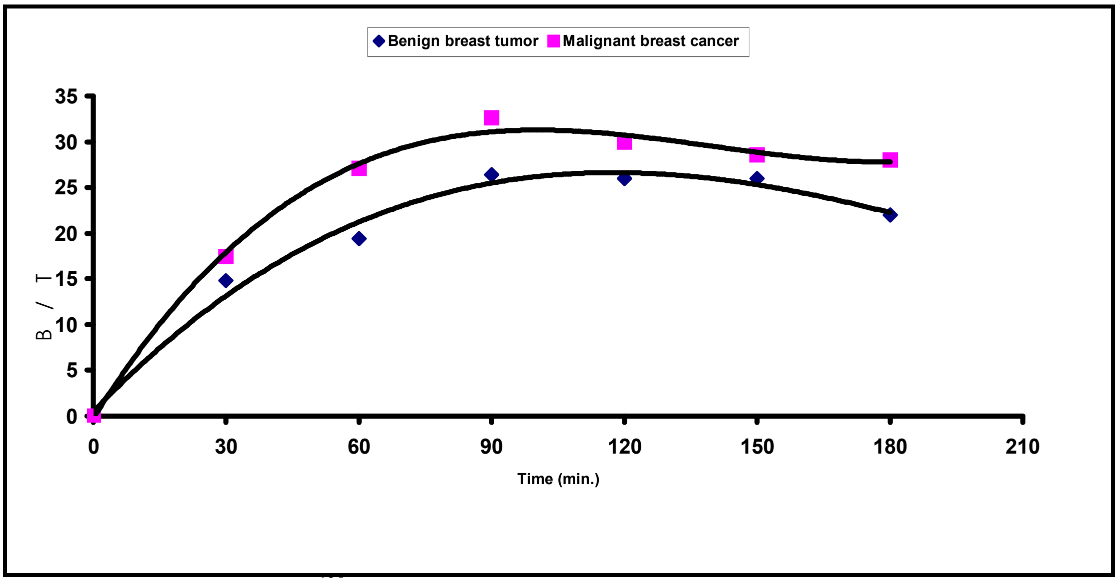


Figure (9): Time dependence of ^{125}I-anti CA15-3 antibody binding with partially purified CA15-3 from breast tumors.

Stability of CA15-3 at –20 °C

The crude and isolated fractions of CA15-3 from malignant breast tumors were stored at –20 °C during the experiments. It was carried out in order to study the stability of CA15-3 and check their efficiencies of the binding through out the storage period. The results showed that CA15-3 of crude fraction was more stable than the isolated fractions as shows in figure (10). This result is in agreement with Al-Atrakchi observations [16].

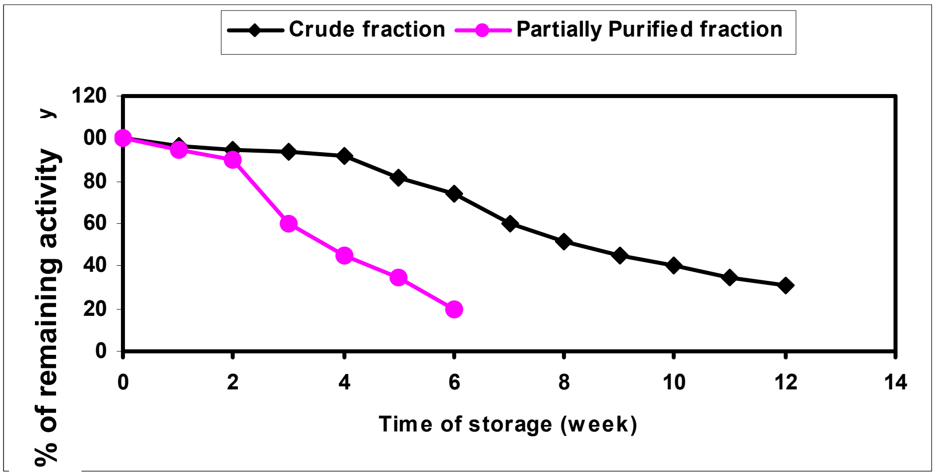


Figure (9): Stability of partially purified and crude CA15-3 upon storage at -20°C.

References:

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