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SUBCELLULAR AND EXTRACELLULAR LOCALIZATION OF SPECIFIC BINDING SITES FOR TRIPHENYLETHYLENE ANTIESTROGENS IN HUMAN BREAST CANCER

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Abstract—MCF-7 human breast cancer cell homogenates and subcellular organelles were submitted to isopycnic centrifugation on Percoll gradients to investigate the subcellular localization of triphenylethylene antiestrogen specific binding sites (AEBS). Electron microscopy revealed that gradient fractions coincident with the migration of [^{3}H]tamoxifen–AEBS complexes were homogeneously represented by rough and smooth endoplasmic reticulum. Eighty percent of AEBS were localized in the endoplasmic reticulum [45,000 ± 4,000 sites/cell, mean ± S.D.), while 20% of these sites were also found in the nuclear fraction (12,000 ± 1,000 sites/cell, mean ± S.D.). A similar subcellular distribution of AEBS was observed in human breast cancer bioptic specimens. No differences in [^{3}H]tamoxifen binding affinity between microsomal and nuclear AEBS were observed in MCF-7 and bioptic breast cancer. No major differences in microsomal AEBS levels were observed in the limited number of estrogen receptor-positive or -negative breast cancer specimens we have studied, whereas estrogen receptor-negative samples had higher levels of nuclear AEBS with respect to estrogen receptor-positive tumors. The presence of AEBS was also detected in the human serum of healthy and tumor-bearing subjects. The affinity and the binding specificity of serum AEBS were similar to those of intracellular AEBS. No differences in the levels of serum AEBS were observed between healthy and tumor-bearing subjects [19 ± 4 and 22 ± 4 pmoles/ml (mean ± S.D.) respectively. Human serum AEBS did not appear to be associated to lipoproteins, whereas it migrated as a 5.5 S sedimenting molecule.

Hormone dependency of mammary cancer has enabled the use of triphenylethylene antiestrogens, such as tamoxifen, as inhibitors of tumor growth [1]. Estrogen receptor (ER)-positive breast cancers have been shown to have a high response rate to tamoxifen compared to ER-negative tumors which rarely respond to this drug [1, 2]. Tamoxifen is able to bind to ER and to either mimic or antagonize some of the estrogen actions [3]. Such properties of antiestrogens have been very useful in understanding the estrogen-regulated processes related to cell growth [4]. Although tamoxifen has been generally shown to inhibit the growth of ER-positive but not ER-negative breast cancer cell lines [5], more recently an inhibitory effect of high concentrations of this antiestrogen on the growth of ER-negative breast cancer cells has also been described [6]. Furthermore, besides binding to the ER, tamoxifen also binds to distinct, specific and high-affinity binding sites (AEBS, antiestrogen specific binding sites) in several experimental models [7–9], suggesting the possibility of additional mechanisms of action of this drug. The attempt to elucidate the role of AEBS in mediating antiestrogen actions awaits their characterization. Contrasting data exist so far on AEBS characteristics, mainly concerning their exact subcellular localization. Several authors first reported AEBS in both the cytosol fraction [7–9], where it sedimented as a heterogeneous 10 to 40 S protein [10–12], and in the nucleus [13–15]. Other authors reported the presence of AEBS only in the microsomal fraction obtained by differential centrifugation [16, 17]. More recently, the antiestrogen specific binding site has been reported as a relatively low molecular weight (sedimentation coefficient 6 S), soluble cytoplasmic protein [14]. We used in this study a fast isopycnic centrifugation of cultured and bioptic breast cancer cell homogenates on self-generating Percoll gradients, which allows a single-step analysis of soluble and particulate drug binders, in order to isolate the subcellular fraction bearing AEBS. Furthermore, since we previously reported a positive correlation between ER and AEBS levels in the guinea pig uterus during development from the fetal to the adult stage [13], we studied the relationships between the ER and AEBS levels in several human breast cancer biopsies. Finally, we investigated the presence and the characteristics of AEBS in the human serum, since AEBS have been detected previously in guinea pig [9] and rat [18] serum.

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MATERIALS AND METHODS

Chemicals

[^{3}H]Tamoxifen (sp. act. 90 Ci/mmole) and [^{3}H]estradiol (sp. act. 100 Ci/mmole) were purchased from Amersham (Buckinghamshire, England). Tamoxifen (ICI 46,474), 4-hydroxytamoxifen (ICI 79,280), 3,4-dihydroxytamoxifen (ICI 77,307), and metabolite A (ICI 46,929, 1-(*p*-

dimethylaminoethoxyphenyl) - 1 - (hydroxy) - 2 - diphenylbutane) were gifts from ICI Pharma (England). Estradiol, diethylstilbestrol, progesterone, testosterone and cortisol were obtained from Sigma (St. Louis, MO, USA). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Hydroxylapatite (Bio-Gel HTP) was purchased from Bio-Rad (Richmond, CA, U.S.A.).

Cells and culture conditions

MCF-7, a cloned cell line derived from a malignant pleural effusion in a female patient with metastatic breast cancer, was provided by Dr. M. E. Lippman, NCI, Bethesda, MD. Cells were grown in monolayer cultures in Eagle's minimum essential medium (MEM) supplemented with 2 mM glutamine, 5% fetal calf serum, non-essential amino acids and antibiotics (Flow Laboratories, Ayrshire, Scotland) at 37° in a humidified atmosphere of 5% CO_2–air.

Cell fractionation

Cultured MCF-7 cells were suspended with trypsin–EDTA and homogenized with a Teflon–glass Potter–Elvehjem homogenizer in 10 mM Tris–HCl, 0.25 M sucrose, pH 7.4 (TS buffer). Fresh human breast cancer bioptic samples were homogenized in TS buffer with an Ultra Turrax TP 18/10. Homogenates were centrifuged at 800 *g* for 10 min at 4°. The 800 *g* supernatant was further centrifuged at 180,000 *g* for 30 min at 4° to obtain the cytosol fraction. In other experiments, the 800 *g* supernatant fraction were either layered on or mixed with a 20% Percoll–TS buffer solution and centrifuged in a Beckman 65 fixed-angle rotor for 40 min at 30,000 rpm at 4° in order to separate the different subcellular fractions according to the method described by Jenkins *et al.* [19]. Neither procedure modified the migration of [^{3}H]tamoxifen–AEBS complexes. The 800 *g* pellet was processed according to the sucrose–calcium procedure described by Chauveau *et al.* [20] in order to obtain a purified nuclear fraction, as assessed by light microscopy. The nuclear pellet was extracted with 10 mM Tris–HCl, 1.5 mM EDTA, 12 mM monothioglycerol, 10% glycerol, 0.6 M KCl, pH 7.4, for 60 min at 4° and centrifuged at 100,000 *g* for 30 min at 4° to obtain a clear supernatant fraction.

Binding studies

AEBS. [^{3}H]Tamoxifen binding to the different subcellular fractions (cytosol, 0.6 M KCl nuclear extract, 800 *g* supernatant) and serum was measured by incubation with 20 nM [^{3}H]tamoxifen (single point assay) or 0.6 to 20 nM [^{3}H]tamoxifen (Scatchard analysis, Ref. 21) plus 1 μM unlabeled estradiol (to prevent the binding to the ER) in the presence or the absence of 1 μM unlabeled tamoxifen in order to evaluate non-saturable binding. [^{3}H]Tamoxifen binding was determined by adsorption of unbound ligand with dextran-coated charcoal (DCC).

ER. Estrogen receptors (occupied plus unoccupied by the endogenous hormone) were measured in the cytosol (prepared in 10 mM Tris–HCl, 1.5 mM EDTA, 12 mM monothioglycerol, 10% glycerol, pH 7.4) and in the 0.6 M KCl nuclear extract according to the hydroxylapatite method described by Garola and McGuire [22]. Briefly, cytosol and nuclear extracts were mixed with hydroxylapatite slurries, and the ER adsorbed to hydroxylapatite were pelleted and incubated with [^{3}H]estradiol (0.05 to 5 nM) in the presence and in the absence of 1 μM unlabeled diethylstilbestrol to evaluate non-saturable binding at 4° overnight followed by a further 4-hr incubation at 30°. At the end of the incubation, the washed hydroxylapatite was extracted with ethanol which was counted for radioactivity content. In other experiments, cytosol was incubated with 5 nM [^{3}H]estradiol plus or minus 1 μM unlabeled diethylstilbestrol for 2 hr at 4°, and unbound ligand was adsorbed by DCC.

Electron microscopy

The fractions recovered from Percoll gradients coincident with the migration of AEBS–[^{3}H]tamoxifen complexes were diluted with TS buffer and centrifuged at 100,000 *g* for 2 hr. By this way, Percoll beads sedimented and formed a cushion stuck at the bottom of the tube, while biological material, which remained collected and lightly adhesive at the surface of this cushion, was gently resuspended and recovered. The biological material was washed twice in phosphate-buffered saline (PBS), fixed in 2.5% glutaraldehyde in PBS (overnight at 4°), post-fixed in 1% osmium tetroxide in veronal acetate buffer, dehydrated in acetone, and embedded in Epon 812. Thin sections were obtained, stained with uranyl acetate and lead hydroxide, and examined under a Philips 300 electron microscope.

Separation of serum lipoproteins

The major serum lipoprotein classes were separated by the density gradient centrifugation method described by Terpstra *et al.* [23]. Serum (1 ml) was added to SW 50.1 tubes containing 114 mg KBr, 25 mg sucrose and 0.1 ml Sudan black solution (1 mg/ml in ethylene glycol) or 0.1 ml 0.9% NaCl. This mixture was overlaid sequentially with 2.4 ml of a solution containing 11.42 mg NaCl/ml and 75.98 mg KBr/ml and then with 2.4 ml of distilled water. Tubes were centrifuged in a Beckman SW 50.1 rotor at 50,000 rpm for 7 hr at 4°. In other experiments, 2 ml of serum was added to SW 41 tubes containing 770 mg KBr, 50 mg sucrose and 0.2 ml Sudan black solution (or 0.2 0.9% NaCl solution) and overlaid sequentially with 2 ml of a solution containing 11.42 mg NaCl/ml and 315.54 mg KBr/ml, 4 ml of 11.42 mg NaCl/ml and 133.48 mg KBr/ml and finally 4 ml of distilled water. The tubes were centrifuged in a Beckman SW 41 rotor at 40,000 rpm for 22 hr at 4°.

Sucrose density gradient analysis

Serum, diluted 1:20 with 10 mM Tris–HCl, 1.5 mM EDTA (pH 7.4) buffer (TE buffer), was incubated for 1 hr at 30° with [^{3}H]tamoxifen (20 nM) plus or minus 1 μM unlabeled tamoxifen. 0.1-ml aliquots of DCC-treated serum were applied on 5–20% linear sucrose gradients in TE buffer and centrifuged at 64,000 rpm for 90 min in a Beckman Vti 65 rotor at 4°. Human γ-globulin (7.1 S) and bovine serum albumin (BSA, 4.6 S) were used as markers.

Protein and DNA determination

Protein concentration was assayed by the method of Lowry *et al.* [24]. DNA was measured on 5% TCA precipitates according to the method described by Burton [25].

RESULTS

Subcellular localization of AEBS in MCF-7 cells

Preliminary studies have shown that most of AEBS of MCF-7 cells was pelleted by centrifugation at 100,000 *g*, in agreement with data reported by other authors [16, 17]. Figure 1A shows the distribution of the saturable binding of [^{3}H]tamoxifen (AEBS) and of [^{3}H]estradiol (ER) in the 800 g supernatant fraction obtained from MCF-7 cell homogenates, after isopycnic centrifugation on self-generating Percoll gradients. ER (which is known to be a soluble cytoplasmic protein) remained in the first fractions of the Percoll gradient, while AEBS migrated towards 1.045 to 1.055 g/ml density fractions. A similar migration of AEBS was observed when subcellular organelles pelleted by centrifugation at 180,000 *g* were submitted to the Percoll density gradient analysis (not shown). Electron microscopic observation of subcellular organelles migrating in the fractions corresponding to the migration of AEBS showed the presence of a homogeneous pool of unaltered microsomal rough and smooth vesicles with a diameter of 100–200 nm (Fig. 2).

Since previous reports have described the presence of AEBS in the 100,000 *g* supernatant fraction of MCF-7 cancer cells [7], we studied the distribution of cytosolic AEBS in Percoll gradients. Figure 1B shows that most of the AEBS remained in the cytosol region of the gradients with only a few AEBS migrating in the region corresponding to the microsomal fraction. However, in these experiments only 10% of the total AEBS found in the 800 *g* supernatant of cell homogenates was observed in the cytosol fraction. These results suggest that AEBS is mainly associated with the microsomal fraction [45,000 ± 4,000 (S.D.) sites/cell] and that during the centrifugation procedures to obtain the cytosol fraction a few AEBS can be solubilized [7,000 ± 500 (S.D.) sites/cell].

A saturable binding of [^{3}H]tamoxifen was also observed in the purified nuclei of MCF-7 cells (Fig. 3). These sites were distinct from ER, since they were observed in the presence of an excess of unlabeled estradiol. The affinity of tamoxifen for nuclear AEBS [K_d 4 ± 0.8 (S.D.) nM] was similar to that of this drug for cytoplasmic sites (K_d 3 ± 0.5 (S.D.) nM], whereas their concentration accounted for 12,000 ± 1,000 (S.D.) sites/cell.

Subcellular localization of AEBS in human breast cancer bioptic specimens

AEBS were also present in all the human breast cancer biopsies studied. Analysis of the subcellular localization of AEBS in these bioptic samples using isopycnic centrifugation on Percoll gradients showed that all the AEBS were associated with endoplasmic reticulum (Fig. 4A). However, we also found the presence of AEBS in the purified nuclear fraction of breast cancer biopsies, with an affinity for tamoxifen [K_d 2.7 ± 0.4 (S.D.) nM] similar to that of cytoplasmic sites [K_d 2 ± 0.3 (S.D.) nM] (Fig. 4B).

Distribution of AEBS in ER-positive and ER-negative human breast cancer biopsies

To investigate whether a relationship exists between AEBS and ER levels, we studied and correlated the concentrations of AEBS and ER in human breast cancer biopsies. Figure 5A shows that

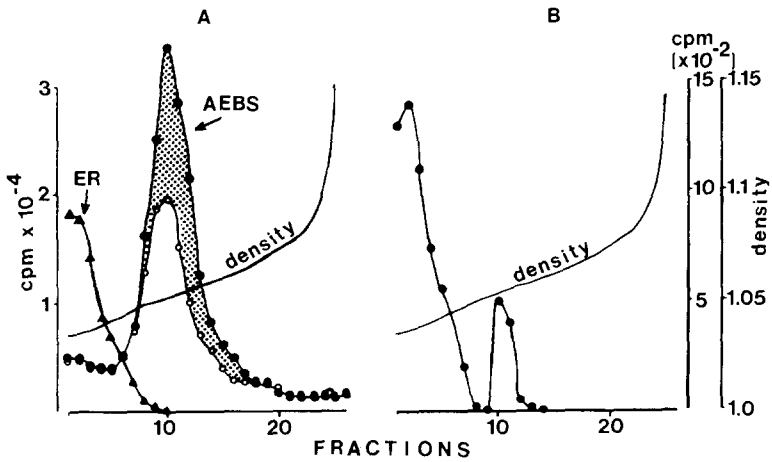

Fig. 1. Isopycnic centrifugation on Percoll gradients of AEBS in the 800 *g* (A) and 180,000 *g* (B) supernatant fractions from homogenates of MCF-7 cells. (A) The 800 *g* supernatant fractions were incubated with 20 nM [^{3}H]tamoxifen plus 1 μM estradiol (●) or with [^{3}H]tamoxifen plus 1 μM estradiol and tamoxifen (○) or with 5 nM [^{3}H]estradiol plus or minus 1 μM unlabeled diethylestilbestrol (▲) for 2 hr at 4°, and DCC-treated aliquots were layered on Percoll gradients and centrifuged as described in Materials and Methods. Saturable binding of [^{3}H]estradiol (▲, ER) is depicted. (B) The 180,000 *g* supernatant fractions were incubated and processed for Percoll gradient analysis as described in A. Saturable binding of [^{3}H]tamoxifen in the presence of unlabeled estradiol (AEBS, ●) is represented. The results are representative of three similar experiments.

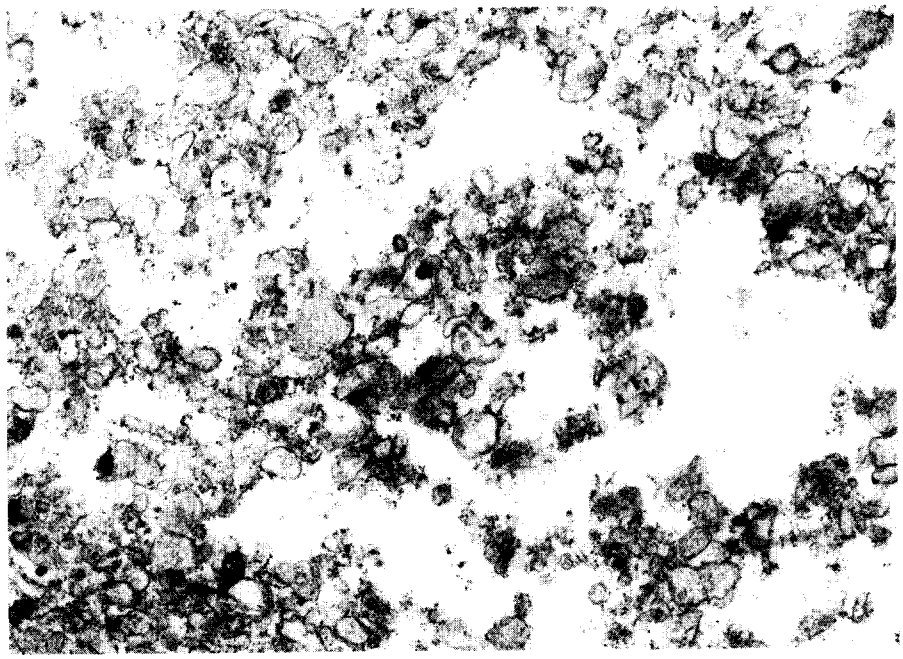

Fig. 2. Electron micrograph of a thin section of biological material obtained from fractions 9–11 of Percoll gradients of 800 g supernatant fractions of MCF-7 cell homogenates as described in Fig. 1A, showing a homogeneous pool of typical, unaltered microsomal vesicles without and with ribosomes attached to the outside surfaces (×67,000).

AEBS was present in both ER-positive and ER-negative human breast cancer biopsies. The range of microsomal AEBS concentration was quite wide, with no significant differences between ER-positive and ER-negative tumor samples. In contrast, higher levels of nuclear AEBS were found in ER-negative versus ER-positive tumors ($P < 0.05$). Higher levels of AEBS were observed in the microsomal than in the nuclear fraction, with no significant difference between ER-positive and ER-neegative tumors (Fig. 5B). No correlation between AEBS levels and ER concentrations was observed within the ER-positive group (not shown).

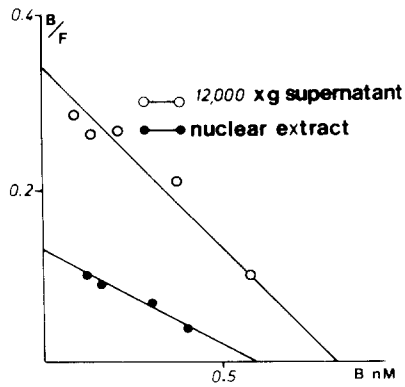

Fig. 3. Scatchard analysis of [^{3}H]tamoxifen binding to AEBS in 12,000 g supernatant fractions (○) or 0.6 M extracts obtained from purified nuclei (●) of MCF-7 cells, carried out as described in Materials and Methods. The results are representative of three similar experiments.

Specific binding of triphenylethylene antiestrogens in the human serum

Besides an intracellular specific binding for triphenylethylene antiestrogens, we have found that [^{3}H]tamoxifen was also able to bind to serum proteins obtained from both normal subjects and tumor-bearing patients. Figure 6 shows that this binding was characterized by high affinity [K_d 2.2 ± 1 (S.D.) nM and 4.0 ± 1.5 (S.D.) nM in normal subjects and tumor-bearing patients respectively]. The binding specificity of the AEBS in the serum was similar to that of intracellular AEBS, since only triphenylethylene antiestrogens, and not other steroids or non-steroidal estrogens, were able to inhibit the [^{3}H]tamoxifen binding to both human serum (Fig. 6B) and intratumor AEBS (not shown). No significant differences in the serum levels of these tamoxifen binding sites were observed either between normal subjects and tumor-bearing patients or between male and female subjects (Fig. 6C). The tamoxifen binding capacity of serum from tumor-bearing patients was 22 ± 4 (S.D.) pmoles/ml while the levels of intratumor AEBS ranged from 60 to 300 pmoles/g of tissue.

Since serum AEBS has been associated previously with low density lipoproteins (LDL) in rats [18] and guinea pigs [11], we studied the binding of [^{3}H]tamoxifen to the major classes of lipoproteins separated by KBr gradients. Figure 7A shows the profile of [^{3}H]tamoxifen binding in the serum after a 7-hr centrifugation through KBr gradients which allowed the separation of very low density lipoproteins (VLDL) and LDL from the other serum proteins. Most of the saturable tamoxifen binding

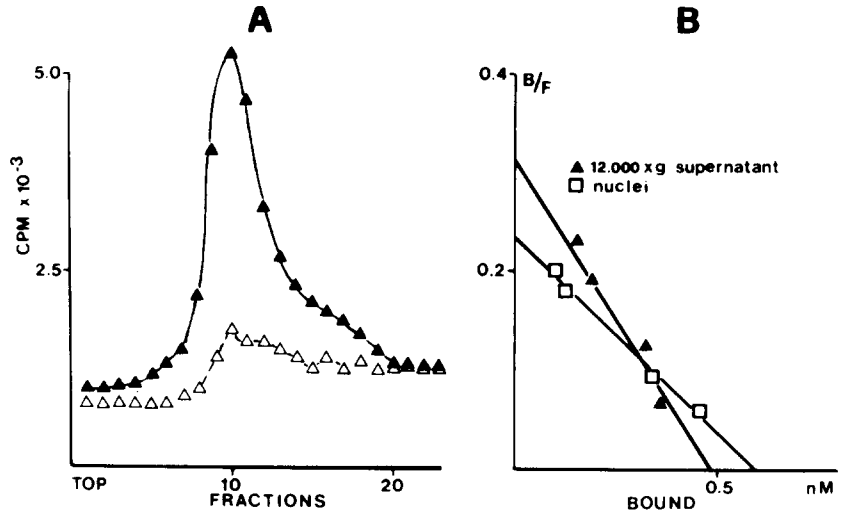

Fig. 4. (A) Isopycnic centrifugation on Percoll gradients of AEBS in the 800 *g* supernatant fraction from homogenates of human breast cancer biopsies, incubated with [^{3}H]tamoxifen plus estradiol without (▲) or with (△) unlabeled tamoxifen. For other details see Fig. 1A. (B) Scatchard analysis of [^{3}H]tamoxifen binding to AEBS of 12,000 *g* supernatant (▲) or nuclear extract (□) of human breast cancer biopsies, carried out as described in Fig. 3. The results are representative of three experiments.

was observed at the bottom of the tube gradient, while only a minimal binding was associated with LDL. To permit a better separation of high density lipoproteins (HDL), we carried out a 22-hr centrifugation KBr gradient study and labeled each fraction with [^{3}H]tamoxifen at the end of the centrifugation period (post-labeling) in order to prevent the dissociation of the labeled drug from serum lipoproteins during the 22-hr centrifugation (Fig. 7B). Similar to the results obtained in Fig. 7A, most of the saturable [^{3}H]tamoxifen binding was observed in high density serum fractions (bottom) with only very low amounts of [^{3}H]ligand bound to LDL. These results suggest that human serum AEBS is not associated to lipoproteins.

To characterize better the human serum AEBS, we examined its sedimentation profile using sucrose gradient analysis. Figure 8 shows that human serum AEBS sedimented as a 5.5 S molecule.

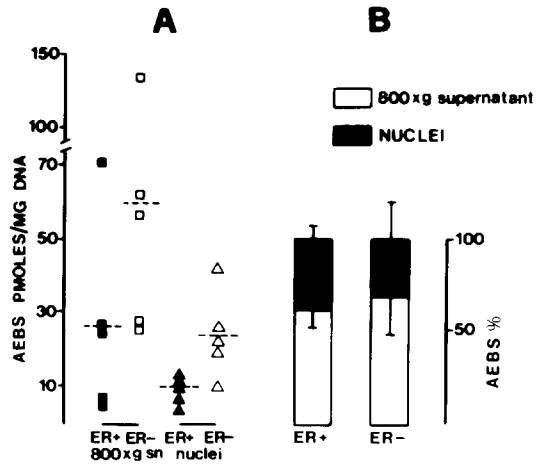

Fig. 5. Concentration (A) and percent subcellular distribution (B) of AEBS in 800 *g* supernatant fractions (■ □) and nuclei (▲ △) of ER-positive (ER levels ≥ 5 fmoles/mg protein) and ER-negative (ER levels < 5 fmoles/mg protein) human breast cancer bioptic specimens. (A) Broken lines represent the means from five samples. (B) The results are the means (±S.D.) from five samples.

DISCUSSION

Confusing data exist in the literature concerning the subcellular localization of AEBS. Several authors have reported the presence of AEBS in the cytosol fraction as either a large (sedimentation coefficient 10 to 40 S) [10, 12] or a relatively small (6 S) [14] molecule, whereas other investigators have described AEBS as associated with the microsomal fraction [16, 17]. The results reported in this study show that most of the AEBS is located in the endoplasmic reticulum of MCF-7 cells. This is suggested by the finding that the fractions recovered from Percoll gradients coincident with the migration of AEBS–[^{3}H]tamoxifen complexes were represented by homogeneous rough and smooth unaltered endoplasmic reticulum, without significant contamination due to other subcellular organelles, as assessed by electron microscopy. This suggests that the finding of soluble cytoplasmic AEBS previously reported is probably a consequence of artifactual release of AEBS from subcellular organelles due to the differential centrifugation procedures used for cell fractionation. This is supported by the presence of soluble AEBS (although in small amounts) observed in the 180,000 *g* supernatant fraction of MCF-7 cell homogenates after fractionation on Percoll gradients (Fig. 1B).

Our study shows that AEBS was also localized in the nuclear fraction of MCF-7 cells, from which it can be extracted by high ionic strength buffer. The affinity of tamoxifen for nuclear AEBS was similar

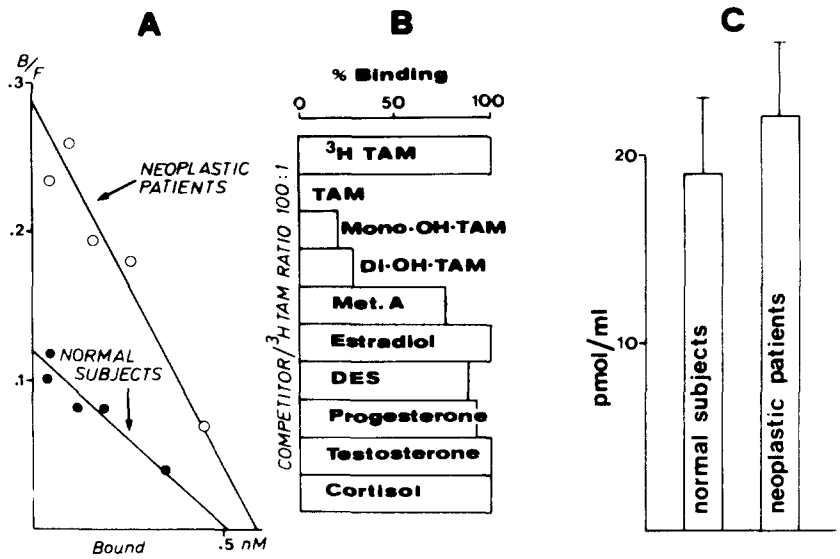

Fig. 6 (A) Scatchard analysis of [^{3}H]tamoxifen saturable binding in the serum (diluted 1:20 with TE buffer) of normal subjects (●) or tumor-bearing patients (○). The results are representative of six to nine experiments. (B) Binding specificity of [^{3}H]tamoxifen binding in the serum. Human serum (diluted 1:20 with TE buffer) was incubated with [^{3}H]tamoxifen alone or with a 100-fold molar excess of several competitors for 1 hr at 30°. Residual binding was measured by the DCC method as the percent saturable [^{3}H]tamoxifen binding. Saturable tamoxifen binding accounted for 20 ± 2 (S.D.) pmoles/ml of serum in the experiments shown. (C) Concentrations of AEBS in the serum of normal subjects and tumor-bearing patients, measured by Scatchard analysis. The results are the means (±S.D.) of six to nine experiments.

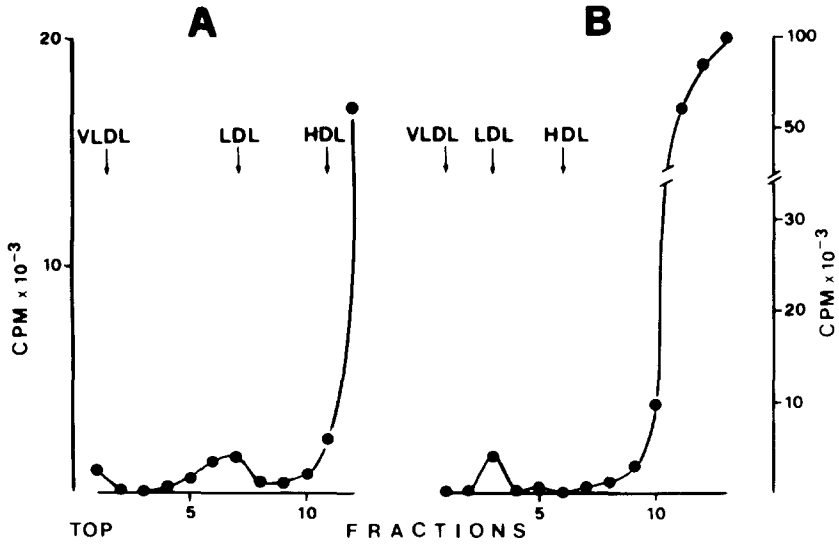

Fig. 7. Pre-labeled (A) and post-labeled (B) KBr gradient analysis of [^{3}H]tamoxifen saturable binding in the human serum. (A) Serum was diluted 1:20 with TE buffer and incubated with [^{3}H]tamoxifen (20 nM) plus or minus unlabeled tamoxifen (1 μM) for 1 hr at 30°, and DCC-treated aliquots were centrifuged in KBr gradients in a Beckman SW 50.1 rotor for 7 hr at 4°. (B) Undiluted serum was centrifuged in KBr gradients in a Beckman SW 41 rotor for 22 hr at 4° as described in Materials and Methods. At the end of the centrifugation the gradients were fractionated and each fraction was incubated with 20 nM [^{3}H]tamoxifen plus or minus 1 μM unlabeled tamoxifen dissolved in 0.1% BSA–TE buffer solution for 1 hr at 30°. [^{3}H]Tamoxifen binding was measured by the DCC method. Saturable binding is represented. Migration of the different classes of lipoproteins was monitored by parallel gradients containing Sudan black stain. The results are representative of three similar experiments.

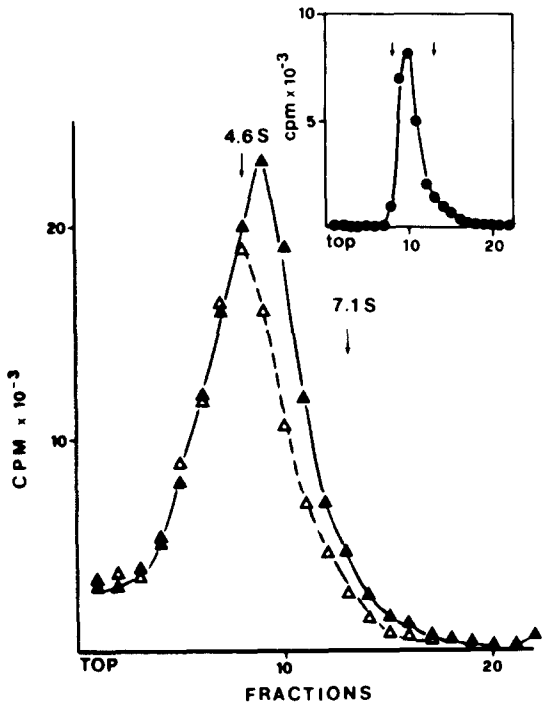

Fig. 8. Sucrose density gradient analysis of [^{3}H]tamoxifen binding in the human serum. Human serum (diluted 1:20 with TE buffer) was incubated with [^{3}H]tamoxifen without (▲) or with 1 μM unlabeled tamoxifen (△) and further processed as described in Materials and Methods. The inset shows the sedimentation profile of the [^{3}H]tamoxifen saturable binding. Arrows show the sedimentation of BSA (4.6 S) and human γ-globulin (7.1 S).

to that for microsomal binding site, although a lower concentration was observed.

AEBS have been mainly studied in animal models or breast cancer cell lines [7–9, 16], while a few data exist about their occurrence and subcellular localization in human breast cancer biopsies [14, 26]. Besides human mammary cancer cell lines, we observed that AEBS were present in both endoplasmic reticulum and nuclear fraction of all the human breast cancer bioptic specimens we studied. We reported previously a positive correlation between ER and AEBS concentrations in the various stages of uterine development [13]. Moreover, the increase in the levels of uterine AEBS by estradiol has been described previously [27, 28]. Consequently, we investigated whether ER and AEBS concentrations were correlated with each other in breast tumor biopsies. AEBS was present in both ER-negative and -positive breast cancer specimens, in agreement with the recent report by Chouvet and Saez [14] but in contrast to data reported by Sutherland and Murphy [26]. Neither microsomal or nuclear concentrations of AEBS correlated with ER levels within the ER-positive group, nor were significant differences in microsomal AEBS levels observed in our study between ER-positive and ER-negative tumors, whereas higher levels of nuclear AEBS were observed in ER-negative samples with respect to the ER-positive ones.

The presence of AEBS in ER-negative tumors suggests the possibility of interactions, of tamoxifen with tumor target cells, which are unrelated to ER-mediated events. Several actions of tamoxifen which are independent of ER or estrogen-mediated events were reported recently, such as estrogen-irreversible growth inhibition and cytotoxic effect [29], calmodulin antagonism [30], and dopamine receptor binding [31]. Interestingly, histamine receptors have more recently been suggested to be associated with intracellular AEBS [32]. Whether intracellular AEBS could participate in mediating the ER-independent actions of tamoxifen remains to be elucidated. Alternatively, microsomal AEBS could represent a site of enzymatic interaction of tamoxifen, since this drug and other triphenylethylene and antiestrogens are known to interact with microsomal cytochrome P-450 and to affect several microsomal enzymes [33].

Besides an intracellular binding site in human breast cancer cells, tamoxifen also has an extracellular binding molecule, since this drug is able to bind to human serum proteins. The finding of serum binding of tamoxifen has been reported previously in guinea pigs [9] and rats [18], but never observed in human serum. The binding specificity of human serum AEBS was similar to that reported for serum AEBS found in other species and to that of intracellular AEBS, being limited to the class of triphenylethylene antiestrogens. In contrast, the affinity of tamoxifen for human serum AEBS was higher than that for the serum AEBS reported in other species [9, 18], but similar to that of intracellular AEBS. Another difference between human serum and rat or guinea pig serum AEBS [11, 18] is represented by the finding that human serum AEBS did not appear to be associated with LDL. This suggests that either species differences concerning the serum proteins binding antiestrogens could exist or serum AEBS is simply associated or linked to LDL in rats and guinea pigs. More detailed studies on the characteristics of the protein domain of the LDL responsible for the binding of tamoxifen in rat and guinea pig serum remain to be done. In this regard, our study shows that the protein binding tamoxifen in human serum appears to be a 5.5 S sedimenting molecule.

In conclusion, the data reported in this study show that tamoxifen is capable of complex interactions with both intracellular and extracellular compartments: specific binding sites were found in the microsomal fraction and to a lesser extent in the nuclei of human breast cancer cells, as well as in human serum. Even though the sedimentation coefficient of human serum AEBS was reminiscent of that of the soluble (or solubilized) intracellular AEBS recently found in human breast cancer cells [14], the relationships between intracellular and extracellular AEBS and the role for these so heterogeneous antiestrogen binding sites in human breast cancer remain to be elucidated.

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