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Rapid and Efficient Purification of Plasma Membrane from Cultured Cells: Characterization of Epidermal Growth Factor Binding†

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ABSTRACT: We have devised a rapid and simple protocol for the purification of the plasma membrane from several lines of transformed cultured cells. A431 or KB plasmalemma was purified in 90 min with a two-step centrifugation cycle after selectively inducing microsomal aggregation by the addition of calcium to homogenized cells. Relative specific activity analysis using membrane marker enzymes on the various fractions indicated that the isolated plasmalemma was purified 8–12-fold over the starting homogenate and contained a high density of epidermal growth factor (EGF) receptors. Transmission electron microscopy showed the final membrane suspension consisted of unilamellar vesicles with an average diameter of approximately 100 Å. The purified membrane vesicles avidly bound to ^{125}I-EGF and reached equilibrium within 30 min. Microfiltration assays indicated more than 90% of the total binding can be displaced by excess unlabeled ligand. Equilibrium binding analysis showed a single class of high-affinity ^{125}I-EGF binding site, with K_d = 0.14 nM and B_{max} = 0.1 pmol/mg of protein for purified KB membrane and K_d = 1.2 nM and B_{max} = 5.26 pmol/mg of protein for purified A431 membrane. Gel electrophoresis of ^{125}I-EGF cross-linked to membrane EGF receptors showed a distinct autoradiographic band at 170 kilodaltons, which could be displaced with excessive amounts of unlabeled EGF. Finally, EGF-dependent autophosphorylation of the EGF receptor was clearly demonstrated with the purified membrane preparation. Membrane vesicles purified in this manner can be stored in liquid nitrogen for several months without losing their biological activity.

Epidermal growth factor (EGF), a single-chain polypeptide with a molecular weight of 6,000, is a potent mitogen for several cell types (Das, 1983; Carpenter & Cohen, 1981; Adamson & Rees, 1981). The transduction of the mitogenic signal across the cell membrane is believed to be mediated by the EGF receptor, a 170-kilodalton (kDa) integral plasmalemma protein (Buhrow et al., 1982; Cohen et al., 1980). Although it is well-known that the EGF receptor is autophosphorylated primarily at the tyrosine-1173 residue upon stimulation with EGF (Downward et al., 1984), neither the role of autophosphorylation nor potential secondary substrates have been clearly identified. The existence of an EGF receptor has been documented in various tissues and in cultured cells (Makku & Stancel, 1985; O&Keefe et al., 1974; Rubin & Earp, 1983; Fernandez-Pol, 1981; Toyota et al., 1986). Due to the unusually high number of EGF receptors found to be present on the A431- and KB-transformed human carcinoma (Cohen, 1983; King & Cuatrecasas, 1982), many previous studies have utilized these two cell lines. We have recently developed a new membrane extraction procedure that allows rapid purification of the cell membrane from these transformed cell lines.

The extraction of plasmalemma from cultured cell lines is an intrinsically difficult process since it is usually limited by the small amount of starting material available and the long lag time needed to grow a sufficient number of cells. Previous methods for cell membrane purification from cultured cells typically required multistep gradient ultracentrifugation ranging from 1 to 16 h (Cohen, 1983; Decker, 1984; Butters & Hughes, 1974; Thom et al., 1977). By selectively inducing

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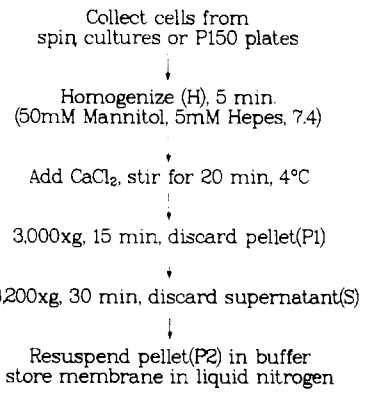

FIGURE 1: Flow chart for the purification of the plasmalemma membrane from cultured cells.

the aggregation of microsomes with calcium (Kamath & Rubin, 1972; Schenkman & Cinti, 1978), we were able to completely eliminate the need for gradient ultracentrifugation and reduce the total centrifugation time to 55 min. This new method significantly shortened the plasma membrane extraction step, and the final preparation showed a comparable degree of purity as in the previous methods. Using the purified membrane preparations from A431 cells, we have already reported a potential substrate that interacted with EGF receptors (Lin et al., 1986a). The detailed procedure for the membrane purification and detection of the active membrane EGF receptor is discussed below.

Materials and Methods

Materials. Purified EGF was purchased from Biomedical Technology (Cambridge, MA). All Sephadex beads and electrophoretic molecular weight markers were from Pharmacia (Piscataway, NJ). Plastic culture ware was from Corning Glass Works (Corning, NY). Culture media and antibiotics were from Grand Island Biologicals (Grand Island, NY). Radiolabeled iodide was obtained from Amersham Corp. Arlington, Heights, IL). [γ-^{32}P]ATP was obtained from ICN Biomedical Co. (Irvine, CA). Bicinchoninic acid and disuccinimidyl suberate were purchased from Pierce Chemical (Rockford, IL). Microfiltration filters were acquired from Millipore Corp. (Bedford, MA). All electrophoretic supplies were from Bio-Rad (Richmond, CA). Triton X-100 was from Sigma (St Louis, MO), and octyl β-glucopyranoside was from Calbiochem (La Jolla, CA). Various other common chemicals were all from major supply houses.

Cell Culture. Both A431 and KB cells were plated at high density in 20–60 P150 (150 mm × 25 mm) culture dishes in α-minimal essential medium (MEM) supplemented with antibiotics and 10% fetal calf serum (FCS) and maintained in a humidified atmosphere of 5% CO_2. The culture medium on the plates was replaced at 4-day intervals until cells reached confluency.

Cell Membrane Purification. The purification procedure is summarized in the flow diagram in Figure 1. All steps used during cell membrane extraction were carried out at 4 °C. Upon reaching confluency, the cells were scraped from the plates with a rubber policeman. The cells were then sedimented with a low-speed spin in a clinical centrifuge (i.e., 10 min at 2500 rpm, IEC HN-S centrifuge), and the weight of the pellet was determined. Typical wet weight of the pellet ranged from 10 to 15 g for 30 plates of P150, pending on the cell type used.

The harvested cell pellet was lysed (1:30 w/v) in a hypotonic homogenization buffer [50 mM mannitol and 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes), pH 7.4] and immediately homogenized in a Waring blender at full speed for 5 min; 1 M calcium chloride solution was then added to the homogenate to a final concentration of 10 mM and the mixture stirred for 10 more min to ensure even distribution of the cation. The calcium-induced membrane aggregate was sedimented with a 3000*g* spin for 15 min. The slightly turbid supernatant solution, which contained the plasmalemma vesicles, was then collected by a 48000*g* spin for 30 min. The round, whitish, translucent membrane pellet at the bottom of the tube was resuspended in the appropriate buffer by triturating with a 1-mL syringe and a 25-gauge needle. The membranes were then aliquoted in polypropylene freeze vials and stored in a liquid nitrogen tank.

Membrane Enzyme Assays. Acid phosphatase (EC 3.1.3.2), glucose 6-phosphatase (EC 3.1.3.9), and 5′-nucleotidase (EC 3.1.3.5) were assayed as in Aronson and Touster (1974). Mg^{2+}-ATPase was assayed as in Eichholz (1968). Alkaline phosphatase was assayed as in Wharton and Goz (1978). The amount of free phosphate released during assays was determined by the procedure of Sanui (1974).

Iodination of EGF. EGF was radiolabeled with sodium [^{125}I]iodide as reported previously (Wakshull & Wharton, 1985). ^{125}I-EGF was separated from the free iodide with a G-15 Sephadex column.

EGF Binding Assay. ^{125}I-EGF binding to the purified membrane vesicles was determined as in Hopfer et al. (1976). The frozen membranes were thawed in a 37 °C water bath for 2 min. A431 or KB cell membranes were then incubated with ^{125}I-EGF in the presence or absence of cold EGF as described in the figure legends. The binding reaction was terminated by vacuum filtration using a 0.22-μm Durapore filter. The filter was then washed once with 3 mL of ice-cold Hanks solution [with 1% bovine serum albumin (BSA) (w/v)]. The complete filtration step required no more than 10 s. The filters were dried, and the amount of radioligand bound to the vesicle was counted in a Packard Autogamma spectrophotometer.

Autophosphorylation of the EGF Receptor. Phosphorylations of the EGF receptor from the purified membrane preparation were performed essentially as described in Carpenter et al. (1979). Thawed membranes were solubilized (1:1) at room temperature for 20 min with a Triton X-100 solution (1% Triton X-100, 1 mM $MnCl_2$, 10% glycerol, and 20 mM Hepes, pH 7.4). Minor amounts of insoluble residue were removed with a Beckman TL-100 ultracentrifuge (360000*g*, 15 min, 4 °C). The supernatant, containing the solubilized EGF receptor, was then further diluted 1:3 (v/v) with 20 mM Hepes and 1 mM $MnCl_2$ (pH 7.4) to reduce the concentration of Triton X-100 to approximately 0.1%.

Unlabeled EGF (2 μg/mL) was added to the solubilized membrane preparation and incubated for 30 min at room temperature. Phosphorylation was initiated by the addition of reaction mixture (1 μCi of [γ-^{32}P]ATP, 10 μM ATP, 20 mM Hepes, and 1 mM $MnCl_2$, pH 7.4) to the solubilized membrane and incubated for 5 min on ice. The reaction was terminated with the addition of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) buffer and heated in a boiling water bath for 5 min.

Transmission Electron Microscopy. Samples of membrane vesicles were concentrated via centrifugation in an Eppendorf microfuge for 10 min; the pellets were fixed for 24 h in 3% glutaraldehyde and postfixed in 1% OsO_3 in 0.1 N phosphate buffer (pH 7.3). Membrane specimens were then dehydrated through a graded alcohol series, embedded in LX-112 resin, and poststained with lead citrate and uranyl acetate. Sections

were observed with a Phillips 410 transmission electron microscope under various magnifications as described in the figure legends.

SDS–Polyacrylamide Gel Electrophoresis and Autoradiography. SDS–PAGE was performed by using 5–15% linear gradient gels in the presence of 0.1% SDS as described in Laemmli (1970). All protein samples were solubilized in standard SDS–PAGE buffer [4% SDS, 5 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) dithiothreitol, 20% glycerol, and 0.125 M tris(hydroxymethyl)aminomethane (Tris), pH 6.8] and heated in a boiling water bath for 5 min. Gels were typically run with circulated cooling at 80 V (constant) overnight. After electrophoresis, gels were stained with Coomassie Blue R-250, destained in 15% methanol and 7.5% acetic acid (v/v), and dried under vacuum in a Bio-Rad slab dryer (Model 483). Autoradiograms of the gels were obtained by using Kodak XAR-5 sheet film with regular intensifying screens at −70 °C.

Disuccinimidyl Suberate (DSS) Covalent Labeling. Covalent cross-linking of ^{125}I-EGF to the membrane EGF receptor was achieved with DSS essentially as described (Pilch et al., 1981). Aliquots of the purified membrane were incubated with ^{125}I-EGF (5×10^5 cpm/tube) for 90 min at 4 °C in Hanks buffer (with 1% BSA, pH 7.4). The unbound ^{125}I-EGF was removed from the solution by washing the membrane with PBS (−Mg^{2+}, Ca^{2+}) 3 times. DSS (20 mM in dimethyl sulfoxide) was then added to the membrane mixture (0.2 mM final concentration) for 15 min on ice to cross-link the receptor to the bound ^{125}I-EGF. The cross-linking reaction was quenched with the addition of 0.5 mL of excess 1 mM EDTA and 10 mM Tris, pH 7.4 (5 min, 4 °C). The treated membranes were pelleted in an Eppendorf centrifuge and processed for SDS–PAGE as described above.

Scatchard Analysis. Binding constants from the purified membrane preparation were measured according to Bylund (1980).

Protein Assay. The amount of protein present was quantified by using the bicinchoninic acid as described in Smith et al. (1985). The presence of neither 1% Triton X-100 nor 1% octyl β-glucopyranoside interfered with the accuracy of the assay.

Results

Relative Specific Activity (RSA) Analysis. The degree of purification for the final membrane pellet was assessed via the specific activities of various known membrane marker enzymes present in the fractions collected. Membrane vesicles purified from KB and A431 cells by using this method showed a high degree of purification (Table I). Of the three cell membrane marker enzymes used, alkaline phosphatase gave the highest folds of purification, with a relative specific activity ratio of 12- and 8-fold over the crude homogenate for A431 and KB cells, respectively. The RSA ratio of 5′-nucleotidase also yielded a similar purification profile, with the final membrane pellet from A431 and KB purified 5- and 9-fold, respectively. A 3-fold purification was also observed with Mg^{2+}-ATPase as a surface membrane enzyme marker.

A nominal amount of contamination was present from endoplasmic reticulum and lysosome, as indicated by the low values of glucose 6-phosphatase and acid phosphatase for A431 and KB membranes.

Attempts to extract BALB/c-3T3 cell membranes by using this method were not as successful. A 5-fold purification was suggested by the RSA value of Mg^{2+}-ATPase, while both alkaline phosphatase and 5′-nucleotidase gave a 2.5-fold purification (Table I). Unlike the A431 and KB cell membranes, however, a major endoplasmic reticulum contamination was indicated by the 14-fold increase of glucose 6-phosphatase activity in the extracted BALB/c-3T3 membrane pellet.

Table I: Relative Specific Activity (RSA) Ratios of Various Membrane Marker Enzymes on Fractions Isolated during the Purification Procedure[a]

enzyme	H	P1	P2	S
alkaline phosphatase				
A431	1	1.5	12.0	
KB	1	0.9	8.3	0.3
Balb	1	0.3	2.6	1.8
Mg^{2+}-ATPase				
A431	1	1.9	3.0	0.3
KB	1	1.0	3.4	1.0
Balb	1	2.1	5.8	1.6
5′-nucleotidase				
A431	1	0.7	5.2	0.8
KB	1	1.5	9.3	0.8
Balb	1	0.7	2.5	2.2
glucose 6-phosphatase				
A431	1	1.0	1.2	2.9
KB	1	0.2	0.3	0.5
Balb	1	1.4	14.0	5.8
acid phosphatase				
A431	1	0.6	2.3	1.0
KB	1	0.7	0.7	0.8
Balb	1			

[a] RSA = specific activity of marker enzyme at each fraction/specific activity of marker enzyme of starting homogenate. Abbreviations indicate various fractions isolated during the purification procedure, as shown in Figure 1. The marker enzyme activity in H (homogenate) is assigned an RSA ratio of 1. P1, being the first pellet collected, contains membrane debris from nuclei, nitochondria, lysosome, and endoplasmic reticulum. P2 is the purified plasma membrane pellet. S is the supernatant fraction, containing the solubilized cellular proteins. Alkaline phosphatase, Mg^{2+}-ATPase, and 5′-nucleotidase were used as enzyme markers for plasmalemma membrane. Glucose 6-phosphatase was used to indicate endoplasmic reticulum membrane, and acid phosphatase was used as a lysosomal membrane marker.

Electron Microscopy. Transmission electron micrographs showed the membrane preparations consisted of a population of unilamellar vesicles with a diameter of roughly 100 Å (Figure 2). Remnants of nuclei, ribosomes, mitochondria, or lysosomes were not detected in the purified membrane sections when viewed under a transmission electron microscope for each of the three cell types.

^{125}I-EGF Binding to the Purified Membrane Vesicles. Binding of ^{125}I-EGF to the purified membrane vesicles of A431 and KB occurred avidly and reached equilibrium rapidly. At 4 °C, greater than 90% of the total amount of ligand bound was seen within 10 min of incubation, and complete binding equilibrium was attained in 30 min for both the A431 and KB cell membranes (Figure 3). ^{125}I-EGF binding to the purified membrane vesicles of A431 and KB was saturable, and greater than 90% of the total amount of ^{125}I-EGF bound to A431 and KB membrane vesicles was specific, as shown by the addition of excess of EGF (Figure 4). Once bound, the level of ligand on the vesicles remained steady for the next 3 h.

Scatchard analysis showed a single class of high-affinity binding sites to ^{125}I-EGF, with K_d = 1.2 nM and B_{max} = 5.26 pmol/mg of protein for the A431 membrane and K_d = 0.14 nM and B_{max} = 0.1 pmol/mg of protein for the KB membrane (Figure 5).

EGF Receptor Autophosphorylation in the Purified Membrane. Using the purified membrane preparation from both A431 and KB cells, we could detect the EGF-dependent phosphorylation of a 170-kDa protein directly via autoradiography without prior immunoprecipitation using an EGF receptor antibody. The phosphorylation of the EGF receptor occurred rapidly and was detected as a single radiolabeled

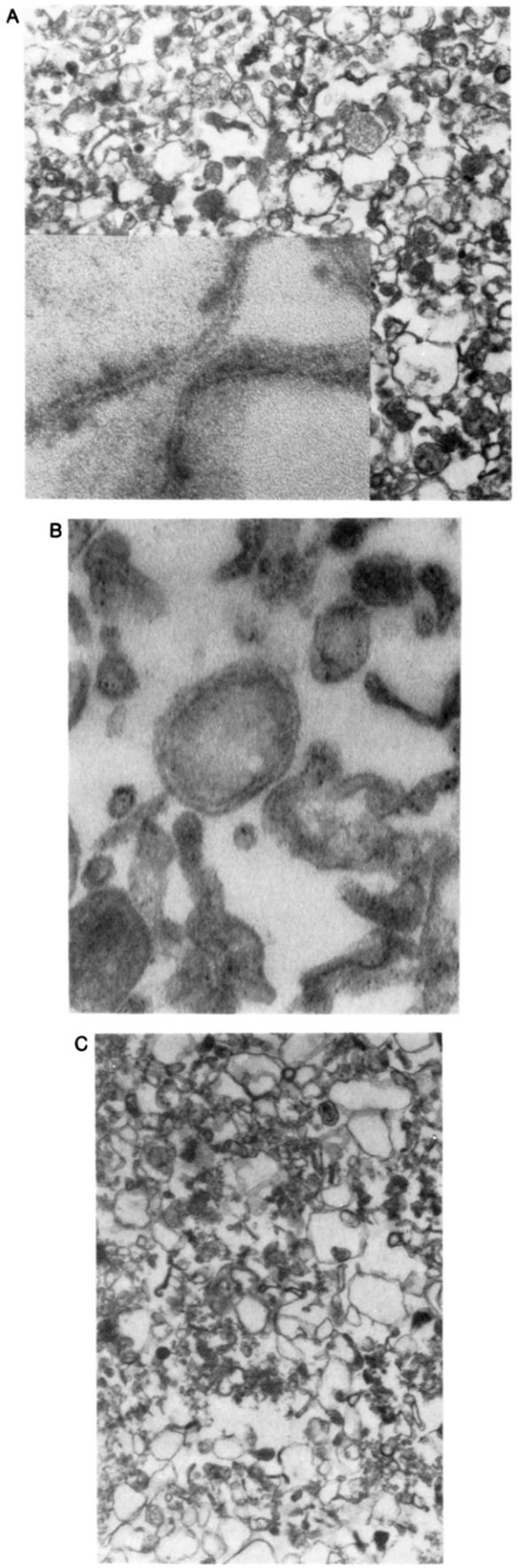

FIGURE 2: Transmission electron micrographs of the purified cell membrane vesicles. (A) A431 cell membrane vesicles at 6600× magnification. The inset demonstrates the unilamellar vesicles at a magnification of 12000×. (B) KB cell membrane vesicle preparation at a magnification of 28000×. (C) BALB/c-3T3 membrane vesicles at 6600× magnification.

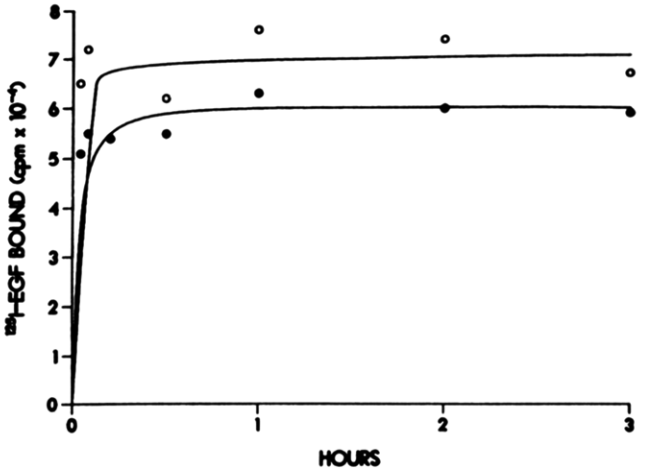

FIGURE 3: Time course of ^{125}I-EGF binding to purified plasma membrane from A431 (open circles) and KB (closed circles) cells. The binding assays were performed as described under Materials and Methods. 5×10^5 cpm of ^{125}I-EGF was added to 35 μg of purified membrane. Incubation lasted for the specified time at 4 °C, and the reaction was terminated via microfiltration as described.

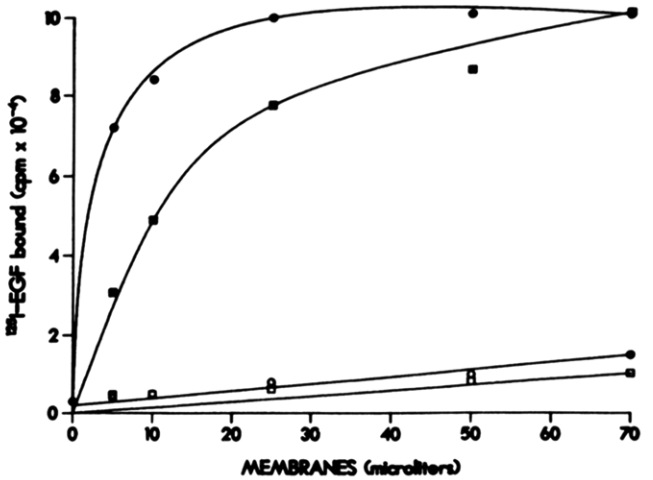

FIGURE 4: Saturation binding curve of ^{125}I-EGF in the presence of increasing amounts of purified plasma membrane from A431 (closed and open circles at 1 μg/μL protein) and KB (closed and open squares at 4 μg/μL protein) cells. Closed symbols denote the total amount of ^{125}I-EGF [specific activity (3–5) $\times 10^4$ cpm/ng] bound to the membrane, and open symbols denote the amount of ^{125}I-EGF non-specifically bound to the membrane (i.e., in the presence of excess cold EGF).

band. The EGF-dependent autophosphorylation of the EGF receptor was seen when the purified membrane was solubilized either with 0.1% Triton X-100 or with 0.5% octyl β-glucopyranoside, although a higher kinase activity for EGF receptor was evident with the Triton X-100 solubilized fraction (Figure 6).

Covalent Labeling of ^{125}I-EGF to Membrane EGF Receptor. Consistent with the autophosphorylation data, the addition of DSS has successfully cross-linked ^{125}I-EGF to the EGF receptor in the purified membrane preparation from A431 cells (Figure 7). Binding to the 170-kDa EGF receptor was specific and could be completely displaced with the addition of excess cold EGF during incubation.

Discussion

Preparative scale purification of cell membranes typically consisted of a series of density gradient centrifugation steps that selectively removes the unwanted constituents, while retaining the desired component. Nuclei, mitochondria, and lysosomes, being of greater density than that of plasma membrane, can be eliminated from the starting homogenate

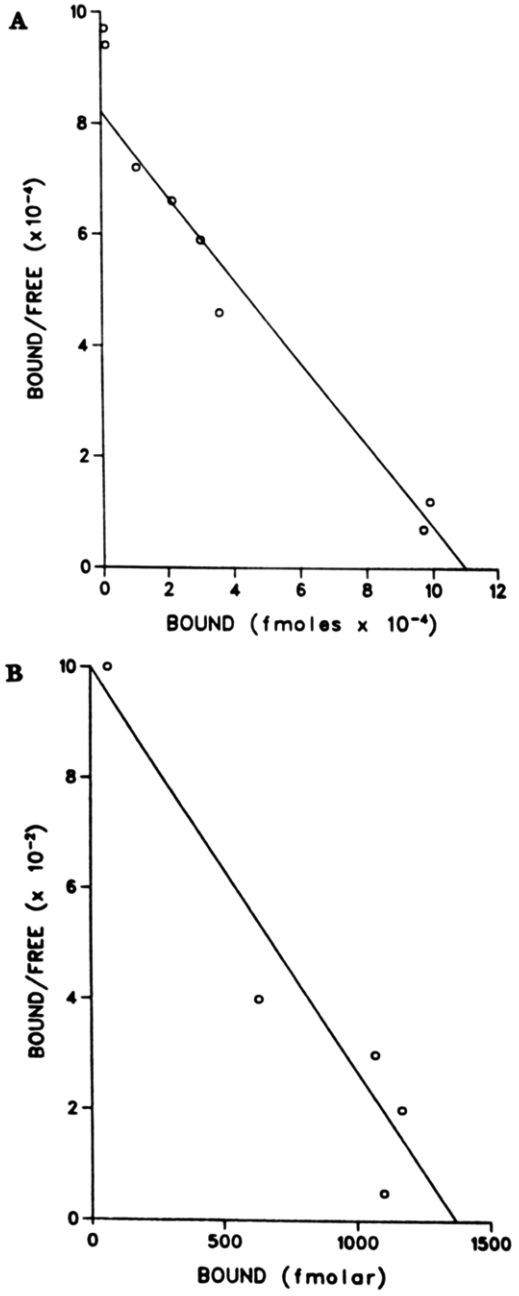

FIGURE 5: Scatchard analysis showing the binding parameters of A431 (A) and KB (B) membranes to ^{125}I-EGF. The lines were fitted by using linear regression analysis from a HP11C calculator. Correlation coefficiencies were greater than 0.95 for both plots.

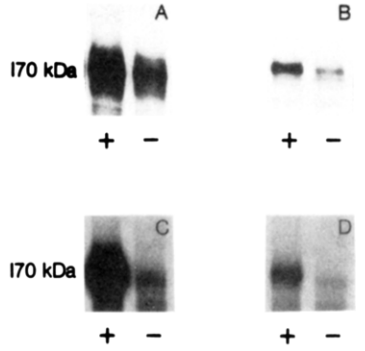

FIGURE 6: EGF-dependent EGF receptor autophosphorylation. Purified membranes were solubilized either with 0.5% octyl β-glucopyranoside (OG) or with 0.1% Triton X-100 and stimulated with EGF as described under Materials and Methods. Phosphorylation was initiated with the addition of 10 μM ATP, 1 μCi of [^{32}P] ATP, and 1 mM $MnCl_2$ for 5 min on ice. Membrane samples were subsequently processed for SDS–PAGE and autoradiography. Molecular weight markers were run concurrently on the gel for size calibration. (A) A431 cell membrane (0.5% OG); (B) KB cell membrane (0.5% OG); (C) A431 cell membrane (0.1% Triton X-100); (D) KB cell membrane (0.1% Triton X-100).

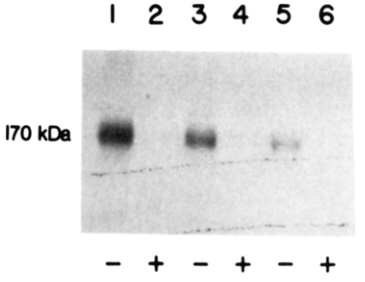

FIGURE 7: Disuccinimidyl suberate cross-linking of ^{125}I-EGF to EGF receptor in purified membrane vesicles [380 μg (lanes 1 and 2), 190 μg (lanes 3 and 4), and 76 μg (lanes 5 and 6)] from A431 in the presence (+) and absence (−) of excessive amounts of unlabeled EGF.

with relative ease [for a review, see Evans (1978)]. The main difficulty in purifying the cell membrane resides in the similarity in density between the endoplasmic reticulum and the plasmalemma fractions which makes the separation between the two membrane types difficult (Evans, 1978). Calcium, at millimolar concentrations, is known to selectively induce microsomal membrane aggregation and has been used to purify the endoplasmic reticulum membrane from liver (Kamath & Rubin, 1972; Schenkman & Cinti, 1978). More recently, its usage has been incorporated into procedures for purifying brush border membranes from various absorptive epithelia (Malathi et al., 1979; Evers et al., 1978). Taking advantage of these observations on the unique role of calcium, we have successfully purified cell membranes from both A431- and KB-transformed human cultured cells.

Transmission electron microscopy of the purified membrane showed that the suspension was a heterogeneous population of unilamellar vesicles with an average diameter about 100 Å. Visual inspection of the purified membrane pellet revealed no contamination from nuclei, mitochondria, or lysosomes. With the use of membrane marker enzymes, relative specific activity analysis has shown that the A431 and KB plasma membrane preparation obtained with this protocol was purified 8–12-fold with respect to the initial homogenate (Table I), a degree of purification that was severalfold greater than the popular rat dermal fibroblast plasmalemma procedure (Thom et al., 1977) and was comparable to the more lengthy procedure for KB membrane purification (Butters & Hughes, 1974). Although alkaline phosphatase, 5′-nucleotidase, and Mg^{2+}-ATPase were documented cell membrane enzyme markers, there was a clear difference in their effectiveness in assessing the folds of purification, as exemplified by the RSA ratio from 3 to 12 obtained in the final membrane preparation for A431. Such a difference may be attributed to the heterogeneous distribution of the membrane marker enzymes on the cell surface, resulting in an uneven extraction of the different marker enzymes during the purification steps, as has been suggested (Evans, 1978).

The degree of membrane purification in A431 and KB was further supported by the binding study. The 1.2 nM K_d of ^{125}I-EGF binding to the purified A431 membrane agreed with the previously reported K_d of 1.5 nM (Fernandez-Pol, 1985). Furthermore, when the EGF receptors from the purified membrane were solubilized with nonionic detergents, such as Triton X-100 or octyl β-glucopyranoside, EGF binding and receptor autophosphorylation activity was preserved, as demonstrated both by the cross-linking experiment (Figure 7) and

by EGF receptor autophosphorylation (Figure 6) in the solubilized system.

The cell membrane pellet extracted from BALB/c-3T3 cells was purified 2.5–5.8-fold as indicated by the plasmalemma enzyme marker. Although high amounts of microsomal contamination as indicated by the RSA ratio of glucose 6-phosphatase were detected, binding of ^{125}I-EGF to the purified BALB/c-3T3 cell membrane was 75% specific as determined by the microfiltration assay.

The addition of calcium ions to the homogenate was critical for the purification procedure. A concentration of 10 mM was chosen on the basis of previous studies for microsomal purification (Kamath & Rubin, 1972) and brush border membrane preparation (Malathi et al., 1979). The addition of calcium caused an immediate and noticeable microsomal aggregation in the cell homogenate, and a large and firmly packed P1 pellet (Figure 1) was collected only in its presence. In the absence of calcium, a small and loosely packed P1 pellet was formed with a majority of the microsomal membrane still in suspension.

The presence of millimolar calcium to the cell homogenate has been shown not to alter the overall structure or integrity of the final membrane pellet (Kamath & Rubin, 1972; Schenkman & Cinti, 1978) and has been recently reported to be superior than magnesium to precipitate endoplasmic reticulum (Aubry et al., 1986). Calcium addition has also been reported to activate Ca-dependent, neutral protease and has resulted in the removal of a 20-kDa fragment from the native 170-kDa EGF receptor (Gates & King, 1985; Ghosh-Dastidar & Fox, 1984). Using Western blot analysis, we have demonstrated that both the 170- and 150-kDa forms of EGF receptor can be detected in the final A431 membrane preparation. The majority of the EGF receptors detected this way, however, existed as the 170-kDa form (Lin et al., 1986b).

In summary, the purification protocol developed here requires no prior enzymatic treatment and can extract 5 mg of purified plasma membrane protein from 20 P150 plates of confluent culture in 90 min. Our method reduced the total amount of centrifugation time from several hours down to 55 min. The speed of the protocol minimizes any nonspecific proteolytic digestion during the membrane isolation procedure and ensured the freshness of the sample. Using the purified membrane preparation, we have demonstrated high-affinity binding to ^{125}I-EGF with K_d value in the nanomolar range. Furthermore, both autophosphorylation and cross-linking experiments have shown that the EGF receptor can be detected without the need for immunoprecipitation prior to SDS–PAGE analysis. Using this protocol, we have successfully scaled the extraction procedure up to 100 P150 plates. The purified membrane pellet can be stored in liquid nitrogen for several months without losing its EGF receptor activity. The simple protocol outlined here should be directly adaptable for the purification of plasma membrane from other cell lines.

Registry No. EGF, 62229-50-9.

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