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Purification and characterization of a bovine acetyl low density lipoprotein receptor

(cell-surface receptors/atherosclerosis/macrophage/scavenger cell pathway/glycoproteins)

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ABSTRACT **The acetyl low density lipoprotein (LDL) receptor is expressed on macrophages and some endothelial cells and mediates macrophage–foam cell formation in culture. A 220-kDa acetyl LDL binding protein was partially purified from bovine liver membranes and was used to make a specific monoclonal antibody. The 220-kDa protein immunoprecipitated by this antibody retained binding activity, and the antibody was used to detect this protein in cells lining bovine liver sinusoids and on the surface of cultured bovine alveolar macrophages. In the human monocytic cell line THP-1, the expression of both acetyl LDL receptor activity and a 220-kDa acetyl LDL binding protein were dramatically induced in parallel after differentiation to a macrophage-like state induced by phorbol ester. The ligand specificity, tissue and cell-type specificity, and coinduction data indicated that this 220-kDa cell-surface binding protein is probably a receptor that mediates acetyl LDL endocytosis. The 220-kDa protein, which was purified 238,000-fold from bovine lung membranes to near homogeneity using monoclonal antibody affinity chromatography, is a trimer of 77-kDa subunits that contain asparagine-linked carbohydrate chains.**

A hallmark of atherosclerosis is the deposition of plasma cholesterol in atherosclerotic plaques in arterial walls (1). Key cellular components of plaques are foam cells, which are derived from monocytes–macrophages (2–4). Chemically modified low density lipoprotein (LDL), such as acetyl LDL, can be rapidly taken up by cultured macrophages via receptor-mediated endocytosis resulting in foam cell formation. This suggested that the acetyl LDL receptor system may play an important role in the formation of foam cells *in vivo* (5–10). Acetyl LDL receptor activity has also been detected in endothelial cells but is usually not expressed at substantial levels in other cell types. This receptor activity is induced during differentiation of cultured human monocytes into macrophages (11) and by the phorbol ester-induced differentiation of the human monocytic cell line THP-1 (12). *In vivo*, high levels of acetyl LDL receptor activity have been detected in the liver, especially in cells lining liver sinusoids, adrenal gland, spleen, and several other organs (13–15). A ligand blotting assay of unreduced specimens has been used by Via and colleagues (16, 17) to detect acetyl LDL binding proteins of 200–260 kDa in a murine macrophage cell line, P388dl, and in rat liver. To obtain further information about the acetyl LDL receptor, we purified and made a monoclonal antibody against a 220-kDa bovine liver protein that exhibited acetyl LDL binding activity. Several lines of evidence, including ligand specificity and cell-type and tissue specificity, indicate that this 220-kDa protein is probably an acetyl LDL receptor. The 220-kDa protein was purified to near homogeneity and was shown to be a trimer of 77-kDa glycoprotein subunits that contain asparagine-linked carbohydrate chains.

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METHODS

Materials. All reagents were obtained as described (ref. 18 or see below) or were from Cappel Laboratories (anti-LDL antibody), Sigma, or Bio-Rad.

Partial Purification from Bovine Liver (Method I). All procedures were performed at 4°C. Membrane proteins prepared from 500 g of bovine liver (19) were resuspended in 500 ml of 10 mM Tris·HCl, pH 8/1 mM $CaCl_2$/0.15 M NaCl/1 mM phenylmethylsulfonyl fluoride (PMSF) (buffer A), sonicated twice, and dissolved by the addition of 55 ml of 20% Triton X-100 with stirring for 30 min. Insoluble material was removed by centrifugation (33,000 rpm, 1 hr; Beckman type 35 rotor). The supernatant (500 ml) was applied (75 ml/hr) to a maleyl–bovine serum albumin (maleyl-BSA) coupled Sepharose 4B column (9.8 × 12 cm; ≈10 mg of maleyl-BSA per ml of gel), which had been equilibrated with buffer A containing 1% Triton X-100. The column was washed overnight with the same buffer and then with two column volumes of buffer A containing 40 mM octyl glucoside. The binding protein was eluted with buffer B (1 M NaCl/20 mM Tris·HCl, pH 8/1 mM $CaCl_2$/1 mM PMFS/40 mM octyl glucoside). The fractions containing acetyl LDL binding activity (100 ml) were pooled and concentrated (35 ml) by ultrafiltration (Diaflo membrane PM30; Amicon). The sample buffer was changed to 25 mM potassium phosphate/40 mM octyl glucoside/1 mM PMSF, pH 6.8, using PD10 columns. The maleyl-BSA affinity-purified fraction (50 ml) was then applied (75 ml/hr) to an Ultrogel-HA (LKB) column (2.5 × 13 cm) and eluted with a gradient of phosphate buffer (25–350 mM) containing 40 mM octyl glucoside. The 220-kDa protein was recovered at phosphate concentrations between 100 and 200 mM and was further purified by nonreducing $NaDodSO_4$/PAGE (3–10% acrylamide gradient) (20). The 220-kDa protein was electroeluted from the gel in 0.1% $NaDodSO_4$/10 mM Tris·HCl, pH 8, using an ISCO 1750 electrophoretic concentrator. Smaller scale purification of the 220-kDa protein from other bovine organs or from cells followed essentially the same procedure.

Immunoaffinity Purification (Method II). All procedures were performed at 4°C. To maleyl-BSA affinity-purified proteins from 500 g of bovine liver or lung in buffer B (100 ml), 100 ml of buffer C (0.1% $NaDodSO_4$/0.1% sodium deoxycholate/1% Nonidet P-40/50 mM Tris·HCl, pH 8/150 mM

Abbreviations: LDL, low density lipoprotein; ^{125}I-acetyl LDL, ^{125}I-labeled acetyl LDL; BSA, bovine serum albumin; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine; PMSF, phenylmethylsulfonyl fluoride.

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NaCl/1 mM PMSF) was added, and the sample was applied (50 ml/hr) to Sepharose 4B coupled with IgG-D1 (see below; 4 mg of antibody per ml of gel; 1 × 2 cm) and recycled overnight. The column was washed consecutively with 50 ml of buffer C, 20 ml of 0.2% Triton X-100/10 mM Tris·HCl, pH 8 (buffer D), 30 ml of buffer D containing 2 M NaCl, 20 ml of 40 mM octyl glucoside, 10 mM Tris·HCl (pH 8) (buffer E), and then bound proteins were eluted with 20 ml of buffer E containing 2 M guanidine thiocyanate. After elution, the buffer was changed to buffer A containing 40 mM octyl glucoside using PD10 columns, and proteins were stored at −70°C.

Monoclonal Antibody. Spleen cells of a mouse immunized with binding protein (15 μg of protein from method I, and 10 μg of booster) were fused (21) with P3X63-Ag8.653 myeloma cells and hybridomas were screened by enzyme immunoassay (22) and immunoblotting (23). The monoclonal antibody IgG-D1 and the control antibody T2D2, which recognizes a distinct and apparently unrelated 287-kDa protein, were isolated from the culture media by Affigel protein A chromatography.

Binding Assays. The filter binding and ligand blotting assays were performed with minor modification, according to methods of Schneider *et al.* (19) and Daniel *et al.* (24), respectively. For the polynucleic acid affinity chromatography assay, the maleyl-BSA purified proteins (150 ng of acetyl LDL binding activity in 4 ml of buffer A containing 40 mM octyl glucoside) was applied to polynucleic acid-coupled agarose columns (AG-POLY series prepacked column; Pharmacia). After washing with the same buffer, the bound proteins were eluted with 5 ml of buffer B.

Immunoprecipitations. The hydroxylapatite purified binding protein from bovine liver or lung (2–10 μg of acetyl LDL binding per mg of protein) was radioiodinated using Iodobeads (Pierce). Immunoprecipitation and sialidase and endoglycosidase F treatments of immunoprecipitates were performed as described (25, 26).

Cell Culture. Bovine alveolar macrophages collected from fresh lung by bronchial lavage with phosphate-buffered saline (PBS; 1 liter per lung) (27) were cultivated with RPMI 1640 medium containing 10% fetal calf serum for 6 hr at 37°C in a CO_2 incubator and washed with PBS six times to remove nonadherent cells. The uptake of fluorescent acetyl LDL labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) was as described (28). For immunoblotting, cells from two 150-mm dishes were incubated with trypsin (0.05%) or PBS for 5 min at 37°C. Attached cells and floating cells were pooled, and the 220-kDa protein was partially purified by maleyl-BSA affinity chromatography prior to immunoblotting. The viability of cells (e.g., trypan blue staining or restoration of DiI acetyl LDL uptake after overnight cultivation) was not significantly affected by the trypsin treatment. THP-1 cells were maintained and induced to differentiate as described (12).

Other Methods. Cellular acetyl LDL receptor activity was assayed by measuring the degradation of ^{125}I-labeled acetyl LDL (^{125}I-acetyl LDL) (2 μg of acetyl LDL protein per ml) in the presence or absence of inhibitor (maleyl-BSA, 500 μg/ml) as described (18). Acetylation and maleylation of proteins and lipoproteins were performed (5) and protein concentrations were determined (19) as described.

RESULTS AND DISCUSSION

A 220-kDa protein with acetyl LDL binding activity was partially purified >2000-fold from bovine liver membranes by a combination of maleyl-BSA affinity chromatography, hydroxylapatite chromatography, and preparative $NaDodSO_4$/PAGE (Table 1, method I; Fig. 1). A broad band (apparent molecular mass, >300 kDa) of binding activity was also detected in the maleyl-BSA fraction (lane D). However, the amount of this material relative to the acetyl LDL filter binding activity varied substantially from preparation to preparation.

Table 1. Purification of the 220-kDa acetyl LDL binding protein from bovine liver and lung

Fraction	Protein, mg per fraction	High-affinity ^{125}I-acetyl LDL binding: Total, μg	Specific activity, μg/mg	Purification factor, -fold
Method I				
Liver membrane	21,580	107.9	0.005	1
Maleyl-BSA purified	72	154.8	2.15	430
Hydroxylapatite purified	6	62.8	10.47	2,094
Preparative PAGE	0.025*	—	—	—
Method II				
Lung membrane	22,800	136.8	0.006	1
Maleyl-BSA purified	45	121.0	2.684	427
Immunoaffinity purified	0.03†	42.0	1430	238,000

Membranes from 500 g of bovine liver or lung were prepared and processed as described. Values for high-affinity binding of acetyl LDL (2 μg/ml) were determined by the filter binding assay. The results shown are from representative preparations. —, Due to the presence of $NaDodSO_4$, the activity could not be determined.
*Estimated from chromogeneity on silver staining of polyacrylamide gels.
†Estimated from amino acid composition analysis.

A mouse monoclonal antibody, IgG-D1, was raised against the unreduced gel-purified 220-kDa protein and recognized the 220-kDa protein on immunoblotting (Fig. 1, lane G). Reduction abolished the binding of IgG-D1 and acetyl LDL ligand blotting activity (data not shown). The results of immunoprecipitation of ^{125}I-labeled specimens by IgG-D1 and control antibody T2D2 are shown in Fig. 2. IgG-D1 specifically precipitated (lane A) three ^{125}I-labeled species (220, 150, and 77 kDa). These related species are described below. The immunoprecipitated 220-kDa protein was recovered and shown to retain acetyl LDL binding activity by ligand blotting (lane E). The acetyl LDL binding protein was also purified 238,000-fold by a combination of maleyl-BSA affinity chromatography and IgG-D1 immunoaffinity chromatography (Table 1, method II). The immunoaffinity-purified activity also exhibited apparent masses of 220, 150, and 77 kDa on nonreducing $NaDodSO_4$/polyacrylamide gels (Fig. 2, lane G; see below).

The cell-type specificity and tissue distribution of the 220-kDa protein and its relation to the acetyl LDL receptor were studied by immunochemical techniques. Previous studies of the acetyl LDL receptor in liver indicated that: (*i*) the activity was associated with cells lining liver sinusoids, (*ii*) an area immediately surrounding the central vein of hepatic lobules exhibited reduced receptor activity, and (*iii*) hepatocytes do not exhibit substantial receptor activity (13–15). Immunohistochemical analysis of frozen liver sections using IgG-D1 (Fig. 3) showed that the distribution of the 220-kDa binding protein matches that of acetyl LDL receptor activity. There was specific immunostaining of cells lining the sinusoids, essentially no staining within the hepatocyte plates (Fig. 3 *B* and *C*), and little staining was observed in the area immediately surrounding the central vein (CV in *B*).

The distribution of the 220-kDa protein and acetyl LDL binding activity in various bovine organs was examined by immuno- and ligand blotting. There was close correlation of

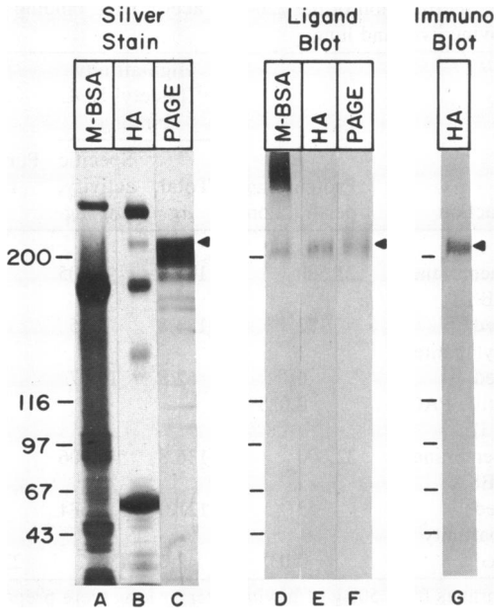

FIG. 1. Silver stain, ligand blot, and immunoblot analysis of partially purified 220-kDa acetyl LDL binding protein. Aliquots of partially purified bovine acetyl LDL binding protein were subjected to nonreducing $NaDodSO_4$/PAGE (3–10% polyacrylamide gradient). The proteins on the gel were either stained with silver (lanes A–C) or electroblotted (at 0.2 A, 4°C, for 16 hr) onto cellulose nitrate paper. For acetyl LDL ligand blotting (lanes D–F), the blot was incubated with acetyl LDL (5 μg/ml) at room temperature for 1 hr, and the acetyl LDL binding was visualized by using rabbit anti-LDL antibody (1:1000 dilution) and goat anti-rabbit IgG conjugated with peroxidase (1:1000 dilution). Lanes A and D, maleyl-BSA affinity-purified fraction (M-BSA, 12.5 μg and 50 μg of protein); lanes B and E, hydroxylapatite-purified fraction (HA, 5 μg and 20 μg); lanes C and E, gel-purified sample (PAGE, 2.5 μg and 5 μg). Lane G, IgG-D1 immunoblotting of hydroxylapatite-purified fraction (20 μg). Arrowheads indicate the 220-kDa protein. Molecular mass standards: myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase B, 97.4 kDa; BSA, 67 kDa; and ovalbumin, 43 kDa.

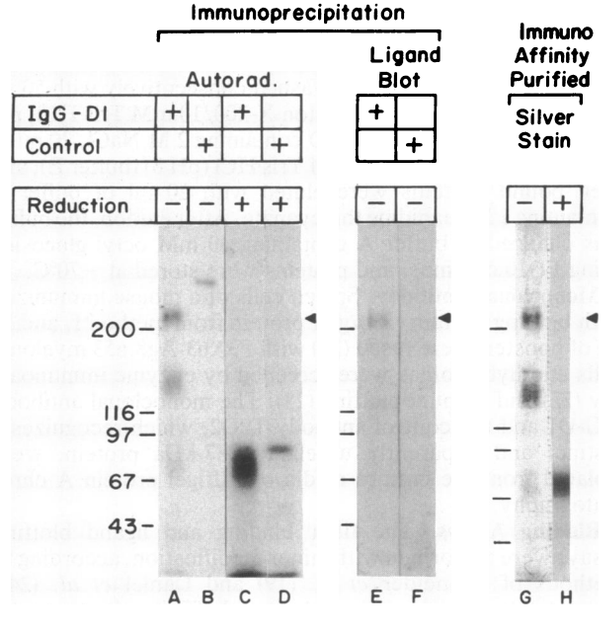

FIG. 2. Immunoprecipitation and immunoaffinity purification of acetyl LDL binding protein. For immunoprecipitation, 120 μl of iodinated hydroxylapatite-purified fraction from bovine lung (10^6 cpm), 300 μl of unlabeled hydroxylapatite-purified fraction (0.6 μg of binding activity), 9 μg of monoclonal antibody IgG-D1 or control antibody T2D2, and salts and detergents [final vol, 600 μl; final concentrations, 0.1% $NaDodSO_4$, 0.1% sodium deoxycholate, 1% Nonidet P-40, 50 mM Tris·HCl (pH 8), 150 mM NaCl, and 1 mM PMSF (buffer C)] were mixed and incubated for 1 hr at room temperature. Then 50 μl of protein A-Sepharose (50% in PBS) was added and the mixture was rotated for 1 hr at 4°C. The precipitate was washed four times with buffer C, dissolved in sample buffer [10% $NaDodSO_4$, 10% (vol/vol) glycerol, 0.1% bromophenol blue, 12.5 mM Tris·HCl (pH 6.8) with or without 5% 2-mercaptoethanol as indicated], subjected to $NaDodSO_4$/PAGE, electroblotted onto cellulose nitrate paper, and visualized by autoradiography (−70°C for 48 hr, lanes A–D). The same cellulose nitrate paper was also subjected to acetyl LDL ligand blotting (lanes E and F are blots of the specimens shown in lanes A and B). The IgG-D1 immunoaffinity-purified samples were dissolved into sample buffer and subjected to $NaDodSO_4$/PAGE and stained with silver (lanes G and H; 870 ng per lane). Arrowheads indicate the 220-kDa protein. Molecular mass standards are the same as in Fig. 1.

the intensities of staining and of the electrophoretic mobilities (220 kDa) of the antibody binding and ligand binding proteins (data not shown). Ligand binding activities and the amounts of 220-kDa protein were high in the liver, spleen, and adrenal gland, which take up acetyl LDL efficiently *in vivo* (13). High levels were also detected in the lung, possibly because of macrophages in the alveolar air space. The intestine, which takes up little acetyl LDL (13), contained very little 220-kDa protein and little binding activity.

Coincident expression of acetyl LDL receptor activity (fluorescent DiI acetyl LDL accumulation; Fig. 4*A*) and the 220-kDa protein (immunoblotting with IgG-D1; Fig. 4*B*, lane A) was also detected in cultured bovine alveolar macrophages. Fluorescent acetyl LDL uptake (and ^{125}I-acetyl LDL degradation; data not shown) was blocked by excess unlabeled acetyl LDL (Fig. 4*A*) and immunodetection of the 220-kDa protein was prevented by mild sublethal trypsin digestion (Fig. 4*B*, lane B). The trypsin sensitivity of this protein in intact cells suggests that some of the 220-kDa protein was exposed at the cell surface. The cell surface location suggests that this 220-kDa protein may be responsible for some or all of the acetyl LDL receptor activity expressed by these cells.

Coincident and coordinately regulated expression of acetyl LDL receptor activity and a 220-kDa acetyl LDL binding protein were also observed when THP-1 human monocytes differentiated into macrophage-like cells (31). Hara *et al.* (12) reported that acetyl LDL receptor activity is induced dramatically in THP-1 cells during phorbol ester-mediated differentiation. Fig. 5 shows that phorbol ester induction of receptor activity (^{125}I-acetyl LDL degradation assay) was accompanied by substantial increases in the ^{125}I-acetyl LDL binding activity of cell membranes (filter binding assay) and in the activity of a 220-kDa acetyl LDL binding protein (ligand blot assay). The IgG-D1 antibody, which apparently detects species-specific epitopes, did not recognize and thus could not be used to study proteins in THP-1 cells or in human liver or lung (data not shown). Addition of IgG-D1 at up to 50 μg/ml to bovine alveolar macrophages did not block acetyl LDL receptor activity (data not shown), suggesting that this antibody may not bind to surface-accessible epitopes or to the ligand binding domain(s) of the acetyl LDL receptor.

We have observed striking coincidence in the tissue and cell-type distribution, the intratissue and intracellular localization, the phorbol ester induction (THP-1 cells), and the ligand binding properties (see below) of the purified 220-kDa acetyl LDL binding protein and acetyl LDL receptor activity. Taken together, these results very strongly suggest that the 220-kDa binding protein purified in the current study and recognized by IgG-D1 is a receptor that mediates the cell-type and tissue-specific endocytosis of acetyl LDL.

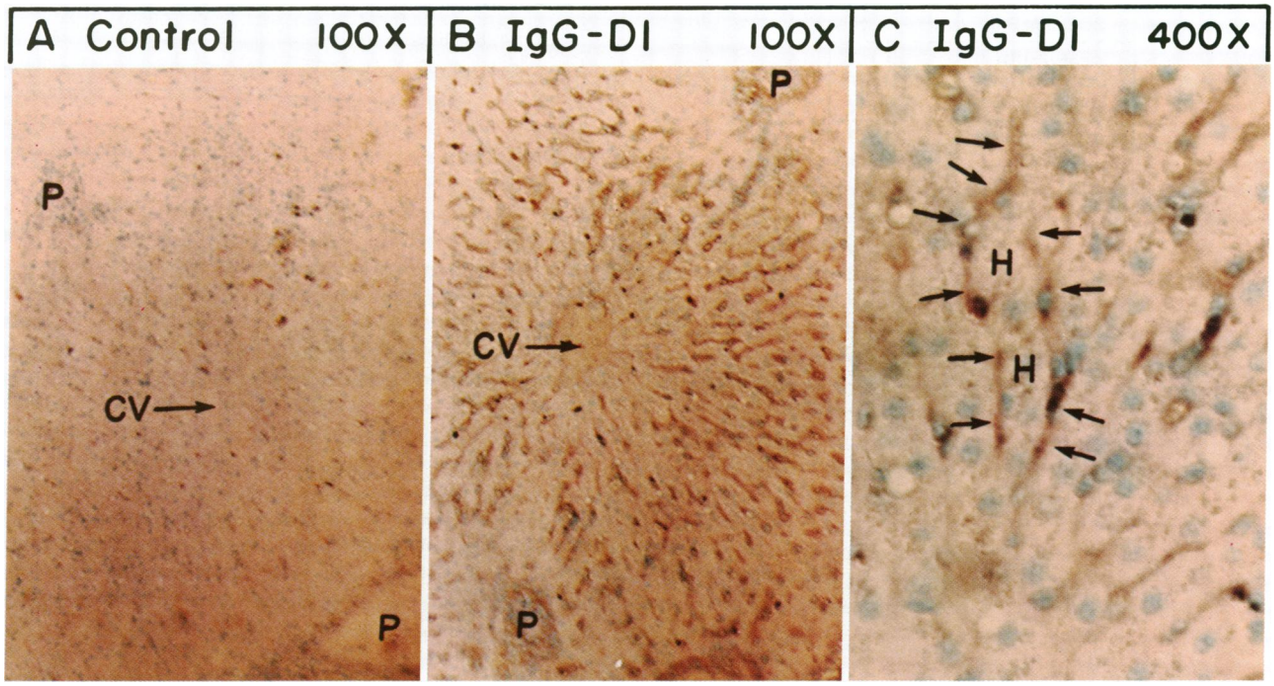

FIG. 3. Immunohistochemical localization of IgG-D1 antigen in frozen bovine liver sections. Bovine liver sections (6 μm) were cut on a cryostat at −20°C, placed on glass slides, air-dried for 1 hr, and fixed in cold 95% methanol. The sections were incubated with IgG-D1 (6 μg/ml) or control mouse IgG, and the binding of IgG was visualized by using peroxidase-conjugated sheep anti-mouse IgG Fab fragment by 3,3′-diaminobenzidine tetrahydrochloride staining (29). Methyl green staining was used to visualize nuclei. (*A*) Control antibody. (*B*) IgG-D1. The brown peroxidase staining was detected along cells lining the sinusoids. (*C*) IgG-D1. Staining is seen along the sinusoidal lumen (arrows) but is not associated with hepatocyte plates (indicated by H). CV, central vein; P, portal area. (*A* and *B*, ×100; *C*, ×400.)

The structure and ligand binding properties of the acetyl LDL binding protein were examined further. The receptor protein purified by immunoaffinity chromatography bound ^{125}I-acetyl LDL with an apparent dissociation constant of 0.5 μg/ml (0.8 nM) (Fig. 6*A*). The maximum binding of ^{125}I-acetyl LDL was ≈1.4 mg/mg. The acetyl LDL receptor has been shown (8) to recognize a broad, but limited, range of negatively charged macromolecules. The ^{125}I-acetyl LDL binding was inhibited by maleyl-BSA, fucoidan, polyvinylsulfate, and poly(IC), but not by LDL (Fig. 6*B*). Among purine polynucleotides, poly(I) and poly(G) can inhibit the receptor-mediated endocytosis and degradation of ^{125}I-acetyl LDL but poly(A) cannot (9). The binding activity of the maleyl-BSA affinity-purified protein from bovine liver could bind to poly(I) and poly(G) columns (67% and 91% of total activity applied, respectively), but did not bind effectively to poly(A) (9%) or poly(C) (18%) columns. Thus, the binding properties of the purified 220-kDa protein and the cellular acetyl LDL receptor (9) are similar, findings consistent with the conclusion that the 220-kDa protein is an acetyl LDL receptor.

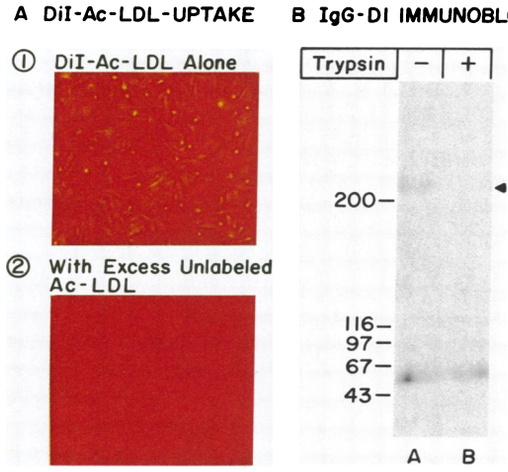

FIG. 4. Uptake of DiI acetyl LDL and immunoblotting of 220-kDa protein from bovine alveolar macrophages. (*A*) Uptake of DiI acetyl LDL (20 μg/ml) by bovine alveolar macrophages was observed (30) after an overnight incubation in the absence (*A1*) or presence of excess unlabeled acetyl-LDL (400 μg/ml; *A2*) in Ham's F-12 medium supplemented with 7% fetal calf lipoprotein-deficient serum. (*B*) Acetyl LDL binding activity was partially purified by maleyl-BSA chromatography from bovine alveolar macrophages treated without (lane A) or with (lane B) trypsin and was subjected (100 μg per lane) to immunoblotting with IgG-D1. The binding of IgG-D1 was visualized by using rabbit anti-mouse IgG (1:2000 dilution) and ^{125}I-labeled protein A (3×10^5 cpm/ml). The blot was exposed to x-ray film at −70°C for 3 days. Arrowhead indicates the 220-kDa protein. Molecular mass standards are the same as in Fig. 1.

The receptor proteins precipitated by IgG-D1 and purified by immunoaffinity chromatography exhibited apparent masses of 220, 150, and 77 kDa on nonreducing NaDodSO$_4$/polyacrylamide gels, and after reduction they collapsed into 77-kDa bands (Fig. 2, lanes C and H). When the 220- and 150-kDa proteins obtained by immunoprecipitation or immunoaffinity chromatography were individually recovered from preparative polyacrylamide gels and then reduced, the apparent mass of each was converted to 77 kDa (data not shown). These results indicate that the 220- and 150-kDa proteins are trimers and dimers of 77-kDa subunits. Sialidase or endoglycosidase F reduced the apparent mass of the immunoprecipitated 77-kDa subunits by 5 and 13 kDa, respectively (data not shown). These results indicate that the 77-kDa protein possesses asparagine-linked carbohydrate chains and sialic acid. The trimeric subunit structure of the bovine acetyl LDL receptor is similar to that of other cell-surface membrane glycoproteins, such as vesicular stomatitis virus G protein and influenza virus hemagglutinin.

200 nM PMA	−	+
Acetyl-LDL Degradation (ng/mg/5hr)	165	1347
Filter Binding Assay (ng/mg)	110	1044

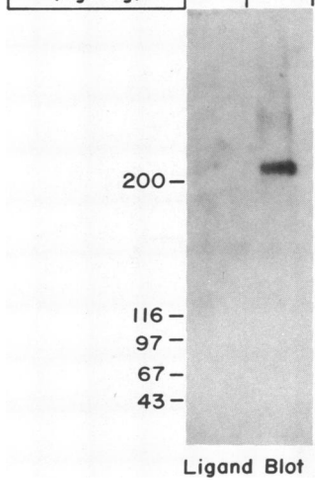

FIG. 5. Coinduction of 220-kDa acetyl LDL binding protein and cellular acetyl LDL receptor-mediated endocytosis in THP-1 cells. THP-1 cells were cultivated with or without 200 nM phorbol 12-myristate 13-acetate (PMA) for 3 days in RPMI 1640 medium containing 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The samples were then subjected to assays for cellular receptor-mediated endocytosis (degradation of ^{125}I-acetyl LDL), and for acetyl LDL binding to maleyl-BSA affinity-purified membrane proteins (≈300 μg of protein per 10^8 cells) using the filter binding (53.3 μg of protein per tube) and ligand blotting (100 μg of protein per lane) methods. For the cellular degradation assay (3×10^5 cells per well), ^{125}I-acetyl LDL (2 μg of protein per ml) degradation was measured with minor modifications as described (18). Each value represents the average of duplicate determinations corrected for nonspecific degradation measured in the presence (single determinations) of competing maleyl-BSA (500 μg/ml). In the ligand blotting assay, acetyl LDL binding was visualized by using rabbit anti-LDL antibody (1:1000 dilution) and ^{125}I-labeled protein A (10^5 cpm/ml). Arrowhead indicates the 220-kDa protein. Molecular mass standards are the same as in Fig. 1.

Trimerization of these viral proteins is critical for intracellular processing and transport (32, 33). Additional experiments focused on the structure and function of the 220-kDa acetyl LDL receptor protein may help provide information about the physiological role of acetyl LDL receptors and their relationship to atherogenesis.

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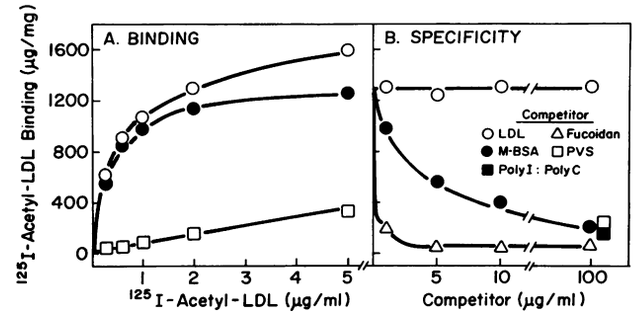

FIG. 6. Characteristics of acetyl LDL binding to the immunoaffinity-purified receptor. The filter binding assay was used to assess the affinity (*A*) and specificity (*B*) of acetyl LDL binding to immunoaffinity-purified receptor (12.3 ng per tube). (*A*) Specific binding (●) was calculated by subtracting the values determined in the presence of excess maleyl-BSA (500 μg/ml; □, single determinations) from those determined in its absence (○, duplicate determinations). (*B*) Competition of acetyl LDL binding was measured by preincubating immunoaffinity-purified receptor–phospholipid complexes with the indicated concentrations of competitors for 30 min prior to addition of ^{125}I-acetyl LDL (2 μg of protein per ml) for 1 hr at 4°C (standard assay). Values represent averages of duplicate determinations. The competitors were as follows: ○, LDL; ●, maleyl-BSA; △, fucoidan; □, polyvinyl sulfate; ■, poly(I·C).

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