

Quantitative analysis of albumin uptake and transport in the rat microvessel endothelial monolayer

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Quantitative analysis of albumin uptake and transport in the rat microvessel endothelial monolayer. *Am J Physiol Lung Cell Mol Physiol* 284: L187–L196, 2003; 10.1152/ajplung.00152.2002.—We determined the concentration dependence of albumin binding, uptake, and transport in confluent monolayers of cultured rat lung microvascular endothelial cells (RLMVEC). Transport of ¹²⁵I-albumin in RLMVEC monolayers occurred at a rate of 7.2 fmol/min/10⁶ cells. Albumin transport was inhibited by cell surface depletion of the 60-kDa albumin-binding glycoprotein gp60 and by disruption of caveolae using methyl-β-cyclodextrin. By contrast, gp60 activation (by means of gp60 cross-linking using primary and secondary antibodies) increased ¹²⁵I-albumin uptake 2.3-fold. At 37°C, ¹²⁵I-albumin uptake had a half time of 10 min and was competitively inhibited by unlabeled albumin (IC₅₀ 1 μM). Using a two-site model, we estimated by Scatchard analysis the affinity (K_D) and maximal capacity (B_{max}) of albumin uptake to be 0.87 μM (K_{D1}) and 0.47 pmol/10⁶ cells (B_{max1}) and 93.3 μM (K_{D2}) and 20.2 pmol/10⁶ cells (B_{max2}). At 4°C, we also observed two populations of specific binding sites, with high (K_{D1} 13.5 nM, 1% of the total) and low (K_{D2} 1.6 μM) affinity. On the basis of these data, we propose a model in which the two binding affinities represent the clustered and unclustered gp60 forms. The model predicts that fluid phase albumin in caveolae accounts for the bulk of albumin internalized and transported in the endothelial monolayer.

capillary permeability; vesicular transport; caveolae; albumin-binding glycoprotein gp60

THERE IS ACCUMULATING EVIDENCE from morphological and functional studies that albumin is continuously exchanged between the microvascular and interstitial compartments by receptor-mediated albumin transcytosis (8, 11, 13, 16–19, 23, 27–29). Recent studies show that albumin molecules can associate with surface glycoproteins such as gp60 (albumin) in endothelial cells, which can activate the transport of albumin (2, 6–8, 13, 19, 20, 23, 25, 28, 29). The signaling pathways mediating transcytosis are not

well understood. The pathway involving the heterotrimeric G protein G_i and the activation of Src kinase signaling may activate albumin endocytosis and its transport from the luminal to the abluminal cell surface (12–14, 27). Interestingly, albumin transcytosis was augmented by antibody-induced cross-linking of gp60 (13, 27–29). These studies suggest that, under physiological conditions, albumin can bind to its cell surface receptors [the albumin-binding proteins (ABPs)] and initiate transcytosis.

However, important issues concerning the mode of albumin uptake and transport by a transcellular route remain unresolved. Fluid phase transport of albumin in vesicles or via the paracellular pathway would not be expected to be a saturable process, whereas transport of albumin, either bound to receptors or regulated by receptors, should be saturable. In addition, the high-affinity nature of ABPs such as gp60 (in the nanomolar range) (19, 25) relative to plasma and interstitial concentrations of albumin (1 mM) implies that 1) albumin uptake and transcytosis should be constitutive processes and 2) bound albumin may not dissociate from its vesicular carriers upon exocytosis. These questions could not be previously addressed with any rigor, because determinations of affinity of albumin to the endothelial plasmalemma were hampered by high nonspecific binding (2, 6, 7, 19). This masked any saturable and specific albumin binding. In the present study, we determined the concentration dependency of albumin binding on the surface of confluent endothelial monolayers and transport of albumin. We specifically used cultured rat lung microvascular endothelial cells (RLMVEC), because they were shown by us to have low nonspecific cell surface adsorption of albumin. We show that 1) binding and uptake of albumin have saturable and fluid phase components, 2) cellular uptake of albumin is submaximal at physiological serum albumin concentration, implying that it can be further increased in response to appropriate signals, and 3) gp60, an ABP, can activate albumin transport.

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METHODS

Endothelial Cell Cultures

RLMVEC (VEC Technologies, Rensselaer, NY) were cultured in high-glucose DMEM (GIBCO BRL, Grand Island, NY) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT) plus 50 U/ml penicillin and 50 g/ml streptomycin, as described previously (4). The cultures were maintained in 5% CO₂-95% room air at 37°C.

Drugs and Reagents

All drugs and reagents were obtained from Sigma Chemical (St. Louis, MO) unless stated otherwise. Hanks' balanced salt solution (HBSS) containing NaHCO₃ (4.2 mM) and HEPES (10 mM) was adjusted to pH 7.4. Bovine serum albumin (fraction V, 99% pure, endotoxin free, cold alcohol precipitated) was freshly dissolved in HBSS in concentrations of 0.01–100 mg/ml. Methyl- β -cyclodextrin was dissolved in HBSS. Rabbit antibody against bovine endothelial gp60 (anti-gp60 antibody) was prepared and labeled with Cy3, as described elsewhere (25, 27). Control isotype-matched antibody was isolated from rabbit preimmune serum. Monoclonal anti-caveolin-1 antibody was obtained from Transduction Laboratories (Lexington, KY). Goat anti-mouse IgG labeled with Alexa 568 or 350, bovine serum albumin-Alexa 488 conjugate, and cholera toxin subunit B-Alexa 594 conjugate were purchased from Molecular Probes (Eugene, OR).

Albumin Iodination

Labeling of albumin with Na-¹²⁵I (ICN Pharmaceuticals) was performed using chloramine T (3). The tracer albumin formed was purified to 0.4% free ¹²⁵I using Sephadex G-25 columns (Sigma Chemical). Specific radioactivity was 0.3–1.0 Ci/g albumin determined by 10% trichloroacetic acid precipitation.

Methyl- β -Cyclodextrin Treatment

Confluent RLMVEC monolayers were incubated with methyl- β -cyclodextrin (0.2 nM–10 mM) in HBSS for 15 min, washed twice with HBSS, and incubated with fresh medium containing tracer albumin for the desired periods.

Immunostaining

Cellular localization of albumin, gp60, and caveolin-1 in the plasma membrane and plasmalemmal vesicles exposed to albumin was determined by immunocytochemical labeling and laser scanning confocal microscopy (LSM 510, Zeiss). RLMVEC were serum deprived for 2 h, washed three times with HEPES-buffered HBSS or phenol red-free DMEM, and exposed to 0.1 mg/ml albumin in the presence of 50 g/ml Alexa 488-conjugated albumin, 20 g/ml cholera toxin subunit B-Alexa 594 conjugate, and/or 3.5 g/ml Cy3-anti-gp60 antibody for up to 30 min (13). Cells were then washed three times with HBSS, fixed with 4% paraformaldehyde in HBSS, and blocked for 30 min in HBSS containing 5% goat serum, 0.1% Triton X-100, and 0.01% NaN₃. Primary antibody labeling was performed overnight at 4°C with anti-caveolin-1 antibody (1 g/ml). Coverslips were then washed three times for 10 min in HBSS, blocked for 30 min with 5% goat serum, and incubated for 2 h at room temperature with fluorescently labeled goat anti-rabbit and goat anti-mouse secondary antibody. In some cases, 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, 1 g/ml) was added to visualize the nucleus. Confocal microscopy was performed using 364-,

488-, and 568-nm excitation laser lines to detect DAPI/Alexa 350 (band pass 385–470 nm emission), FITC/Alexa 488 (band pass 505–550 nm emission), and rhodamine/Alexa 568 fluorescence (long pass 585 nm emission) in optical sections 1 μ m thick (pinhole set to achieve 1 Airy unit).

Cell Surface gp60 Cross-Linking

RLMVEC monolayers were washed twice with HEPES-buffered DMEM at 4°C and incubated for 30 min at 4°C with anti-gp60 antibody (20 g/ml) and then with secondary antibody (goat anti-rabbit, 20 g/ml) for 30 min to cross-link gp60. Controls were treated with the preimmune antibody (20 g/ml) at 4°C for 30 min. Cells were rewarmed to 37°C to activate endocytosis (27). To deplete ABP gp60 (27), anti-gp60 antibody was preincubated with cells for 2 h at 37°C.

Binding of ¹²⁵I-Albumin

Confluent cell cultures in six-well plates (1.0–1.2 $\times 10^6$ cells/35 mm) were washed twice in 10 mM HEPES-buffered DMEM, pH 7.4, and then serum deprived for 2 h by incubation in DMEM. Cells were precooled to 4°C for 30 min, and ¹²⁵I-albumin in the presence or absence of unlabeled albumin in HBSS was added for 30 min at 4°C. Unbound ligand was removed by three washes with ice-cold HBSS, and cells were lysed with 1 ml of 50 mM Tris HCl buffer, pH 7.4, containing 1% Triton X-100 and 0.5% SDS (13). Cell-associated ¹²⁵I-albumin counts were measured in a gamma counter (Packard Instruments, Downers Grove, IL).

Uptake of ¹²⁵I-Albumin

Serum-deprived confluent RLMVEC monolayers (see above) were pretreated with indicated reagents at 37°C for required periods before addition of ¹²⁵I-albumin in the presence or absence of unlabeled albumin in HBSS. Uptake at 37°C was allowed to proceed for times indicated and then terminated by chilling on ice and washing three times with ice-cold HBSS. To remove cell surface-bound ¹²⁵I-albumin, cells were washed three times with 1 ml of acid wash buffer (0.5 M NaCl and 0.2 M acetic acid, pH 2.5) (26). Cells were then lysed and counted as described above.

Transendothelial ¹²⁵I-Albumin Transport

RLMVECs were grown on clear microporous polyester Transwell membranes (12 mm diameter, 1 cm² growth area, 0.4 μ m pore size; Corning Costar, Cambridge, MA). The membrane inserts were filled with a total of 0.5 ml of incubate containing ¹²⁵I-albumin in the presence or absence of unlabeled albumin. The lower well was filled with 1.5 ml of incubate of the same osmolarity as the inner well. Thus fluid levels and osmotic pressure in the "upper" and "lower" wells were equalized to minimize hydrostatic and osmotic effects. Labeled albumin and test agents were added to the upper well in equimolar solution, and transendothelial ¹²⁵I-albumin permeability was measured at 37°C. Aliquots of 50 μ l were sampled from the lower chamber every 10–15 min, and gamma radioactivity was measured. Albumin permeability was calculated for radiolabeled albumin flux across the cell monolayer, as described elsewhere (5, 22). Trichloroacetic acid precipitation analysis and SDS-PAGE of the tracer albumin (27) in the abluminal chamber showed that the radioactivity measured on the abluminal side of the cell monolayer remained attached to albumin and that albumin was not hydrolyzed in the uptake or transport process.

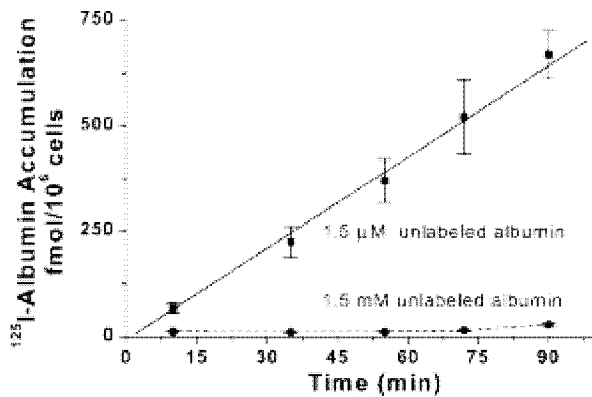


Fig. 1. Time course of transendothelial ^{125}I -albumin permeability. ^{125}I -Albumin transport was determined in rat lung microvascular endothelial cells (RLMVEC) grown to confluence in Transwell inserts. Tracer albumin (68 nM ^{125}I -albumin) was applied to the apical chamber together with 1.5 μM or 1.5 mM free albumin, which was also present in the basal chamber to equalize osmotic pressure, and samples from the basal chamber were taken every 10–15 min for 90 min (4 wells per incubation time). Specific transport of ^{125}I -albumin (amount inhibited by 1.5 mM albumin) was 80% of total ^{125}I -albumin clearance. ^{125}I -Albumin transport was cumulative, increasing linearly for up to 90 min. Data are from 1 experiment, which is representative of 3 experiments performed.

Statistical Analysis

Data were analyzed by the nonlinear least-squares curve-fitting programs LIGAND (Elsevier Biosoft) and Microcal Origin (Microcal Software, Northampton, MA). Student's *t*-test was used to compare results at significance level of *P* 0.05. Multiple comparisons were made by ANOVA. All statistical tests were made using GraphPad Prism InStat software (San Diego, CA).

RESULTS

Albumin Transport in Endothelial Monolayers

Time course of transendothelial ^{125}I -albumin transport. For studies of transendothelial ^{125}I -albumin transport in the apical-to-basolateral direction, RLMVEC were grown to confluence on Transwell filter inserts (1-cm² surface area). Experiments began when the liquid in the upper well was replaced by a warm (37°C) solution containing ^{125}I -albumin plus unlabeled albumin (1.5 μM or 0.1 mg/ml). During the 90-min period, aliquots of media (0.05 ml) in the lower well were sampled and analyzed for gamma radioactivity (see METHODS). Appearance of tracer albumin in the basal chamber vs. time described a straight line (Fig. 1), as predicted if the backflux of tracer (into upper chamber) was negligible. From the slope of the fitted line in Fig. 1, we calculated the ^{125}I -albumin flux of 7.2 fmol min⁻¹ 10⁶ cells⁻¹. Excess unlabeled albumin (1.5 mM or 100 mg/ml) blocked ^{125}I -albumin flux (Fig. 1), implying competition between labeled and unlabeled albumin at the apical endothelial plasmalemmal surface.

Cell pathway regulates albumin transport. The effect of methyl- β -cyclodextrin, the cholesterol-binding agent that disrupts caveolae (10), on transendothelial ^{125}I -albumin permeability was assessed. RLMVEC mono-

layers grown on Transwell filter inserts received vehicle (without drug) or methyl- β -cyclodextrin (0.2 nM–10 mM) for 15 min in the upper chamber, and tracer albumin was added immediately thereafter. Methyl- β -cyclodextrin blocked transendothelial ^{125}I -albumin permeability with an IC₅₀ of 1.0 μM (Fig. 2). The highest concentration of the inhibitor (10 mM) reduced permeability of the albumin tracer by 80% (from 7.7 to 1.5 fmol min⁻¹ 10⁶ cells⁻¹), indicating the importance of the cellular pathway in albumin transport in RLMVEC.

Regulation of transendothelial albumin transport by gp60. Inasmuch as activation of gp60 can stimulate albumin endocytosis in endothelial cells via caveolae (13, 21, 27), we determined whether gp60 activation also increased the transport of tracer albumin. To prevent internalization of the antigen-antibody complex, gp60 on the apical cell surface was cross-linked, as described elsewhere (27), using an anti-gp60 antibody for 30 min in cold conditions (4°C). Cells were rapidly rewarmed to 37°C with a solution containing ^{125}I -albumin (2.5 nM) plus unlabeled albumin (1.5 μM) to activate uptake of the tracer. Control cultures received isotype-matched antibody in lieu of anti-gp60 antibody. Cross-linking of gp60 significantly increased ^{125}I -albumin permeability 2.3-fold above the control value of 38 \pm 1.5 nl min⁻¹ 10⁶ cells⁻¹. Excess unlabeled albumin (1 mM) reduced ^{125}I -albumin permeability to 1.2 \pm 0.6 nl min⁻¹ 10⁶ cells⁻¹ (Fig. 3) in the cells subjected to gp60 cross-linking. To address the dependence of basal transport of tracer albumin on the cell surface gp60, RLMVEC monolayers were incubated with anti-gp60 antibody for 2 h at 37°C to deplete cell surface gp60 (9, 27). This procedure reduced basal transendothelial ^{125}I -albumin permeability to 7.3 \pm 0.6 nl min⁻¹ 10⁶ cells⁻¹ (80% inhibition; Fig. 3).

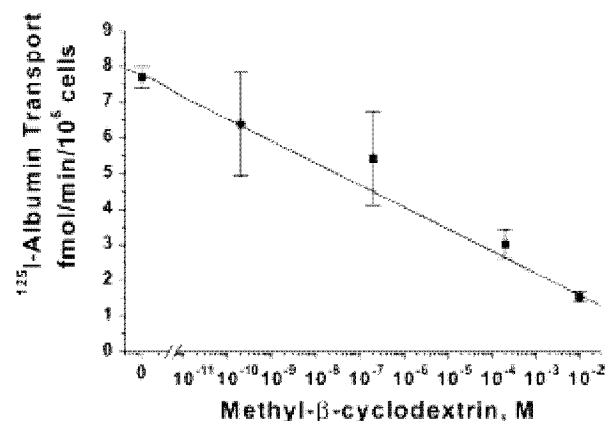


Fig. 2. Inhibitory effect of methyl- β -cyclodextrin on flux of ^{125}I -albumin. RLMVEC on Transwell inserts were preincubated for 15 min with 0.2 nM–10 mM methyl- β -cyclodextrin or vehicle and then with ^{125}I -albumin in Hanks' balanced salt solution (HBSS) containing 1.5 μM free albumin for 15 min. A dose-dependent inhibition of ^{125}I -albumin flux from a control value of 7.7 \pm 0.3 fmol min⁻¹ 10⁶ cells⁻¹ was observed. Values are means \pm SE from 4 replicates for each point in 1 experiment, which is representative of 4 experiments performed.

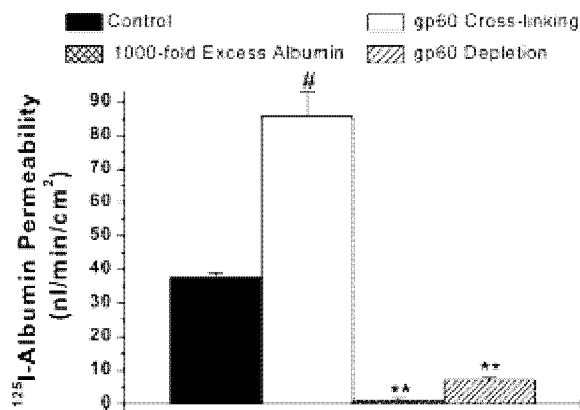


Fig. 3. 60-kDa albumin-binding protein (gp60)-dependent ^{125}I -albumin transport in RLMVEC. RLMVEC monolayers were washed twice with HEPES-buffered DMEM at 4°C and incubated for 30 min with anti-gp60 antibody (10 $\mu\text{g/ml}$) at 4°C and with secondary antibody (goat anti-rabbit, 10 $\mu\text{g/ml}$) for 30 min to induce cross-linking. Controls were treated with preimmune antibody (10 $\mu\text{g/ml}$) and secondary antibody. Cells were rewarmed to 37°C to activate uptake of ^{125}I -albumin and 1.5 M unlabeled albumin. Cross-linking of gp60 increased ^{125}I -albumin permeability 2.3-fold from a control value of $38 \pm 15 \text{ nl min}^{-1} \text{ cm}^{-2}$. To deplete cell surface gp60, RLMVEC monolayers were washed twice with HEPES-buffered DMEM and incubated with anti-gp60 antibody (10 $\mu\text{g/ml}$) for 2 h at 37°C . Depletion of gp60 reduced ^{125}I -albumin transport by 85%. ^{125}I -Albumin flux was also blocked by 95% when cells were coincubated with 1.5 mM unlabeled albumin (100 $\mu\text{g/ml}$). Values are means \pm SE ($n = 4$). #, ** $P < 0.01$ vs. control by ANOVA.

Albumin Uptake in Endothelial Cell Monolayers

Time course of tracer albumin uptake. RLMVEC monolayers were incubated for various periods with ^{125}I -albumin in the presence of 1.5 M unlabeled albumin. ^{125}I -albumin accumulation within the cells was determined by counting gamma radioactivity in cell lysates. Lysates were prepared from acid-washed cells to avoid contamination by tracer albumin associated with the cell exterior. The time course of cellular ^{125}I -albumin uptake was biphasic (Fig. 4). Specific tracer uptake (^{125}I -albumin uptake in the presence of 1.5 M albumin minus that in 1.5 mM albumin) reached an initial peak (60 fmol/ 10^6 cells) within 15 min, approached a minimum (50 fmol/ 10^6 cells) at 30–45 min, and finally rose to a second peak (75 fmol/ 10^6 cells) at 75 min. The initial slope of the uptake curve was used to estimate the rate of tracer albumin uptake, which was $6 \text{ fmol min}^{-1} 10^6 \text{ cells}^{-1}$; this value was within the same range as ^{125}I -albumin transendothelial transport of $7.2 \text{ fmol min}^{-1} 10^6 \text{ cells}^{-1}$ (see above). Endothelial ^{125}I -albumin uptake was markedly inhibited at each time point by excess (1.5 mM) unlabeled albumin (Fig. 4).

Concentration dependence of albumin uptake. Submaximal concentrations of unlabeled albumin produced biphasic inhibition of tracer albumin uptake (Fig. 5A; $\text{IC}_{50} = 1.15 \text{ M}$). Scatchard analysis (Fig. 5B) used to parse the sigmoidal function into its two components showed a 0.87 M high-affinity component, with maximal binding capacity (B_{max}) of $0.47 \text{ pmol}/10^6$ cells, and a 93.3 M low-affinity component, with

B_{max} of $20.2 \text{ pmol}/10^6$ cells. From these data, we determined the total albumin uptake (labeled + unlabeled forms) as a function of albumin concentration (Fig. 5C). The cellular uptake of albumin was dependent on albumin concentration; the steepest region of concentration dependency was between 10^{15} and 10^{13} M albumin. The smooth curve in Fig. 5C predicts an EC_{50} of 100 M and saturation of transport at 3 mM albumin.

Confocal fluorescence imaging of albumin uptake. We visualized the uptake of albumin in caveolae by colocalization of fluorescent forms of albumin, cholera toxin subunit B, and anti-gp60 antibody. Cholera toxin subunit B was used as a specific marker for caveolae, because it labels the caveolae-specific ganglioside GM1 (14); gp60 was previously shown to be associated with endothelial caveolae (13). After 30 min of incubation with these fluorescent probes at 37°C , cells were acid washed to remove residual cell surface probe, fixed, and stained with the nuclear marker DAPI (1 $\mu\text{g/ml}$) or anti-caveolin-1 antibody plus Alexa 350-labeled secondary antibody. High-resolution confocal images (1.0 μm optical thickness) in Fig. 6A show internalized Alexa 488-albumin and Cy3-gp60 antibody, together with caveolin-1 immunostaining near the apical cell surface. Coincident staining of gp60 and albumin and colocalization of all three probes were apparent in the overlay. In other experiments, fluorescent albumin was coincubated with fluorescent cholera toxin subunit B. Figure 6B shows the confocal images of internalized Alexa 594-cholera toxin subunit B and Alexa 488-albumin, together with DAPI. The fluorescence of albumin and cholera toxin was coincident in the merged image, indicating albumin's association with caveolae.

To address the effects of methyl- β -cyclodextrin on albumin uptake and caveolin-1 distribution, endothelial cells were pretreated with 10 mM methyl- β -cyclodextrin and incubated with HBSS containing Alexa

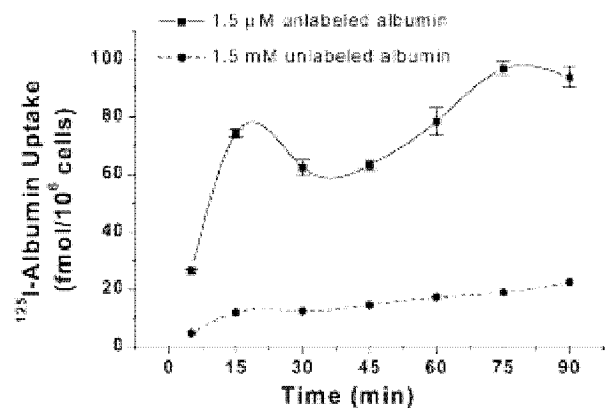


Fig. 4. Time course of endothelial ^{125}I -albumin uptake. Confluent RLMVEC were incubated for 5–90 min (6 wells per incubation time) with 22.8 nM ^{125}I -albumin and 1.5 M albumin to quantify total uptake (\square) or with 1.5 mM unlabeled albumin to quantify nondisplaceable ^{125}I -albumin uptake (\circ). Total uptake of ^{125}I -albumin increased with time, peaking initially at 15 min and again at 75 min (half time = 10 min). Data are representative of 4 separate experiments.

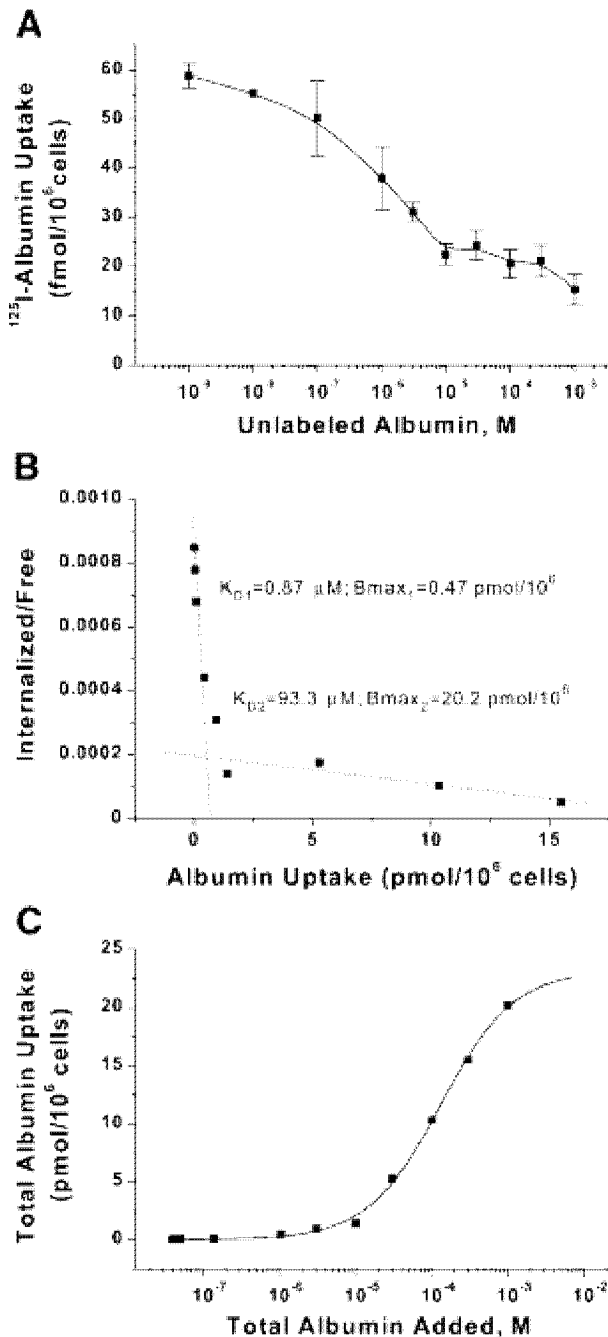


Fig. 5. Competition kinetics of ^{125}I -albumin uptake by unlabeled albumin. Increasing concentrations of unlabeled albumin (10^{10} – 10^{13} M) were added to RLMVEC monolayers followed by 34 nM ^{125}I -albumin, and monolayers were incubated at 37°C for 1 h. **A:** uptake of ^{125}I -albumin was inhibited in the presence of increasing concentrations of free albumin. IC_{50} was calculated to be $1.15 \mu\text{M}$; near-maximal displacement was achieved with 1 mM albumin. **B:** Scatchard analysis of ^{125}I -albumin displacement was best fit by a 2-site model, represented by K_{D1} and $B_{\text{max}1}$ (site 1) and K_{D2} and $B_{\text{max}2}$ (site 2), where K_D is affinity constant and B_{max} is maximal binding capacity. **C:** total albumin uptake calculated from Scatchard analysis of combined tracer ^{125}I -albumin plus unlabeled albumin uptake shows saturation of albumin uptake with 3 mM added albumin. Data are representative of 4 separate experiments.

488-tagged albumin (50 $\mu\text{g/ml}$) plus unlabeled albumin (1.5 mM) for 30 min. Cells were acid washed, fixed, and stained for nuclei (DAPI) and for caveolin-1 (i.e., anti-caveolin-1 monoclonal antibody at 1 $\mu\text{g/ml}$ followed by goat anti-mouse Alexa 568-labeled secondary antibody). Methyl- β -cyclodextrin abolished albumin internalization by plasmalemmal vesicles (Fig. 7). In addition, methyl- β -cyclodextrin markedly reduced the cell surface-associated caveolin-1 immunostaining, consistent with its effect in disrupting cell surface caveolae.

Albumin Binding to Endothelial Cell Surface

Competition between endothelial cell surface labeled and unlabeled albumin. Agonist competition binding studies were made using RLMVEC in cold conditions (4°C) to abolish tracer endocytosis and internalization. Cells were incubated with ^{125}I -albumin in the presence or absence of unlabeled albumin ($10^{11.2}$ – 10^{13} M) for 30 min. Unlabeled albumin reduced the specific binding of ^{125}I -albumin from 17.5 to 2.7 – $0.4 \text{ fmol}/10^6$ cells. The IC_{50} and Hill coefficient fitted to the data were 4.5 nM and 0.28 , respectively, suggesting the existence of more than one albumin binding site on the RLMVEC surface (Fig. 8A). Scatchard analysis of the binding data (Fig. 8B) demonstrated two albumin binding sites, with affinity (K_D) values of 13.5 nM and 1.6 M , respectively, and corresponding B_{max} values of $24.2 \text{ fmol}/10^6$ cells and $2.4 \text{ pmol}/10^6$ cells. The lower-affinity site accounted for 98% of the binding.

Displacement of albumin from cell surface binding sites by anti-gp60 antibody. Pretreatment of RLMVEC monolayers for 1 h at 4°C with a 1:200 dilution of anti-gp60 antibody decreased ^{125}I -albumin binding by $47.8 \pm 5.1\%$ ($n = 3$) relative to control antibody. The tracer albumin and the specific anti-gp60 antibody competed for the same membrane binding sites. Thus high- and low-affinity binding sites detected on RLMVEC may represent different states of gp60.

DISCUSSION

RLMVEC monolayers exhibited a high degree of specific ^{125}I -albumin binding, because 80% of total binding was inhibited by excess unlabeled albumin. Although a saturable low-affinity binding site for albumin has been described in bovine pulmonary artery endothelial cells (23) and rat fat tissue microvessel endothelial cells (20), the finding of a second site of much higher affinity is a novel observation. Competition studies of ^{125}I -albumin binding under cold conditions (4°C) to prevent tracer internalization showed that albumin bound to the endothelial cell surface with affinity constants of 13.5 nM (K_{D1}) and 1.6 M (K_{D2}). On the basis of the corresponding B_{max} values, the high-affinity site represented 1% of the specific albumin binding and averaged 5×10^5 sites/cell. Schnitzer and Oh (21) and Tirupathi et al. (25) demonstrated that an anti-gp60 antibody displaced the specific binding of ^{125}I -albumin from rat and bovine pulmonary microvascular endothelial cells, consistent with our

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