Journal of Cellular Biochemistry 68:427–435 (1998)

Uptake of ^{125}I-Labelled α_2-Macroglobulin and Albumin by Human Placental Syncytiotrophoblast In Vitro

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Abstract We have investigated the binding and internalization of α_2-macroglobulin and serum albumin by human placental syncytiotrophoblast cells in vitro. The time course (obtained at 4°C) of α_2-macroglobulin binding indicated that an equilibrium was reached after 4 h. The binding of ^{125}I-labelled α_2-macroglobulin to syncytiotrophoblast cells was competitively reduced in the presence of excess unlabelled α_2-macroglobulin. When the concentration-dependence of binding was examined over a wide concentration range, non-linear regression analysis yielded a K_d of 6.4 nM. In the case of albumin, binding was weak and ligand dissociated from the cell surface during aqueous washing making it impractical to analyze the binding reaction. In other experiments, syncytiotrophoblast cells were incubated with ^{125}I-labelled α_2-macroglobulin at 37°C. Under these conditions, trypsin-resistant cell-associated radioactivity increased with time consistent with ligand internalization. ^{125}I-Labelled-ligand was internalized with a $t_{1/2}$ of about 5 min. After a lag period some radioactivity was released back into the incubation medium. When measured at times up to 210 min, this was found to consist of mostly TCA-precipitable material that had been lost from the cell surface. However, when the incubation was extended to 24 h, almost 15% of the initial cell-associated radioactivity was released to the extracellular medium as TCA-soluble material, consistent with a slow rate of ligand degradation. The specific binding of ^{65}Zn-labelled α_2M was similar to that of the ^{125}I-labelled ligand and trypsin-resistance measurements provided evidence of α_2M-mediated ^{65}Zn uptake. These results support a role for syncytiotrophoblast in the metabolism of α_2-macroglobulin during pregnancy and are also consistent with a role for α_2-macroglobulin in the maternal-fetal transport of zinc. J. Cell. Biochem. 68:427–435, 1998.

Key words: α_2-macroglobulin; albumin; placenta; zinc

INTRODUCTION

In humans, the placental syncytiotrophoblast interfaces directly with maternal blood and plays a critical role in regulating maternal-fetal transport processes. Syncytiotrophoblast is known to express receptors for many serum proteins including transferrin, IgG, LDL, insulin, IGF, and α_2-macroglobulin [King, 1991; Jensen et al., 1988, 1989]. In vitro studies have demonstrated that isolated trophoblast cells are able to internalize transferrin (and transferrin-bound iron) and LDL by receptor-mediated endocytosis [Douglas and King, 1990b; Malassine et al., 1990; Kennedy et al., 1992; Bierings et al., 1992, 1991]. The serum protein α_2-macroglobulin (α_2M) is of interest because of its protease scavenging activity and because of its zinc binding activity. Plasma zinc is mostly (>98%) found associated with α_2-macroglobulin and serum albumin [Giroux, 1975; Cousins, 1988], suggesting possible roles for these proteins in zinc transport processes. Since the human fetus is dependent on an adequate supply of zinc in order to achieve normal pre- and postnatal development [Swanson and King, 1987; Apgar, 1992], it is likely that these proteins play a role in maternal-fetal zinc transport.

An α_2M receptor has been purified from a human placental membrane preparation [Jensen et al., 1989] and Gafvels et al. [1992] found that α_2M receptor antigenic material and α_2M receptor mRNA were present in isolated trophoblast. While it is likely that the tropho-

Contract grant sponsor: NIH; Contract grant numbers: HD26759, HD11658.

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Received 30 April 1997; Accepted 8 October 1997

blast receptor functions to bind and internalize serum α_2M, as has been shown for macrophages, hepatocytes and fibroblasts [Borth, 1992], this has not been demonstrated. Information about the uptake and metabolism of both α_2M and albumin by syncytiotrophoblast is required to provide a better understanding of their proposed roles in maternal-fetal zinc transport. Accordingly, in the present paper we have studied the binding, internalization and intracellular processing of α_2M and albumin in highly purified primary cultures of human syncytiotrophoblast cells.

MATERIALS AND METHODS

Reagents

Ham's F12 medium, Waymouth's MB 752/1 medium (HWM), bovine serum albumin (BSA; essentially globulin free), ovalbumin, human serum albumin (albumin), and methylamine (Me) were purchased from Sigma Chemical Company (St. Louis, MO). Human plasma α_2-macroglobulin (α_2M) was purchased from Biodesign International (Kennebunk, ME). Bovine serum albumin and transferrin were obtained from Sigma and Jackson Immunoresearch Laboratories, Inc. (West Grove, PA), respectively. Keratinocyte Growth Medium (KGM) was obtained from Clonetics Corporation (San Diego, CA) and fetal calf serum (FCS) from Intergen (Purchase, NY). Sephadex PD-10 columns were from Pharmacia Fine Chemicals (Piscataway, NJ) and Bio-Gel P-6 from Bio-Rad Laboratories (Richmond, CA). Iodo-Gen was purchased from Pierce Chemical Co. (Rockford IL).

Trophoblast Isolation and Primary Culture

A detailed description of the procedure used to isolate and characterize cytotrophoblast cells from term human placentas has been described previously [Douglas and King, 1989].

All experiments described here were performed using syncytiotrophoblast. Cytotrophoblast cells differentiate to form multinucleated syncytiotrophoblast when cultured in KGM [Douglas and King, 1990a] supplemented with 10% fetal calf serum (FCS). By day 2 more than 90% of the cells were multinucleated as assessed by desmosome and nuclear immunocytochemistry [Douglas and King, 1990a, 1993]. Day 3 cells were used for all experiments.

Cells were plated into 96-well culture dishes (Costar, Cambridge, MA) and maintained in an air/CO_2 incubator at 37°C. Plating efficiency was usually about 80% and cell numbers were adjusted so that the plating density was between 250,000 and 300,000 cells per well.

Iodination

Prior to iodination, α_2M was reacted with methylamine using a method modified from that described by Imber and Pizzo [1981]. Hereafter, α_2M refers to protein that has been reacted with methylamine. α_2M (104-152 pmols) or human serum albumin (hereafter referred to as albumin; 6.2–9.2 nmols) were reacted with carrier-free $Na^{125}I$ (150–250 µCi) in PBS to a final volume of 1 ml in a glass vial coated with 100 µg Iodo-Gen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril). The reaction was allowed to proceed at room temperature for 10 min (α_2M) and 15 min (albumin), and then stopped by removing the solution from the vial. Radiolabelled albumin was passed down a PD-10 column previously equilibrated with 0.02% ovalbumin. α_2M was passed down a Bio-Gel P-6 column equilibrated with PBS, pH 7.0. The specific activities for the final products were generally about 800 cpm/ng (580,000 cpm/nmol) for α_2M and 550 cpm/ng (35,700 cpm/nmol) for albumin.

Preparation of ^{65}Zn-Labelled α_2M

Methylamine-modified α_2M (152 pmols) was incubated with ^{65}Zn (10 µCi) for 18 h at 4°C and then passed down a PD-10 column equilibrated with PBS to remove unbound zinc. This produced material with a specific activity of 28,000 cpm/nmol that was stable for at least 1 week.

Binding Studies

Syncytiotrophoblast cultures were washed twice and preincubated for 1 h at 37°C in serum-free Ham's/Waymouth's medium (HWM) containing 5 mg/ml BSA (for ^{125}I-labelled α_2M studies), 5 mg/ml lactalbumin (for ^{65}Zn-labelled α_2M studies), or 2 mg/ml ovalbumin (for albumin studies) in order to remove endogenous bound α_2M or albumin. The addition of BSA, ovalbumin, or lactalbumin was also found to reduce non-specific binding of radiolabelled ligands to the cells and to the plastic. Concentrations below 2 mg/ml were less effective. The cells were then placed on ice for 5 min before the addition of fresh medium containing radiolabelled protein (ligand concentrations are given in the figures) and BSA, ovalbumin, or lactalbumin as

described above. Parallel incubations were set up containing 200-fold molar excess unlabelled α_2M to measure nonspecific binding. Cells were incubated for various times (actual times are given in the figure legends) at 4°C and then washed three times with ice-cold HWM and once with PBS to remove unbound radioactivity. The cells were then solubilized by the addition of 0.5% SDS and the radioactivity was counted using an LKB gamma counter. HWM was selected for these studies in order to reduce usage of the more expensive KGM that is used for long-term trophoblast culture. Although these media have different zinc concentrations, trophoblast cells and ligand binding are not affected by switching the culture medium for the short duration of these experiments (results not shown).

Specific binding/uptake is defined as total binding minus nonspecific binding (i.e., radioactivity associated with cells in the presence of excess unlabelled ligand).

Internalization and Degradation Studies

α_2M. Cells were incubated with ^{125}I-labelled-α_2M for 4 h at 4°C and then washed three times with ice-cold HWM to remove unbound radioactivity. They were subsequently reincubated in fresh KGM at 37°C for different time periods. The culture medium was removed and counted for radioactivity. The cells were treated with 0.1% trypsin in PBS containing 5 mg/ml BSA for 30 min at 4°C in order to remove surface-bound α_2M (trypsin-sensitive counts) not removed by the previous washes. This treatment removed 75–80% of the surface radioactivity. Cells were then solubilized and counted to determine the intracellular radioactivity (trypsin-resistant counts).

In order to determine whether there was any intracellular degradation of the internalized α_2M, cells were incubated with ^{125}I-labelled α_2M at 4°C, washed, and reincubated at 37°C. The spent culture medium was mixed with an equal volume of ice-cold 20% (w/v) TCA. After 30 min the resulting precipitate (TCA-precipitable fraction) and supernatant (TCA-soluble fraction) were collected and counted for radioactivity.

For experiments with ^{65}Zn-labelled α_2M, cells were incubated with ligand (6.9 nM) at 37°C for different times. The incubation medium contained α-lactalbumin (5 mg/ml) to minimize nonspecific binding. Separate experiments showed that unlike BSA, α-lactalbumin does not bind ^{65}Zn. At each time point the cells were washed and subjected to trypsinization as described above. Total and trypsin-resistant radioactivities were determined as described above.

Albumin. A different protocol was used to measure the internalization and processing of albumin. This was necessitated by the rapid dissociation of albumin from the cells (see text). Cells were incubated with ^{125}I-labelled albumin for 1 h at 37°C (without prior incubation at 4°C), cooled to 4°C and washed twice with ice-cold HWM containing 2 mg/ml ovalbumin and once with PBS containing 2 mg/ml ovalbumin. They were then reincubated at 37°C in KGM containing 2 mg/ml ovalbumin for different time periods. The culture medium was removed, counted to determine total radioactivity released from the cells and then subjected to TCA precipitation. The TCA-precipitable and soluble fractions were counted for radioactivity. The cells were washed three times with ice-cold HWM to remove unbound radioactivity, solubilized, and counted to determine cell-associated radioactivity.

Degradation and Release Studies

In order to determine whether there was any intracellular degradation of the internalized α_2M and albumin, the following experiments were performed. Cells were incubated with ^{125}I-labelled α_2M (6.9 nM) at 4°C, washed to remove unbound radioactivity, and reincubated in fresh medium at 37°C. The spent medium was then mixed with an equal volume of 20% TCA, centrifuged at 1,560*g* and the resulting precipitate and supernatant were counted for radioactivity.

In another protocol, syncytiotrophoblast cells cultured in 4-well plates ($1x10^6$ cells/well) were incubated with ^{125}I-labelled α_2M or ^{125}I-labelled albumin at 37°C for 2 h (i.e., there was no incubation with ligands at 4°C), rinsed three times to remove unbound radioactivity, and reincubated in fresh medium for 24 h at either 4°C or 37°C. TCA precipitation was then performed as described above and fractions counted for radioactivity.

Protein Determination

One hundred µl aliquots of solubilized cell extracts from each well were used to determine cell protein content. The Lowry method, modified by inclusion of sodium dodecyl sulphate in

the alkaline copper reagent [Bennett, 1982], was used.

Treatment of Results

The results that are presented are the means ± S.D., generally from at least three separate experiments. Each experiment represents cells from a different placenta. Within an experiment, mean values were calculated from at least three replicates. Actual *n* values are given in the figure legends. Statistical analyses were performed using InStat software (Graphpad Software, San Diego, CA). When required, statistical significance was determined using Student's *t*-test. Values of $P > 0.05$ were considered not statistically significant.

RESULTS

Binding at 4°C

A series of experiments were performed to characterize the binding kinetics of ^{125}I-labelled-α_2M and ^{125}I-labelled-albumin to syncytiotrophoblast. Critical assumptions for kinetic binding studies are that unbound ligand is efficiently removed and that surface-bound ligand does not dissociate during the washing steps. Preliminary studies were therefore performed in order to ensure that these assumptions held in the present case. Cells were incubated with radiolabelled proteins for 2 h at 4°C and then subjected to sequential washes (Table I). For ^{125}I-labelled α_2M, most of the unbound radioactivity was removed by the first wash and the cell-associated counts decreased only slightly after the third wash. Three washes were therefore used routinely in all experiments. Subsequent treatment with trypsin was effective in removing almost 80% of the remaining cell-associated (surface-bound) radioactivity. When the nonenzymatic washing protocol was used on cells that had been incubated at 4°C with ^{125}I-labelled albumin, the pattern was similar to α_2M for the first two washes. However, in contrast to α2M, subsequent washes continued to remove ^{125}I-labelled albumin from the cells (Table I). No attempt was made to use trypsin to remove surface-bound ligand. The relatively rapid dissociation of albumin from the cells negated further analysis of binding.

The time course (obtained at 4°C) shown in Figure 1 indicates that for α_2M, the cell-associated counts reached an equilibrium at about 4 h. The decrease in α_2M binding at 5 h was not significantly different from the 4 h value ($p > 0.05$). Figure 2 shows the concentration-dependence of α_2M binding following a 4 h incubation at 4°C. Specific binding increased but then started to level off at a free ligand concentration of about 2 nM. The degree of nonspecific binding showed variation from placenta to placenta and accounted for between 50–80% of total binding. Nonlinear regression analysis of the specific binding data yielded a mean K_d of 6.4 nM. The goodness of fit (R^2) was 0.933.

TABLE I. Cell-Associated Radioactivity Remaining After Washing*

Wash	Albumin cells (cpm/well)	%	α_2-Macroglobulin cells (cpm/well)	%
0	16,565 ± 7,413	100	14,985 ± 4,904	100
1	1,537 ± 289	9.2	1,897 ± 356	12.7
2	657 ± 147	4.0	1,042 ± 202	6.9
3	408 ± 76	2.5	804 ± 89	5.4
4	285 ± 80	1.7	713 ± 151	4.8
5	200 ± 83	1.2	676 ± 144	4.5

*Cells were incubated with ^{125}I-labelled albumin (308 nM) or ^{125}I-labelled $\alpha_2$macroglobulin (3.1 nM) for 2 h at 4°C. The culture medium was then removed using a multichannel pipette and the cell-associated radioactivity was determined (initial cell-associated radioactivity, wash 0). The cells were then washed by the addition of 200 µl ice-cold edium for 2 min followed by removal of the medium. This procedure was repeated as indicated above. The wash supernatants and the cells were counted for radioactivity. The cell-associated radioactivity remaining after each wash (expressed as cpm/well and as a percentage of the initial cell-associated radioactivity) are shown above. Data are means ± S.D. from three experiments.

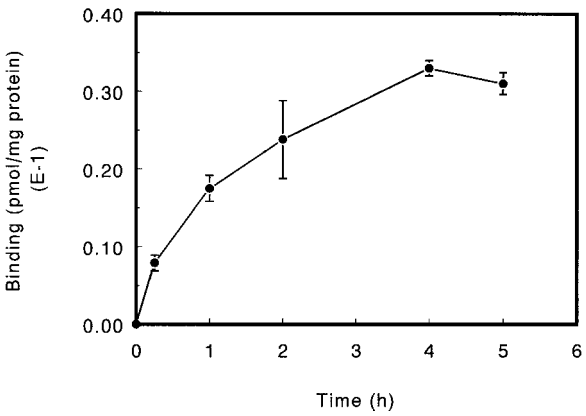

Fig. 1. Cells were incubated with ^{125}I-labelled α_2M (3.1 nM) at 4°C for the times indicated above, then washed and counted for radioactivity as described in Materials and Methods. Mean values ± S.D. from a total of three experiments are shown.

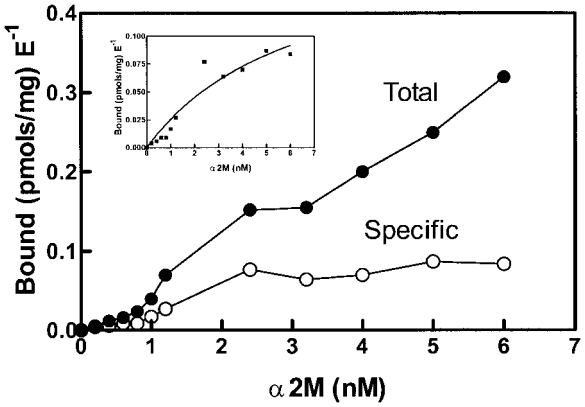

Fig. 2. Cells were incubated with different concentrations of ^{125}I-labelled α_2M for 4 h at 4°C. After washing to remove unbound radioactivity, the cells were solubilized and counted for radioactivity. Specific binding was measured as described in Materials and Methods. Mean values from five experiments are shown. Standard deviations have been omitted for clarity. The inset shows the nonlinear regression analysis of the specific binding data.

Internalization of ^{125}I-Labelled Ligands

Several approaches were used in an attempt to obtain evidence for ligand internalization. In the case of α_2M, cells were incubated with radiolabelled protein at 4°C to allow surface binding, then washed and reincubated in isotope-free medium at 37°C. Internalization was assessed by measuring the trypsin-resistance of the cell-associated radioactivity at different times. From Figure 3A it can be seen that the distribution of radioactivity within the trypsin-resistant, trypsin-sensitive, and extracellular compartments changed with time. At the start of the reincubation, most of the cell-associated radioactivity could be removed by trypsin treatment. However, during the first 10 min there was a progressive and rapid loss of these trypsin-sensitive counts concomitant with the appearance of trypsin-resistant counts and the appearance of radioactivity in the culture medium. After 30 min, the levels of radioactivity remained constant in all compartments. The $t_{1/2}$ for the appearance of trypsin-resistant radioactivity was about 5 min. When the released radioactivity was analyzed by TCA precipitation, most was found to consist of TCA-precipitable material. The small amount of TCA-soluble radioactivity was not above background levels at any time point (0–210 min).

The same protocol could not be used with ^{125}I-labelled albumin because of the weak binding exhibited by this ligand (see above). Instead, cells were incubated with radiolabelled albumin at 37°C (with no prior incubation at 4°C) to allow fluid-phase uptake. The cultures were then washed and reincubated at 37°C. At different times, the cell-associated and released radioactivity was measured. Figure 3B shows that increasing amounts of TCA-soluble counts were released into the medium with time.

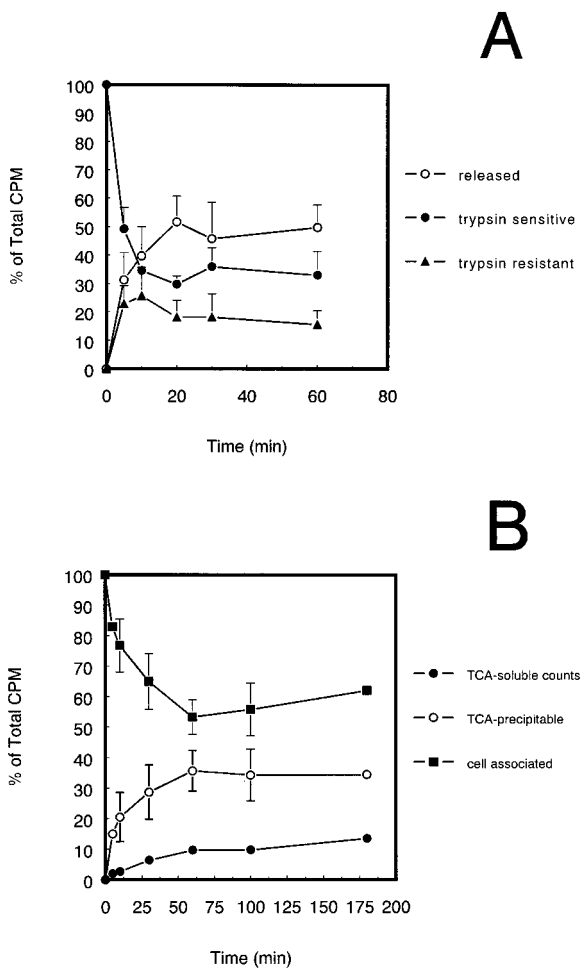

Fig. 3. Internalization of ligands by trophoblast. **A:** Cells were incubated with ^{125}I-labelled α_2M (6.9 nM) for 4 h at 4°C. The cells were then washed and reincubated at 37°C. At the times indicated above, the culture medium was removed and counted for radioactivity. The cells were treated with trypsin to determine the distribution of trypsin-resistant and trypsin-sensitive radioactivities. Results are means ± S.D. from three experiments. **B:** Cells were incubated with ^{125}I-labelled albumin (308 nM) at 37°C for 1 h then washed and reincubated at 37°C. At each time point, the culture medium was removed and subjected to TCA precipitation as described in Materials and Methods. Results are means ± S.D. from four experiments.

Binding and Internalization of ^{65}Zn-Labelled α_2M

In addition to investigating the uptake of ^{125}I-labelled α_2M we also examined the uptake of α_2M that had been labelled with ^{65}Zn. The

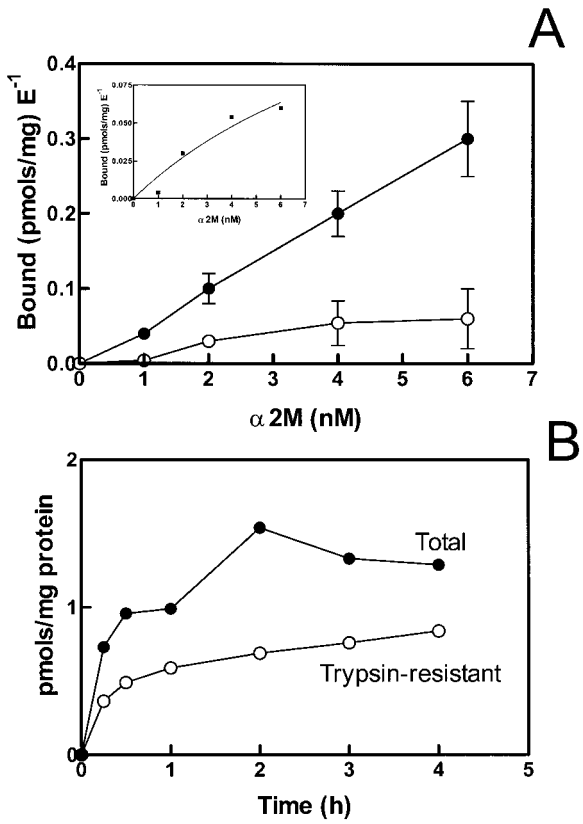

Fig. 4. Binding and internalization of ^{65}Zn-labelled α_2M. **A:** Cells were incubated with different concentrations of ^{65}Zn-labelled α_2M for 4 h at 4°C in the presence of a 200-fold excess of unlabelled ligand. Specific binding was determined as described in Materials and Methods. The inset shows the nonlinear regression analysis of the specific binding data. Results are means ± S.D. (n = 3). **B:** To demonstrate internalization, cells were incubated with ^{65}Zn-labelled α_2M (at a final concentration of 6.9 nM) at 37°C and the incubation continued for the times shown above. At each time point, cells were washed to remove unbound radioactivity and total and trypsin-resistant radioactivity was determined as described in Methods. Results are mean values of quadruplicate determinations from one experiment.

binding of ^{65}Zn-labelled α_2M to cells at 4°C (Fig. 4A) was competitively blocked by excess unlabelled ligand in a similar manner to the ^{125}I-labelled ligand (c.f., Fig. 2). Nonlinear regression analysis of these specific binding data yielded a K_d of 11.6 nM. To measure internalization, cells were incubated with ^{65}Zn-labelled α_2M at 37°C for different times. Total and trypsin-sensitive radioactivity was determined at each time point. The results showed that the trypsin-resistant counts increased rapidly during the first 30 min and then started to level off (Fig. 4B). At all time points examined the trypsin-resistant counts accounted for at least 50% of the total cell-associated radioactivity.

Intracellular Degradation of α_2-Macroglobulin

In the experiments described above, evidence for albumin degradation was obtained. However, no evidence for intracellular degradation of α_2M was seen. The following experiments were performed to more fully investigate these observations. Syncytiotrophoblast cells were incubated with labelled protein for 2 h at 37°C, washed and then reincubated at 37°C or 4°C for 24 h. From Figure 5 it can be seen that of the initial cell-associated radioactivity almost 15% was released as TCA-soluble material in 24 h at 37°C. It should be noted that the radiolabelled ligand used here contained 5–8% residual TCA soluble radioactivity that cannot be removed. This background TCA-soluble radioactivity has not been subtracted from the data presented in Figure 5. If the reincubation was carried out at 4°C the release of TCA-soluble material was reduced to background levels. It can also be seen that about 40% of the radioactivity remained cell-associated after reincubation for 24 h at 37°C. Incubation of ^{125}I-labelled ligand with spent trophoblast culture medium for 24 h at 37°C did not increase TCA-soluble radioactivity above the residual background levels, indicating that degradation was not mediated by proteases released into the incubation medium (Fig. 5).

DISCUSSION

The data presented here provide evidence for the receptor-mediated binding and internalization of α_2M by human syncytiotrophoblast cells. Binding of radiolabelled ligand was time and ligand concentration-dependent, was competitively reduced by unlabelled α_2M and showed saturation. Nonlinear regression analysis of the specific binding data for the ^{125}I-labelled ligand provided a K_d of 6.4 nM with a good fit. Receptors for α_2M have previously been demonstrated using ligand binding assays on a variety of human and rodent cells [Borth, 1992] and kinetic analyses have sometimes yielded relatively low specific binding or nonlinear Scatchard plots. Assuming a single class of binding sites, the reported K_d for the α_2M receptor from a variety of cells lies between 0.12 and 12 nM [see Ashcom et al., 1990]. The purified placental receptor had a K_d of 400 pM [Jensen et al., 1989]. Analysis of α_2M binding can be complicated by various factors. In the present case, the nonspecific binding was high and varied

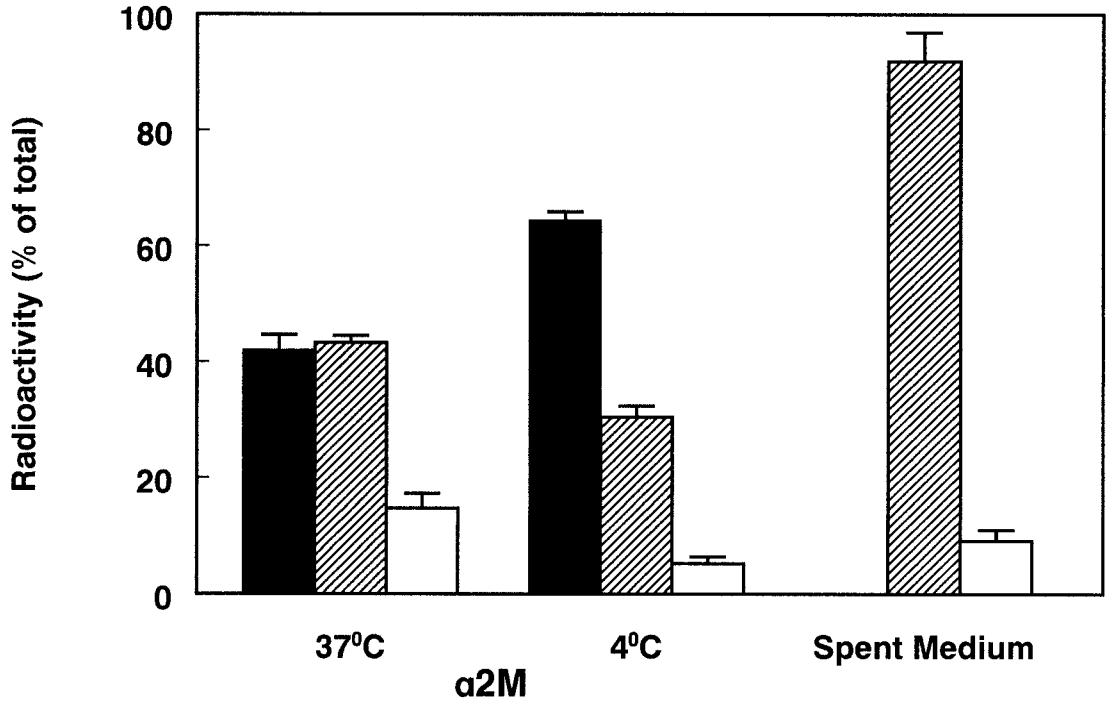

Fig. 5. Degradation of α_2M. Cells were incubated with ^{125}I-labelled α_2M (6.9 nM) for 2 h at 37°C then washed and reincubated for 24 h at either 37°C or 4°C. The culture medium was then analyzed by TCA-precipitation (see Methods section). To control for possible extracellular degradation, spent medium from 3-day syncytiotrophoblast cultures was collected, centrifuged at 1,300*g* for 1 min and aliquots of the supernatant were mixed with ^{125}I-labelled α_2M (final concentration was 6.9 nM). After 24 h at 37°C, the mixture was analyzed by TCA precipitation. Results are expressed as means ± S.D from three experiments. Open bar, TCA-soluble fraction; Cross-hatched bar, TCA-precipitable fraction; Solid bar, cell-associated fraction.

from placenta to placenta. Other factors such as the presence of more than one binding site, receptor aggregation or the multivalent nature of α_2M [Van Leuven et al., 1986; Ashcom et al., 1990] might also have contributed to the variable results that can be found in the literature.

As mentioned above, the previous evidence for the existence of trophoblast α_2M receptors was based on receptor isolation and on the detection of receptor mRNA [Jensen et al., 1989; Gafvels et al., 1992]. Jensen et al. [1988] found saturable binding of α_2M to placental villous tissue explants. However, the extent of specific binding (compared to nonspecific binding) was not described in this case. It should also be noted that this in vitro system has several inherent problems. Since villous tissue consists of many different cell types (syncytiotrophoblast, cytotrophoblasts, fibroblasts, macrophages, and endothelial cells) each of which could have access to radiolabelled ligand under in vitro conditions, this makes it difficult to determine where the observed binding activity resides. The results presented here therefore represent a significant improvement over these data since highly purified syncytiotrophoblast cells were used and internalization and degradation have been demonstrated. In addition we have also analyzed the binding and internalization of ^{65}Zn-labelled α_2M (see below).

The mechanism of internalization for serum albumin is controversial. Some studies have led to the proposal that albumin receptors exist on certain cells while other studies refute this claim [Stremmel et al., 1983; Jones et al., 1985]. Compared to α_2M, the binding of ^{125}I-labelled albumin to syncytiotrophoblast at 4°C was weak and ligand was readily removed by simple aqueous, nonenzymatic washing. These results do not support the existence of specific, high affinity albumin binding sites on trophoblast but, rather, suggest that what binding there is may be nonspecific. Our results do show that albumin is internalized and degraded by syncytiotrophoblast but this most likely occurs as the result of fluid-phase pinocytosis. However, the possibility that trophoblast expresses a low affinity albumin receptor cannot be ruled out. On the basis of these findings it is hard to see how albumin uptake by trophoblast could contrib-

ute to the regulated transport of zinc to the fetus. This view is strengthened by the fact that serum albumin binds zinc with relatively low affinity and in no particular stoichiometric ratio, while each molecule of α_2M binds four zinc atoms with high affinity [Prasad and Oberleas, 1970; Giroux, 1975].

For ^{125}I-labelled α_2M, evidence of internalization was obtained from groups of experimental data. Firstly, the amount of ligand that became cell-associated at 37°C exceeded the surface binding capacity and, secondly, cell-associated radioactivity rapidly became trypsin-resistant at 37°C. The information that is available suggests that the processing of α_2M varies with the cell type [Borth, 1992]. In some cells, internalization and degradation is rapid while in others it is slow, with no evidence of degradation for many hours. In other instances, internalized α_2M is eventually released intact back to the extracellular medium [Maxfield et al., 1981; Yamashiro et al., 1989; Willingham and Pastan, 1995]. We found little evidence for the latter in the present studies. Although significant amounts of intact α_2M (as determined by TCA-precipitable radioactivity) were released to the incubation medium most of this appeared to simply have dissociated from the cell surface and did not represent previously internalized ligand. However, our results indicate that of the α_2M that was cell-associated at the start of the incubation, about 40% remained cell-associated after 24 h at 37°C and only 15% had been degraded. Thus, syncytiotrophoblast seems to metabolize α_2M slowly. Jensen et al. [1988] were unable to detect any internalization or degradation of α_2M using chorionic villous tissue explants. However, in addition to the inherent problems with this system described above, their incubation time was only 1 h which, in our hands, is insufficient to detect any proteolysis.

It is tempting to speculate that this accumulation of α_2M by syncytiotrophoblast cells in vitro may be related to the observed accrual of zinc by the third trimester placenta [Simmer et al., 1985; Page et al., 1988; Shennan and Boyd, 1988]. For each molecule of α_2M endocytosed, 2–4 atoms of zinc will be taken up which, under the appropriate conditions, may then be available for further transport to the fetal circulation. Our present studies with ^{65}Zn-labelled α_2M support the idea that α_2M mediates the internalization of zinc by syncytiotrophoblast. We found that when cells were incubated with ^{65}Zn-labelled α_2M at 37°C almost half of the cell-associated radioactivity became resistant to trypsin within 15 min, consistent with α_2M-mediated internalization of zinc. The fact that uptake appeared to level off after 30 min could suggest dissociation of the zinc from the α_2M followed by release to the culture medium. The factors which control the intracellular release of zinc from α_2M and the intracellular compartment(s) involved are unknown but may play an important role in the regulation of zinc transport to the fetus. Future studies will be aimed at obtaining detailed information on the intracellular pathways and compartments that are involved in the processing of α_2M as well as more studies with zinc-labelled protein.

Binding of α_2M to its receptor may be affected by zinc status but control of receptor expression may be quite complex and regulated by factors other than those directly related to zinc. First, α_2M is an acute phase reactant and infection/inflammation may affect placental uptake of α_2M. Second, the amount of protease-modified α_2M will affect the amount of ligand bound to receptor. Third, the α_2M receptor has been shown to be identical to the low density lipoprotein-receptor related protein (LRP) and belongs to a family of "giant" receptors that mediate endocytosis of multiple ligands [Moestrup, 1994]. Thus, subclinical infection, extent of tissue catabolism and lipid metabolism may all affect the rate of α_2M uptake and/or degradation by the placenta. This, in turn, may explain previous difficulties in interpreting changes in zinc metabolism during pregnancy.

ACKNOWLEDGMENTS

Tissue was made available to us through the cooperation of the medical and nursing staff at Sutter Memorial Hospital, Sacramento, CA. We thank Jianyi Huang, Twanda Thirkill, and Jinjie Hu for expert technical assistance.

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