Metabolic fate of pancreas-derived cholesterol esterase in intestine: an in vitro study using Caco-2 cells

Yan Huang and David Y. Hui[1]

Department of Pathology and Laboratory Medicine, University of Cincinnati, College of Medicine, 231 Bethesda Avenue, Cincinnati, OH 45267-0529

Abstract Bile salt-stimulated cholesterol esterase is synthesized in the pancreatic acinar cells and is released into the intestinal lumen where it catalyzes cholesterol absorption. In the current study, Caco-2 cells were used as an in vitro model to study the interaction between the pancreatic cholesterol esterase with intestinal cells. Results showed that addition of increasing concentrations of cholesterol esterase in the incubation medium increased the uptake of micellar cholesteryl oleate by Caco-2 cells. The cholesterol esterase also increased the cellular uptake of the nonhydrolyzable cholesteryl linoleoyl ether. However, maximum uptake of the cholesteryl ether analog was 50% of that for cholesteryl oleate. The initial interaction of cholesterol esterase with Caco-2 cells was mediated by binding of the protein to a low affinity and high capacity binding site on the cell surface. Cholesterol esterase bound to the cell surface could be internalized via a monensin-sensitive mechanism. The cholesterol esterase taken up by the cells had a short residence time and was either degraded or was rapidly re-secreted from the cells. Chloroquine had no effect on the degradation or re-secretion of cholesterol esterase by Caco-2 cells, indicating that lysosomes were not involved with these processes. The cholesterol esterase taken up by the cells was not available to mediate further cholesterol uptake. These results indicated that the bile salt-stimulated cholesterol esterase secreted from pancreas could facilitate intestinal lipid absorption only transiently. The data suggest that the regulation of cholesterol esterase synthesis and secretion by the pancreas may be important for regulation of cholesterol absorption.—**Huang, Y., and D. Y. Hui.** Metabolic fate of pancreas-derived cholesterol esterase in intestine: an in vitro study using Caco-2 cells. *J. Lipid Res.* 1990. **31:** 2029–2037.

Supplementary key words cholesterol absorption • protein degradation

Bile salt-stimulated cholesterol esterase is one of the most abundant proteins synthesized by the pancreas (reviewed in ref. 1). The enzyme is made in the acinar cells and is released into the intestinal lumen as a component of the pancreatic juice (2). In the lumen of the small intestine, and in the presence of bile salt, the cholesterol esterase catalyzes the hydrolysis of dietary cholesteryl esters and the absorption of free cholesterol through the intestinal mucosa (3). Additionally, the bile salt-stimulated cholesterol esterase also catalyzes the hydrolysis of vitamin esters and may be important for intestinal absorption of fat-soluble vitamins (1).

The role of the cholesterol esterase in lipid absorption was initially suggested by observations that intact cholesteryl esters were not effectively absorbed (4). Furthermore, sterically hindered cholesteryl esters, which were resistant to cholesterol esterase hydrolysis in vitro, were also absorbed poorly in vivo (1). The rates of absorption for the various esters of cholesterol have also been correlated to their rates of hydrolysis by the pancreatic cholesterol esterase (1). Thus, these studies strongly implied the necessity of cholesteryl ester hydrolysis for cholesterol absorption to occur.

Although cholesterol esterase appears to be important for the absorption of dietary cholesterol, the precise mechanism by which cholesterol esterase facilitates lipid transport in the intestine remains controversial. Gallo and associates (5) have proposed that the function of cholesterol esterase is to promote esterification of the cholesterol in intestinal mucosa. This hypothesis was challenged by evidence implicating ACAT as the enzyme that was responsible for this process (6–9). However, several other studies have shown that ACAT inhibitors either reduced (10) or had no effect (11, 12) on cholesterol absorption. While this discrepancy remains unresolved, an alternative hypothesis for the role of cholesterol esterase in mediating cholesterol absorption was proposed. Bhat and Brockman (13) have suggested that pancreatic cholesterol esterase catalyzes the esterification of cholesterol on the cell surface and its hydrolysis in the inner leaflet of the plasma membranes. The free cholesterol liberated from intracellular hydrolysis can then be re-esterified by ACAT. This hypothesis favors the role of both cholesterol esterase and ACAT in dietary cholesterol absorption and lymphatic transport.

The pancreatic cholesterol esterase was shown to be present in endocytic vesicles of enterocytes (14). More recently, Bosner

[1]To whom correspondence should be addressed.

et al. (15) demonstrated the interaction of cholesterol esterase with heparin-like molecules on the surface of intestinal cells. However, the fate of cholesterol esterase subsequent to its interaction with intestine remains unknown. The present study was undertaken to address this issue. The adenocarcinoma cell line Caco-2 was utilized as the model system to determine the metabolic pathway of cholesterol esterase in intestine. Other investigators have shown that this cell line is a good model for the study of intestinal lipid metabolism. The Caco-2 cells possess many characteristics of intestinal cells including the ability to synthesize lipoproteins containing apolipoprotein B-48 (16, 17).

EXPERIMENTAL PROCEDURES

Enzyme preparation

Bile salt-stimulated cholesterol esterase was purified from porcine pancreas by the method as described by Rudd, Mizuno, and Brockman (18) with minor modifications. Freshly frozen porcine pancreas was ground over dry ice and homogenized in buffer containing 0.1 M sodium acetate, pH 4.8, 150 mM NaCl, 0.2% Triton X-100, 3 mM sodium taurocholate, 2 mM benzamidine hydrochloride, 0.2 mM phenylmethyl sulfonyl fluoride, 2 mM hydrocinnamic acid, and 0.5 mM N^{α}-benzoyl-D, L-arginine (18). The sample was centrifuged for 20 min at 14,000 *g* and filtered to remove the floating fat. The cholesterol esterase was partially purified by precipitation with ammonium sulfate and concentrated with *tert*-butanol (18). Purified cholesterol esterase was obtained by applying the partially purified sample to an immunoaffinity column containing antibodies against the porcine pancreatic cholesterol esterase as described previously (19). In a typical experiment, an 8-ml IgG-Sepharose column containing 20 mg IgG per g of gel was used. The column was washed initially with 100 ml of buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% Triton X-100 followed by 100 ml of the same buffer without Triton X-100. The cholesterol esterase bound to the IgG-Sepharose column was eluted with 0.2 M glycine-HCl, pH 2.8, into centrifuge tubes containing 1.1 M Tris-HCl, pH 8.3. The cholesterol esterase obtained by this technique included both the high and low molecular weight form of the enzyme as judged by SDS-polyacrylamide gel electrophoresis. The purified enzyme was dialyzed against 150 mM NaCl, pH 7.5, and was stored at − 20 °C until use.

Iodination of the cholesterol esterase was performed by the iodine monochloride method (20). The radiolabeled cholesterol esterase was separated from unincorporated ^{125}I by gel filtration on a PD-10 column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) and was dialyzed exhaustively in 150 mM NaCl. Greater than 95% of the ^{125}I associated with the cholesterol esterase was precipitable with 10% trichloroacetic acid. A typical iodination resulted in the incorporation of one atom of ^{125}I per molecule of cholesterol esterase.

Cell culture

The human colonic adenocarcinoma cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD: ATCC HTB 37). The cells were cultured at 37 °C in a 5% CO_2/95% air atmosphere in RPMI 1640 media supplemented with 2 mM glutamine, 50 μg/ml gentamycin, and 20% fetal bovine serum. Stock cultures were maintained in 75-cm^2 flasks and cell media were changed every 2 days. When the cell culture reached 80% confluency, the cells were dissociated with 0.25% trypsin and 0.05% EDTA and replated into new flasks at a split ratio of 1:3. Experiments were performed with cells at the 30th to 40th passages. The Caco-2 cells were seeded in multiwell tissue culture plates at a density of approximately 2–3 × $10^4/cm^2$. Cell media were changed every 2 days and experiments were performed 5–6 days after plating when the cells reached confluency.

Binding and uptake of cholesterol esterase by Caco-2 cells

Cell surface binding of cholesterol esterase was determined by incubation at 4 °C. The Caco-2 cells were plated on 12-well tissue culture plates as described above. On the day of experiment, the cells were washed once with RPMI 1640 media, without serum, and were incubated for 90 min at 4 °C with ^{125}I-labeled cholesterol esterase in the presence or absence of 500 μg/ml of unlabeled cholesterol esterase. At the end of the incubation period, the cells were washed three times with RPMI 1640 containing 5% lipoprotein-deficient serum followed by one wash with serum-free RPMI 1640. The amount of ^{125}I-labeled cholesterol esterase bound to the cells was determined by counting in a gamma counter after dissolving the cells in 0.5 ml of 1 N NaOH. All data reported herein represented the average of duplicate dishes from at least three different experiments. The amount of nonspecific binding of ^{125}I-labeled cholesterol esterase, as determined in the presence of unlabeled cholesterol esterase, was approximately 20–25% of the binding observed in the absence of unlabeled enzyme. Specific binding was calculated as the difference between ^{125}I-labeled cholesterol esterase bound to the Caco-2 cells in the presence or absence of unlabeled cholesterol esterase. Scatchard analysis (21) was performed to calculate the apparent equilibrium dissociation constant K_d and to estimate the maximum binding of cholesterol esterase to Caco-2 cells.

Cellular uptake of ^{125}I-labeled cholesterol esterase was determined by incubation at 37 °C. At the end of the incubation period, the cells were washed extensively and harvested as described above to determine the amount of cholesterol esterase associated with the cells. In experiments to differentiate surface-bound enzyme from the enzyme taken up by the Caco-2 cells, the cholesterol esterase on the cell surface was released by incubating the cell culture with 0.25% trypsin and 0.05% EDTA for 3 min at 37 °C. At the end of incubation, 1 ml of RPMI 1640 containing 10% fetal bovine serum was added to each well and the cells were scraped

into test tubes. The cell suspension was centrifuged at room temperature at 1,000 *g* for 10 min. Radioactivity in the supernatant (trypsin-sensitive) and in the pellet (trypsin-resistant) was then determined by gamma counting.

Degradation of cholesterol esterase by Caco-2 cells

The Caco-2 cells were incubated with 25 μg/ml of ^{125}I-labeled cholesterol esterase in the presence or absence of 50 μM of chloroquine for 1 h at 37 °C. This concentration of chloroquine was shown to inhibit 75% of ^{125}I-labeled LDL degradation by Caco-2. The cells were washed exhaustively and one set of culture was harvested to determine cell-associated radioactivity. The incubation was continued for the remaining duplicate cultures in media without ^{125}I-labeled cholesterol esterase. Radioactivity remaining associated with the Caco-2 cells at various times after the initial incubation period was then determined as described above. The ^{125}I-labeled cholesterol esterase degraded and released by the cells was determined by assaying for radioactivity in the culture media.

Cellular uptake of [^{3}H]cholesteryl esters

Radiolabeled neutral lipids were obtained from Amersham Corp. (Arlington Hts, IL) and the purity was determined by thin-layer chromatography on silica gel. Only samples with greater than 97% purity were used for experiments. Micellar substrate containing radiolabeled cholesteryl esters was prepared by adding 10 μCi of [^{3}H]cholesteryl oleate (55 mCi/mmol), [^{3}H]cholesteryl linoleoyl ether (46.6 mCi/μmol), or [^{3}H]cholesterol (45 mCi/μmol) to chloroform containing 0.75 μmol of egg phosphatidylcholine and 0.15 μmol of unlabeled cholesteryl oleate. The solvent was evaporated to dryness and the lipids were resuspended in 1.6 ml of 50 mM potassium phosphate, pH 6.6, containing 2 μmol of sodium taurocholate. The sample was sonicated at 46 °C for 30 min using a Branson Sonifier. The clear solution containing the mixed micelles was isolated after low speed centrifugation and was stored at 4 °C under nitrogen.

On the day of the experiment, the micellar solution containing radiolabeled neutral lipids was diluted 20-fold with RPMI 1640 containing 10% lipoprotein-deficient serum. One ml of the substrate solution was then added to confluent Caco-2 cells in RPMI 1640 containing 10% lipoprotein-deficient serum in the presence or absence of cholesterol esterase. Incubation was carried out at 37 °C for 5 h. After incubation, the media were removed and the cell monolayer was washed with RPMI 1640 with 5% lipoprotein deficient serum once and media without serum three times. The cells were harvested by addition of 1 ml of 1 N NaOH. Radioactivity taken up by the Caco-2 cells was determined by liquid scintillation counting.

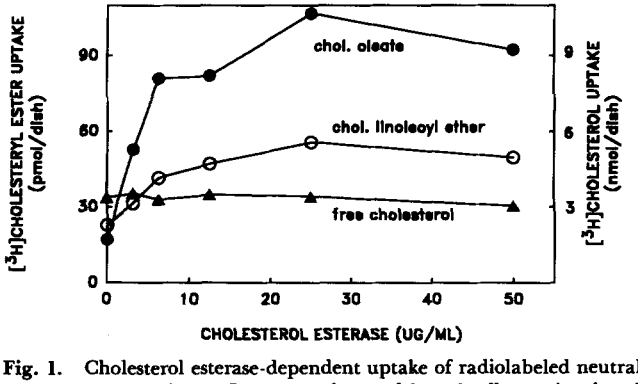


Fig. 1. Cholesterol esterase-dependent uptake of radiolabeled neutral lipids by Caco-2 cells. Confluent monolayers of Caco-2 cells were incubated with various concentrations of cholesterol esterase in the presence of micellar substrates containing either [^{3}H]cholesteryl oleate (●), [^{3}H]cholesteryl linoleoyl ether (○), or [^{3}H]cholesterol (▲). Incubations were carried out at 37 °C for 5 h. After the incubation period, the media were removed, the cell monolayers were washed extensively, and after scraping the cells from the dishes, radioactivity incorporated into the cells was determined by liquid scintillation.

RESULTS

Cholesterol esterase-mediated transport of cholesteryl esters to Caco-2 cells

Initial studies were designed to determine whether Caco-2 cells can be a model for cholesterol esterase-catalyzed lipid transport. The Caco-2 cells incubated with lipid micelles containing [^{3}H]cholesteryl oleate, in the absence of exogenously added cholesterol esterase, resulted in the cellular uptake of [^{3}H]cholesteryl oleate at a rate of 6 pmol/h per 10^6 cells. The addition of purified bile salt-stimulated cholesterol esterase to the incubation medium resulted in an increase of cellular uptake of [^{3}H]cholesteryl oleate in a dose-dependent manner. Maximum induction of lipid transport was observed when the extracellular cholesterol esterase concentration was greater than 25 μg/ml (**Fig. 1**). This result, consistent with observation by Bosner et al. (15), documented that Caco-2 cells can be used as model for studying cholesterol esterase interaction with intestinal cells.

Experiments were also performed to determine whether prior hydrolysis of the cholesteryl esters was required for cellular uptake. The ability of cholesterol esterase to mediate cellular uptake of [^{3}H]cholesteryl oleate was compared with the uptake of unesterified [^{3}H]cholesterol or [^{3}H]cholesteryl linoleoyl ether. The latter compound is a nonhydrolyzable analog of cholesteryl linoleate and is not a substrate for cholesterol esterase. The incubation of Caco-2 cells with cholesterol esterase resulted in a concentration-dependent increase in cellular uptake of the cholesteryl ether analog (Fig. 1). The uptake of the nonhydrolyzable cholesteryl ether was saturated at a level approximately 50% of that observed with cholesteryl oleate (Fig. 1). In contrast, the cellular uptake of the unesterified cholesterol was several orders of magnitude higher than the uptake of the cholesteryl esters. Since the latter experiment was performed by following cellular association of [^{3}H]cholesterol, the result may include both cellular uptake and cholesterol exchange. Nevertheless, it

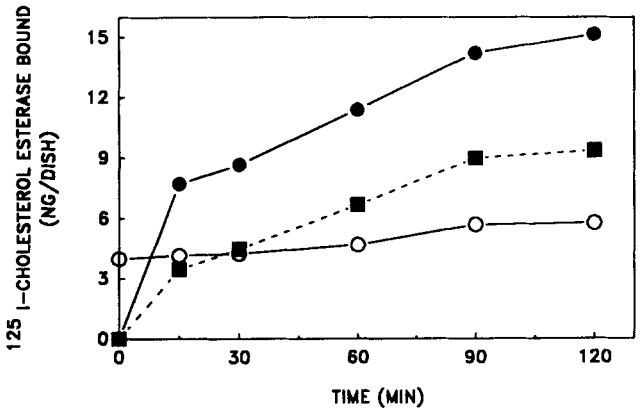


Fig. 2. Time-dependent binding of cholesterol esterase to Caco-2 cells. The Caco-2 cells were plated on 12-well tissue culture plates and incubated at 4 °C with 25 μg/ml of ^{125}I-labeled cholesterol esterase in the presence (○) or absence (●) of 500 μg/ml unlabeled cholesterol esterase. After incubation for the indicated time, the cells were washed extensively and the cell-associated ^{125}I-labeled cholesterol esterase was then determined. The specific binding (■) was determined as the difference observed in cholesterol esterase binding in the presence or absence of excess unlabeled enzyme. The data reported herein represented average of triplicate experiments.

is noteworthy that addition of cholesterol esterase to the medium had no effect on [^{3}H]cholesterol association with the Caco-2 cells (Fig. 1).

Interaction of cholesterol esterase with Caco-2 cells

The interaction of bile salt-stimulated cholesterol esterase with the Caco-2 cells was investigated initially by incubating the cells with ^{125}I-labeled enzyme at 4 °C. Results showed that cholesterol esterase associated with the cells increased with increasing time of incubation, reaching an equilibrium after 90 min of incubation (**Fig. 2**). The binding of cholesterol esterase to Caco-2 cells also increased with increasing concentration of ^{125}I-labeled cholesterol esterase (**Fig. 3**). The binding of ^{125}I-labeled cholesterol esterase was inhibited by addition of excess unlabeled cholesterol esterase, suggesting the existence of specific binding sites for the enzyme on intestinal cells. Specific binding was then determined by subtracting the amount of ^{125}I-labeled cholesterol esterase bound to the cells in the presence of excess unlabeled enzyme from the total binding observed in the absence of unlabeled protein. The results showed that the specific binding component was saturable with maximum binding at 25 μg/ml (Fig. 3A). Scatchard analysis of the specific binding curve revealed a linear plot (r = 0.95) indicative of a single class of binding site on the Caco-2 cells for cholesterol esterase (Fig. 3B). Scatchard analysis from three separate experiments showed maximum binding of 9 to 16 ng of cholesterol esterase per 10^6 cells. Since the molecular weight of the porcine pancreatic cholesterol esterase is 74,000, each Caco-2 cell contains 7.9 to 13 × 10^5 binding sites for cholesterol esterase. The affinity of interaction between cholesterol esterase and Caco-2 cells, based on the slopes of Scatchard plots from three different experiments, was shown to be between 54 and 123 nM. Therefore, results in this experiment suggest that cholesterol esterase interacts with Caco-2 cells through a low affinity and a high capacity binding site.

The next set of experiments was performed to examine the fate of the ^{125}I-labeled cholesterol esterase subsequent to its interaction with intestinal cells. In these experiments, the Caco-2 cells were incubated with ^{125}I-labeled cholesterol esterase at either 4 °C or 37 °C. The cells were washed extensively at various time intervals after the incubation. The amount of ^{125}I-labeled cholesterol esterase associated with the Caco-2 cells was then determined. Parallel experiments were performed by addition of trypsin after the incubation to remove ^{125}I-labeled cholesterol esterase bound to the cell surface. Results showed that > 95% of the ^{125}I-labeled cholesterol esterase bound to the cells at 4 °C was trypsin-sensitive (**Table 1**). These results suggest that the cholesterol

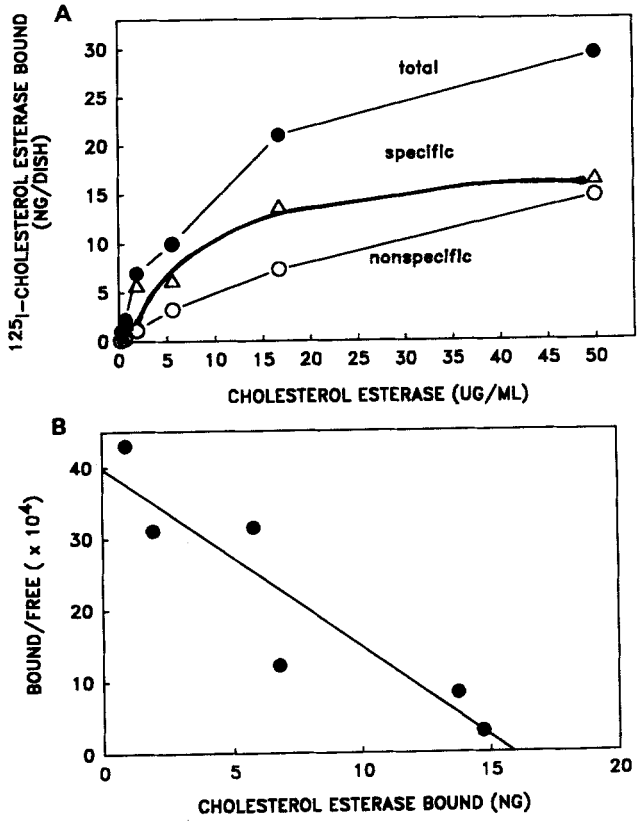


Fig. 3. Concentration-dependent binding of cholesterol esterase to Caco-2 cells. A: The Caco-2 cells were plated on 12-well tissue culture plates and incubated with various amounts of ^{125}I-labeled cholesterol esterase in the presence (○) or absence (●) of 500 μg/ml of unlabeled cholesterol esterase. After incubation for 90 min at 4 °C, the cells were washed extensively and uptake of ^{125}I-labeled cholesterol esterase was then determined. The amount of specific binding (△) was determined by subtracting the nonspecific binding observed in the presence of excess unlabeled cholesterol esterase from the total binding data. The data reported represent the average of triplicate experiments. B: The Scatchard plot analysis of the specific binding data.

TABLE 1. Effect of trypsin on the 4°C-binding of cholesterol esterase to Caco-2 cells

Samples	^{125}I-Labeled Cholesterol Esterase
	ng/dish
Without trypsin	
Supernatant	< 0.1
Pellet	15.3
With trypsin	
Supernatant	15.1
Pellet	0.3

The Caco-2 cells were plated onto 12-well tissue culture dishes for 8 days until confluency. The cell monolayer was washed with RPMI 1640 media and then incubated for 90 min at 4°C with 25 μg/ml of ^{125}I-labeled cholesterol esterase in the presence or absence of 500 μg/ml of unlabeled cholesterol esterase. At the end of the incubation period, the media were removed, the cell monolayer was washed extensively, and the cells were further incubated for an additional 3 min in the presence or absence of 0.25% trypsin and 0.05% EDTA. The cells were then scraped into test tubes and sedimented by centrifugation at 1,000 *g* for 10 min. The radioactivity in the supernatant and in the pellet was determined by gamma counting.

esterase was located at the cell surface at 4°C. In contrast, when the binding experiments were performed at 37°C, approximately 60% of the cell-associated ^{125}I-labeled cholesterol esterase was susceptible to trypsin hydrolysis at each time point after the incubation (**Fig. 4**). The remaining enzyme was trypsin-insensitive, suggesting that approximately 40% of the cholesterol esterase was internalized into the cell interior (Fig. 4).

The mechanism of cholesterol esterase uptake by Caco-2 cells was further investigated using metabolic inhibitors. Results indicated that 50 μM chloroquine had little effect on the total amount of cholesterol esterase associated with the cells (**Fig. 5**). In contrast, the cell-associated cholesterol esterase was reduced by approximately 50% with monensin (Fig. 5). The mechanism of monensin inhibition of cholesterol esterase uptake was investigated by determining the effect of the inhibitor on the trypsin-sensitive and trypsin-resistant pool of cholesterol esterase. In this experiment, the Caco-2 cells were incubated with ^{125}I-labeled cholesterol esterase at 37°C in the presence of inhibitors. The cell monolayer was then washed and treated with trypsin as described above. Results demonstrated that while chloroquine did not have any significant effect on the cellular distribution of the cholesterol esterase, the enzyme associated with the Caco-2 cells in the presence of monensin was almost entirely trypsin-sensitive (Fig. 5). Thus, monensin appeared to affect the cellular uptake of the cholesterol esterase and not the binding of cholesterol esterase to the surface of Caco-2 cells. The binding and uptake of cholesterol esterase by Caco-2 were insensitive to chloroquine inhibition.

In an experiment to further characterize the fate of the internalized cholesterol esterase, Caco-2 cells were preincubated with ^{125}I-labeled cholesterol esterase for 2 h at 37°C. The amount of radiolabeled enzyme that remained associated with the cells at various times after incubation was determined. The amount of cell-associated ^{125}I-labeled cholesterol esterase was found to decrease rapidly with approximately 50% of the radioactivity released from the cells after 4 h of incubation (**Fig. 6**). The inclusion of the lysosomal inhibitor chloroquine in the media had no effect on the release of the internalized cholesterol esterase from Caco-2 cells (Fig. 6).

Consideration was given to the possibility that the enzyme may be released from the cells via a retroendocytosis pathway. Experiments were then performed to analyze the radioactive products released from Caco-2 cells after various period of incubation with ^{125}I-labeled cholesterol esterase. Results showed that approximately 50% of the radioactivity in the cell media was trichloroacetic acid-soluble representing degradation of the protein (**Fig. 7**). However, the remaining radioactivity could be precipitated by trichloroacetic acid (Fig. 7). Examination of the trichloroacetic acid-precipitated radioactivity by SDS-polyacrylamide gel electrophoresis revealed the association of the radioactivity with intact cholesterol esterase (**Fig. 8**). Taken together, these results indicated that while half of the internalized cholesterol esterase could be degraded via a lysosomal-independent pathway, the undegraded cholesterol esterase could also be released from the cells.

Experiments were also performed to determine whether the enzyme released from the cells could facilitate lipid transport. The Caco-2 cells were preincubated with unlabeled cholesterol esterase for 2 h at 4°C. Unbound enzyme was removed by extensive washing and the incubation was continued for an additional 4 h to allow the release of cell-associated cholesterol esterase. Radiolabeled cholesteryl oleate was then added to the incubation media to determine cellular uptake. The amount of radiolabeled lipids taken up by the Caco-2 cells was similar to the cholesteryl ester uptake by cells without

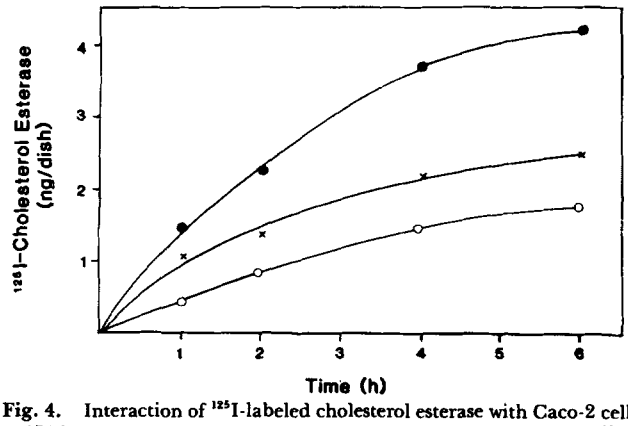
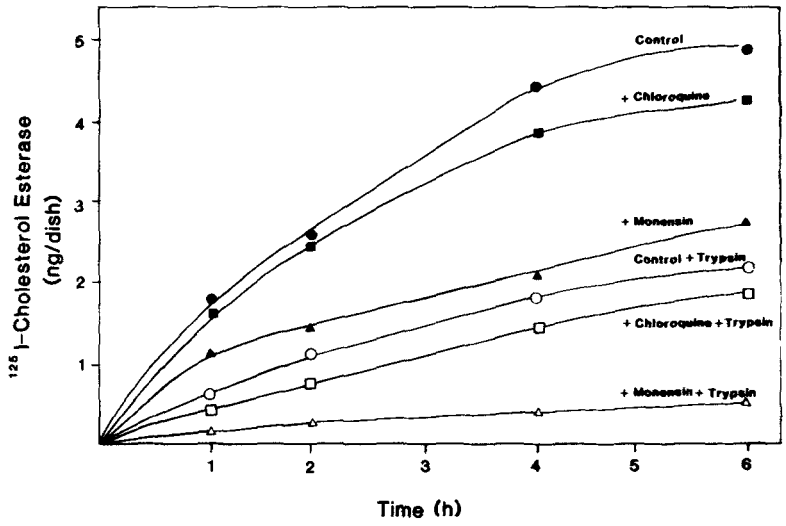


Fig. 4. Interaction of ^{125}I-labeled cholesterol esterase with Caco-2 cells at 37°C. The Caco-2 cells were incubated at 37°C with 25 μg/ml of ^{125}I-labeled cholesterol esterase for the time as indicated. The cells were washed exhaustively and total cell-associated radioactivity (●) was determined from one set of duplicate dishes. In another duplicate set of cultures, the cells were incubated with trypsin (0.25%) and EDTA (0.05%) for 3 min at 37°C to remove surface bound enzymes. The trypsin-sensitive (x) and trypsin-resistent (○) counts associated with the cells were then determined.

Fig. 5. Effect of metabolic inhibitors on cholesterol esterase uptake by Caco-2 cells. The Caco-2 cells were incubated at 37 °C with 25 μg/ml of ^{125}I-labeled cholesterol esterase for the indicated time in the absence of inhibitor (●, ○) or in the presence of 50 μM chloroquine (■, □), or 10 μM monensin (▲, △). The cells were then washed and cell-associated radioactivity was determined (closed symbols). Duplicate cell cultures were treated with trypsin-EDTA as described in the legend for Fig. 4 to determine trypsin-resistant radioactivity (open symbols).



cholesterol esterase (**Table 2**). This result is not unexpected since only a small fraction of the added cholesterol esterase was bound and then released by the cells. The low level of the cholesterol esterase was not sufficient to catalyze uptake of cholesteryl esters. Thus, continuous infusion of cholesterol esterase to the extracellular environment appeared to be essential for lipid transport.

DISCUSSION

Physiological studies in rats have shown that depancreatectomized animals have very low cholesterol esterase activity in their intestine and had defective cholesterol absorption (22). Recent studies using cDNA hybridization techniques have confirmed that cholesterol esterase mRNA was not present in rat intestine (23). Taken together, these observations suggest that the cholesterol esterase in intestine may be derived entirely from the pancreas. The initial mechanism for cholesterol esterase transport to the intestine involves the binding of the enzyme to heparin-like macromolecules on the intestinal cell surface (15). In an effort to further understand the interaction between cholesterol esterase and intestinal cells, this research utilized the Caco-2 cells as a model system to determine the metabolic fate of the en-

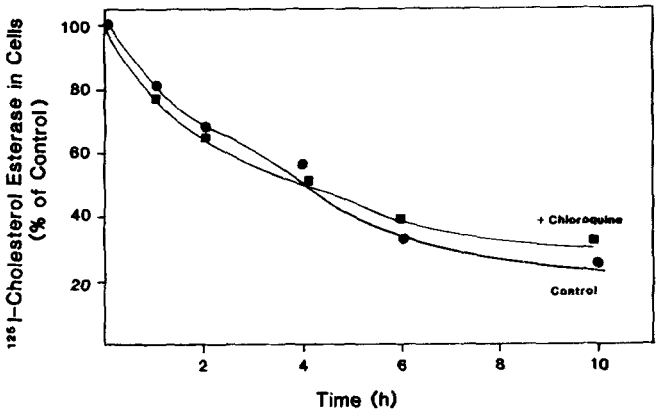


Fig. 6. Metabolic fate of cell-associated cholesterol esterase. The Caco-2 cells were incubated with 25 μg/ml of ^{125}I-labeled cholesterol esterase for 2 h at 37 °C. The cells were washed exhaustively and one set of cultured cells was harvested to determine cell-associated radioactivity. The incubation was continued for the remaining cultures in media without ^{125}I-labeled cholesterol esterase in the presence (■) or absence (●) of chloroquine for the time indicated. ^{125}I-labeled cholesterol esterase remaining associated with the cells at each time point was then determined from triplicate cultures.

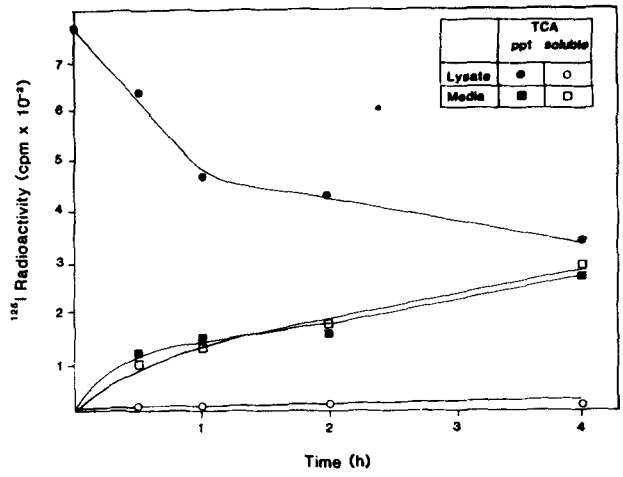


Fig. 7. Degradation and release of ^{125}I-labeled cholesterol esterase from Caco-2 cells. The Caco-2 cells were incubated with 25 μg/ml of ^{125}I-labeled cholesterol esterase for 1 h at 37 °C. The cells were washed exhaustively and incubation was continued in the absence of exogenous cholesterol esterase at 37 °C for the indicated time. Radioactivity secreted from the cells was determined by counting of the media (■, □) and radioactivity remaining in the cells (●, ○) were determined as described in the legend to Fig. 7. Cell lysate was prepared by making the cell suspension 1% in Triton X-100 and centrifuging briefly to sediment insoluble debris. Trichloroacetic acid was then added to the media and to cell lysate to a final concentration of 10%. The acid-soluble (open symbols) and -insoluble counts (closed symbols) were then determined from the samples.

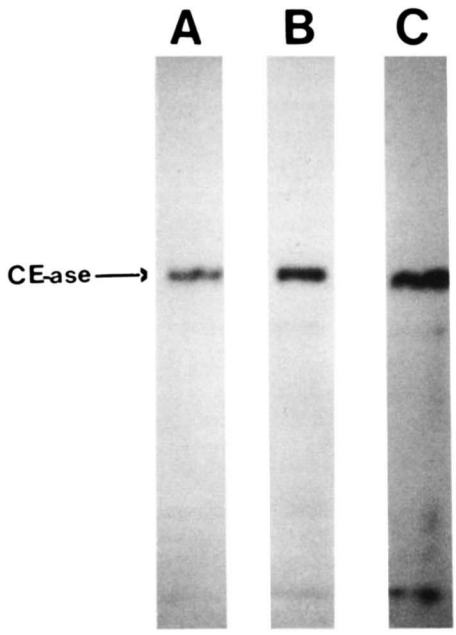


Fig. 8. SDS-polyacrylamide gel electrophoresis of cholesterol esterase released by Caco-2 cells. The Caco-2 cells were incubated with 25 μg/ml of ^{125}I-labeled cholesterol esterase for 1 h at 37 °C. The cells were washed exhaustively and then incubated for an additional 4 h in the absence of cholesterol esterase. The ^{125}I-labeled cholesterol esterase released from the cells was then precipitated with 10% trichloroacetic acid and subjected to SDS-polyacrylamide gel electrophoresis. Lane A shows the radiolabeled cholesterol esterase (CE-ase) before incubation with Caco-2 cells. Lanes B and C show the ^{125}I-labeled cholesterol esterase released from the Caco-2 cells 2 or 4 h after the incubation period, respectively.

zyme in intestine. Results of this study showed that the binding of cholesterol esterase to the cell surface was mediated by low affinity and high capacity binding site(s). The interaction of cholesterol esterase with these binding sites resulted in the partial internalization of the protein. The uptake of cholesterol esterase by Caco-2 cells involved specific endocytosis and was sensitive to monensin inhibition. These biochemical data are in agreement with previous immunolocalization studies that demonstrated the presence of cholesterol esterase in endocytic vesicle-like structures in enterocytes (14).

Cholesteryl ester hydrolytic activity has also been demonstrated previously in brush-border membranes (24) and in the soluble fraction (25) of an homogenate of rat intestine. Immunocytochemical studies have localized the cholesterol esterase in enterocytes with an occasional staining of brush border membranes (26). This dichotomy was partially resolved by results of the current study. The data presented in Fig. 4 indicated that approximately half of the cholesterol esterase was located on the cell surface while the remaining enzyme was internalized by the cells at each time point after incubation. Therefore, it is likely that the cholesterol esterase on the cell surface in this in vitro experiment was similar to the enzyme observed on brush border membranes in vivo. The cytosolic cholesterol esterase in enterocytes was probably derived from endocytosis of the protein as observed in this in vitro study.

A unique finding in the current study is the short residence time of cholesterol esterase in intestinal cells. The results showed that the endocytosed cholesterol esterase was degraded to trichloroacetic acid-soluble products and/or rapidly secreted from the cells. The degradation of cholesterol esterase in Caco-2 cells was not sensitive to chloroquine inhibition, suggesting that hydrolysis occurred independent of lysosomal activity. Although the mechanism by which cholesterol esterase is degraded within intestinal cells remains unknown, it is tempting to speculate that the rapid degradation of the enzyme is inherent in the structure of the protein. Previous analysis of the protein structure, deduced from nucleotide sequencing of the cDNA, has revealed the presence of four repetitive sequences riched in prolyl, aspartyl, seryl, and threonyl residues (23). According to the PEST hypothesis of Rogers et al. (27), proteins with regions enriched with these residues are susceptible to hydrolysis and have short half-lives in vivo. It is possible that the PEST region of cholesterol esterase provided the signal for the rapid extralysosomal degradation of this protein in intestinal cells.

The rapid disappearance of cholesterol esterase from intestinal cells is noteworthy. This result documented that, regardless of whether the enzyme is degraded intracellularly or secreted from the cells, the cholesterol esterase secreted from pancreas could facilitate intestinal lipid absorption only transiently. This hypothesis is in agreement with the time course of lipid absorption observed in intact animals. Previous studies by Vahouny and Treadwell (28) have shown that the cholesterol level in lymph increased rapidly after cholesterol administration to the rats. Maximum level of lymphatic

TABLE 2. Cholesterol esterase-mediated uptake of [^{3}H]cholesteryl oleate by Caco-2 cells

Cholesterol Esterase Added	[^{3}H]Cholesteryl Oleate Uptake	
	With Washing	Without Washing
μg/ml	*pmol/dish*	
0	24.0	24.8
3.13	24.1	41.4
6.25	23.4	43.5
12.50	22.8	54.9
25.00	24.4	59.4
50.00	24.6	66.3

Confluent Caco-2 cells were incubated in RPMI 1640 media containing 10% lipoprotein-deficient serum in the presence of cholesterol esterase at the indicated concentrations. Incubation was carried out at 37 °C for 2 h. Cell culture media were then removed from half of the samples and the cells were washed with media without cholesterol esterase and fresh media were added. [^{3}H]Cholesteryl oleate was then added to the cells (with or without removal of the cholesterol esterase) and incubation was continued for 5 h. Uptake of the radiolabeled lipids was then determined by scraping the cells and counting in a liquid scintillation counter. The results reported are the average of two duplicate experiments.

cholesterol was observed between 3–6 h followed by a gradual decline to the preabsorptive level at 9–12 h of after cholesterol administration (28). A comparable time course of cholesterol absorption was also demonstrated in human subjects. After administration of radioactive cholesterol to patients with thoracic duct fistula, the maximum level of radioactive cholesterol in the lymph was observed after 8–9 h (29). Thus, cholesterol absorption may be related to the level of cholesterol esterase in the intestine. Since cholesterol esterase is degraded rapidly by the intestine, the hypothesis would predict a requirement for a rapid increase in cholesterol esterase synthesis after food ingestion. In this regard, it is interesting that pancreatic cholesterol esterase biosynthesis could be stimulated by gastric hormones such as cholecystokinin and secretin (30). The level of these hormones is regulated by food components delivered to the intestinal tract (31).

The results of this study also provided additional clues toward understanding the mechanism of cholesterol absorption. First, the data showed that cholesterol esterase does not catalyze the intestinal uptake of free cholesterol. This observation is consistent with the observation that infused [^{3}H]cholesterol was absorbed and esterified with equal efficiency in the presence or absence of pancreatic flow (6). These results also supported the concept that the uptake of free cholesterol and its lymphatic transport as cholesteryl esters were mediated by ACAT and were independent of the pancreatic cholesterol esterase (8, 32). Second, the results of this study demonstrated that cholesterol esterase was important in mediating intestinal uptake of esterified cholesterol. This conclusion supports the results of Fernandez and Borgström (9). These investigators have shown that the lipase and esterase inhibitor tetrahydrolipstatin prevented the absorption of cholesteryl esters but not that of free cholesterol by rat intestine. Our results also indicated that cholesterol esterase-mediated uptake of cholesteryl esters is partially independent of the hydrolysis of the cholesteryl esters. Although the precise mechanism of cholesteryl ester uptake remains to be determined, it is possible that the cholesteryl esters interacted with the enzyme and were internalized to the cell interior as a lipid-protein complex. The lower level of cholesteryl ether uptake, in contrast to the uptake of cholesteryl oleate, may be due to the hydrolysis of the internalized cholesteryl oleate before its transport to an intracellular compartment for re-esterification by ACAT (13). The inability of the cholesterol esterase to hydrolyze the cholesteryl ether analog may result in its accumulation and saturation at the cell surface membrane. Thus, our results support the concept that cholesterol esterase is not required for intestinal uptake and lymphatic transport of free cholesterol. However, cholesterol esterase appears to be important for the cellular uptake, and possibly the hydrolysis, of esterified cholesterol. This protein may then act in concert with ACAT to mediate the transport of the cholesterol component associated with cholesteryl esters through the digestive tract.

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