Continuous monitoring of cholesterol oleate hydrolysis by hormone-sensitive lipase and other cholesterol esterases

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Abstract Hormone-sensitive lipase (HSL) contributes importantly to the hydrolysis of cholesteryl ester in steroidogenic tissues, releasing the cholesterol required for adrenal steroidogenesis. HSL has broad substrate specificity, because it hydrolyzes triacylglycerols (TAGs), diacylglycerols, monoacylglycerols, and cholesteryl esters. In this study, we developed a specific cholesterol esterase assay using cholesterol oleate (CO) dispersed in phosphatidylcholine and gum arabic by sonication. To continuously monitor the hydrolysis of CO by HSL, we used the pH-stat technique. For the sake of comparison, the hydrolysis of CO dispersion was also tested using other cholesteryl ester-hydrolyzing enzymes. The specific activities measured on CO were found to be 18, 100, 27, and 3 μmol/min/mg for HSL, cholesterol esterase from *Pseudomonas* species, *Candida rugosa* lipase-3, and cholesterol esterase from bovine pancreas, respectively. The activity of HSL on CO is ~4- to 5-fold higher than on long-chain TAGs. In contrast, with all other enzymes tested, the rates of TAG hydrolysis were higher than those of CO hydrolysis. The relatively higher turnover of HSL on CO observed in vitro adds further molecular insight on the physiological importance of HSL in cholesteryl ester catabolism in vivo. Thus, HSL could be considered more as a cholesteryl ester hydrolase than as a TAG lipase.—Ben Ali, Y., F. Carrière, R. Verger, S. Petry, G. Muller, and A. Abousalham. **Continuous monitoring of cholesterol oleate hydrolysis by hormone-sensitive lipase and other cholesterol esterases. *J. Lipid Res.* 2005.** 46: **994–1000.**

Supplementary key words cholesterol ester hydrolase • triacylglycerol lipase • cholesterol oleate emulsion

FFAs are an important source of energy in mammals. Hormone-sensitive lipase (HSL) is thought to play a crucial role in the mobilization of FFAs from the triacylglycerols (TAGs) stored in adipocytes (for review, see 1). In vivo, HSL is activated by phosphorylation via cAMP-dependent kinase in response to various lipolytic hormones, such as catecholamines. Phosphorylation of HSL leads to its translocation from the cytoplasm to the lipid droplet (2). Insulin acts as an antilipolytic hormone by phosphorylating and activating phosphodiesterase 3B, which hydrolyzes cAMP and thus reduces the hydrolysis of TAG (1) by HSL. In addition to adipocytes, HSL is expressed in other tissues (3), including skeletal muscle, heart, brain, pancreatic β cells, adrenal gland, ovaries, testes, and macrophages (1).

HSL also plays an important role in the mobilization of cholesterol from cholesteryl ester. Cholesteryl esters provide the cholesterol required for steroid synthesis in steroidogenic tissues, such as the adrenal cortex. The level of cholesteryl ester hydrolase activity in the adrenals of HSL-null mice has been found to be reduced by more than 98% compared with that in wild-type mice, which suggests that HSL is mainly responsible for the hydrolysis of most of the cholesteryl esters present in this tissue and that it is involved in the intracellular processing of the cholesterol required for adrenal steroidogenesis (4, 5). Furthermore, evidence has been presented in various studies (6, 7) that HSL contributes importantly to the hydrolysis of most of the cholesteryl esters present in macrophage foam cells. However, Contreras (8) has reported that cholesteryl esters from macrophages of HSL-null mice are mobilized similarly to wild-type macrophages. One possible explanation for this finding is that in the absence of HSL, a compensatory induction of other cholesteryl ester hydrolases may occur (9, 10).

In previous kinetic studies, it was established that HSL

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Abbreviations: β-CD, β-cyclodextrin; CO, cholesterol oleate; CRL-3, *Candida rugosa* lipase-3; DAG, diacylglycerol; DGL, dog gastric lipase; FID, flame ionization detection; GA, gum arabic; HPL, human pancreatic lipase; HSL, hormone-sensitive lipase; MAG, monoacylglycerol; NaTDC, sodium taurodeoxycholate; PC, phosphatidylcholine; PCE, cholesterol esterase from bovine pancreas; PI, phosphatidylinositol; PsCE, cholesterol esterase from *Pseudomonas* species; TAG, triacylglycerol.

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has broad substrate specificity, because it hydrolyzes long-chain TAG, diacylglycerol (DAG), and monoacylglycerol (MAG) as well as cholesteryl and retinyl esters (for review, see 1). HSL activity on long-chain acyl esters is generally determined by measuring the release of radiolabeled oleic acid from phospholipid-stabilized emulsions of various substrates in a discontinuous assay system. In a recent study (11), we further documented the kinetic behavior of pure recombinant HSL, using emulsions of medium- and long-chain vinyl esters and TAG as substrates. In all cases investigated, the hydrolytic activity of HSL was monitored using the pH-stat method, and the FFAs released were measured potentiometrically. Under standardized in vitro conditions, HSL activity on DAG was ~10- and 5-fold higher than that on TAG and MAG, respectively (11, 12).

Although the activity of HSL on acylglycerols has been characterized in detail, little attention has been paid to the kinetics of cholesteryl ester hydrolysis by this important enzyme. This gap in the kinetic data available on HSL is mainly attributable to the lack of an easy, specific, and continuous assay for monitoring cholesteryl ester hydrolysis. In previous studies, the activity of HSL on cholesteryl esters generally has been determined by measuring the release of radiolabeled fatty acid from cholesteryl ester emulsions (for review, see 13). This method is not a continuous one and is time-consuming, in that it requires a separation step to evaluate the fatty acids released.

In previous assays, cholesterol oleate (CO) has been used in the form of an emulsion stabilized by gum arabic (GA) (14), dispersed with phosphatidylcholine (PC) in glycerol (15), dispersed in PC and sodium taurocholate (16), dispersed in PC/phosphatidylethanolamine liposomes (17), or emulsified with PC/phosphatidylinositol (PI; 3:1, w/w) (12). It turns out that, in addition to the buffer composition and pH, the hydrolysis of cholesteryl esters by cholesteryl ester-hydrolyzing enzymes depends strongly on the mode of organization of the lipid substrate present in interfacial structures, such as monolayers, micelles, liposomal dispersions, or oil-in-water emulsions. These lipid-water interfaces are characterized by a very different "interfacial quality," as estimated by the interfacial tension, which is one of the most decisive parameters to be considered when working with lipolytic enzymes (18).

The natural substrates of lipolytic enzymes, such as TAGs and cholesteryl esters, are usually the esters of long-chain fatty acids. Upon hydrolysis, they generate poorly water-soluble lipolysis products, which remain transiently at the lipid-water interface. The progressive interfacial accumulation of these products can induce a change in the physicochemical properties of the interface, associated either with product inhibition or enhancement of the reaction rate, as characterized by lag periods (19, 20). To solubilize the lipolysis products, tensioactive BSA has been widely used as an acceptor of long-chain FFAs (21, 22). We recently described the use of the nontensioactive compound β-cyclodextrin (β-CD) as an acceptor for long-chain FFAs as well as MAG, resulting from lipase hydrolysis of TAG and DAG (23), and lysolecithin, resulting from the phospholipase A_2 hydrolysis of lecithin (24, 25). β-CD is a cyclic oligosaccharide (seven glucopyranoside units) that has a polar surface and a hydrophobic cavity. These molecules not only can form inclusion complexes with FFA, MAG, and lysolecithin but have also been found to solubilize cholesterol (26). The aim of the present study was to monitor, continuously, the rate of hydrolysis by HSL of CO dispersed in GA and PC using the pH-stat technique. Under the same experimental conditions, the specific activity of HSL was compared with that of other known cholesterol esterases.

MATERIALS AND METHODS

Materials

Egg yolk PC, soybean PI, CO, β-CD, GA, sodium taurodeoxycholate (NaTDC), and BSA were obtained from Sigma. Crude olive oil was purchased from Lesieur (Aix-en-Provence, France). All other chemicals and solvents were of reagent or better quality and were obtained from local suppliers.

Proteins

Recombinant human HSL was expressed in insect cells and purified as described by Ben Ali et al. (11). Cholesterol esterase from *Pseudomonas* species (PsCE) and cholesterol esterase from bovine pancreas (PCE) were obtained from Sigma. Cholesterol esterase from *Candida cylindracea* [known as *Candida rugosa* lipase-3 (CRL-3)] was obtained from Roche Diagnostics. The purity of PsCE, PCE, and CRL-3 was checked by performing SDS-PAGE (10% acrylamide) followed by Coomassie blue staining (**Fig. 1**) and by densitometric analysis. Using various known concentrations of BSA as standard in SDS-PAGE, the purity of CRL-3 and PCE was found to be more than 95% and that of PsCE was found to be ~90–95% (Fig. 1). Recombinant human pancreatic lipase (HPL) was expressed and purified from insect cells as described by Thirstrup et al. (27). Recombinant dog gastric lipase (DGL) was kindly provided by Meristem Therapeutics (Clermont-Ferrand, France). Recombinant *Thermomyces lanuginosus* lipase was kindly provided by Dr. S. Patkar (Novo Nordisk, Copenhagen, Denmark). Porcine colipase devoid of pancreatic phospholipase A_2 contamination was purified by J. De Caro [Enzymology at Interfaces and Physiology of Lipolysis (EIPL), Marseille, France]. The protein concentrations were determined using Bradford's procedure (28), with Bio-Rad dye reagent and BSA as the standard.

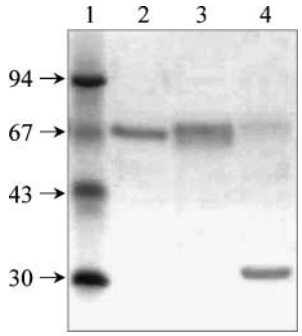

Fig. 1. SDS-PAGE (10% acrylamide) of cholesterol esterase from bovine pancreas (PCE), cholesterol esterase from *Candida cylindracea* (CRL-3), and cholesterol esterase from *Pseudomonas* species (PsCE). The gel was stained with Coomassie blue to reveal the proteins. Lane 1, molecular mass markers; lane 2, PCE (3 μg); lane 3, CRL-3 (3 μg); lane 4, PsCE (3 μg).

Preparation of substrate dispersion

To prepare the CO dispersion, 90 mg of CO was dissolved in 40 μl of a PC solution (100 mg/ml in chloroform) and mixed in a 20 ml glass vial, and the solvent was evaporated under a stream of N_2. The dried lipid mixture was resuspended in 5 ml of GA solution (10%) (29) and then subjected to ultrasonic treatment in an ice bath using a Branson Sonifier (type II, standard microtip; 2 × 1 min at setting 2 followed by 2 × 1 min at setting 4 with 1 min intervals). Olive oil emulsion was prepared as described previously (11).

Lipolytic activity measurements

Lipolytic activity was assayed by continuously measuring the FFA released from mechanically stirred emulsions of either CO or olive oil, using 0.1 N NaOH with a pH-stat apparatus (TTT80 radiometer; Radiometer, Copenhagen) adjusted to a constant end point value. Each kinetic assay was performed in a thermostated (37°C) vessel in a final assay volume of 10 ml containing 0.25 ml of substrate emulsion and 9.75 ml of 0.25 mM Tris-HCl buffer, 150 mM NaCl, and 2 mM $CaCl_2$. Lipolysis of long-chain TAGs or cholesteryl esters is inhibited by the free fatty acids (or their soaps) released, and this inhibition can be prevented by adding $CaCl_2$ and NaCl to the reaction medium (30). When required, BSA (0.6 μM final concentration), β-CD (3 mM final concentration), or NaTDC (4 mM final concentration) was added to the assay medium. Because of the presence of 1 mM β-mercaptoethanol in all of the buffers used for the purification of HSL, its final concentration ranged between 3 and 5 μM in the pH-stat reaction medium when either CO or olive oil was used as substrate. This final concentration of β-mercaptoethanol was sufficient for optimal HSL activity (data not shown).

The activity of HPL was measured in the presence of porcine colipase at a colipase-lipase molar excess of 5 and NaTDC (4 mM final concentration). For the DGL activity measurements, incubations were performed at pH 5 during 1–2 min, and the FFA released were back-titrated at the end of the incubation period by shifting the pH from 5 to 9 (22). Specific lipolytic activities are expressed in international units per milligram of enzyme. One unit corresponds to 1 μmol of fatty acid released per minute.

Extraction and analysis of lipolysis products

Lipid extraction was performed immediately after the sampling procedure from the pH-stat reaction medium using the method of Folch, Lees, and Sloane Stanley (31). A 1 ml sample of the pH-stat reaction medium containing CO dispersion undergoing lipolysis was mixed vigorously with 200 μl of 0.1 N HCl and 5 ml of chloroform-methanol (2:1, v/v) in a 15 ml glass tube with a screw cap. After phase separation, the lower organic phase was collected using a Pasteur pipette and transferred to a 15 ml test tube, where it was dried over anhydrous $MgSO_4$. Once $MgSO_4$ had precipitated, 1–2 ml of the clear organic phase was transferred to a 2 ml vial with a screw cap, and the vial was kept at −20°C until analysis was performed.

Quantitative analysis of CO, cholesterol, and FFA was performed by TLC coupled to flame ionization detection (FID) using Iatroscan MK5 equipment (Iatron Laboratories). To separate the neutral lipids, 1 μl of each lipid extract was spotted onto a quartz rod coated with silica (0.9 mm diameter Chromarod™ SIII; Iatron Laboratories), and sample migration was determined with heptane-diethyl ether-acetic acid (55:45:1, v/v/v). After ~20 min, the chromarod holder (10 chromarods) was removed from the TLC tank, and the chromarods were dried at 150°C for 15 min (Rod dryer TK8; Iatron Laboratories). The chromarod holder was then transferred to the Iatroscan MK5, and each chromarod was scanned by FID for detection and quantification of the compounds separated on silica. Known amounts (0.1–10 μg) of standard compounds (CO, cholesterol, and oleic acid) were used to calibrate the mass detection by FID. For each class of compound analyzed (CO, cholesterol, and oleic acid), a response factor (peak area vs. mass) was estimated and used to quantify the lipid masses in the various samples. The mass detection data were converted into moles using the following molar masses: CO, 651 Da; cholesterol, 386.66 Da; 1(3)-monoolein, 335.4 Da; FFA, 282.47 Da.

RESULTS AND DISCUSSION

Various methods of dispersing CO by sonication were initially tested as a means of determining HSL activity (data not shown). Only in the presence of PC and GA did we obtain stable CO dispersions. Therefore, the lipolytic activity of HSL was measured using CO as substrate (4 mM final concentration) dispersed in 10% GA and PC (0.4 mM final concentration). Although GA increases the physicochemical complexity of the system, it is the most commonly used emulsifier of long-chain TAGs such as natural olive oil and triolein (29, 32). The HSL activity remained unchanged when this CO dispersion was kept for several hours at 4°C, and reproducible results were obtained when the dispersion was stored at −20°C for up to 1 week. Based on ^{13}C-NMR spectroscopic data (33, 34), it was previously reported that cholesteryl esters are slightly soluble in phospholipid vesicles. Furthermore, the single carbonyl group of CO was present near the aqueous interface of the phospholipid bilayer and was thus favorably oriented for enzymatic hydrolysis (33). In a recent study (11), we established that the kinetics of the hydrolysis of diolein emulsions by pure recombinant HSL were nonlinear, whereas adding BSA (0.6 μM final concentration) resulted in linear kinetic recordings and therefore probably prevented HSL from undergoing interfacial denaturation at the lipid/water interface (11). It has been established in the case of gastric lipase (22) that surface denaturation can be prevented by adding proteins such as BSA. As shown in Fig. 1, kinetic recordings of the process of CO hydrolysis by HSL were linear for at least 3 min in the presence of BSA (0.6 μM final concentration) after a short lag period lasting a few seconds. The final BSA concentration in the assay (0.6 μM) was optimized by measuring the rate of hydrolysis of CO and olive oil emulsions by HSL (data not shown). Under these experimental conditions, the specific activity of HSL was 18 ± 2 U/mg, and the rates of hydrolysis were found to vary linearly with the amounts of recombinant human HSL (data not shown). However, adding BSA after HSL injection failed to reactivate the enzyme (data not shown). HSL activity was reduced by ~50% when the nontensioactive β-CD instead of BSA was added to a system containing CO dispersion to sequester the lipolysis products, FFA and cholesterol (**Fig. 2**). We checked that the combined addition of β-CD and BSA did not increase the hydrolysis rate above the value measured in the presence of BSA alone (data not shown).

Various mixed liposomes were prepared with CO concentrations ranging from 0 to 9 mM and used as substrate for recombinant human HSL. The steady-state reaction

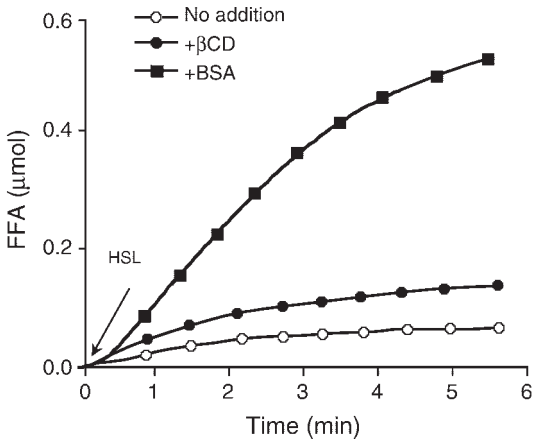

Fig. 2. Kinetic recording of the hydrolysis of cholesterol oleate (CO) dispersion by hormone-sensitive lipase (HSL) using the pH-stat technique. The experiments were carried out at 37°C in a final assay volume of 10 ml containing 0.25 ml of CO dispersion (see Materials and Methods) and 9.75 ml of 0.25 mM Tris-HCl buffer, 150 mM NaCl, and 2 mM $CaCl_2$. BSA (0.6 μM final concentration) or β-cyclodextrin (β-CD; 3 mM final concentration) was added to the assay system before enzyme injection. HSL was added at time 0 (arrow) after recording the background hydrolysis level for 5 min. The kinetic recordings shown here are typical of those obtained in three independent experiments.

rates were plotted versus the CO concentration (**Fig. 3**). The reaction rate increased quasilinearly with the CO concentration from 0 to ~4 mM and then reached a plateau at higher CO concentrations. Based on the above results, a final CO concentration of 4 mM was selected.

The chemical composition of the mixed liposomes was found to significantly affect the rate of CO hydrolysis by HSL. As shown in **Fig. 4A**, the highest rates of lipolysis (100% of the maximum activity) were obtained when PC liposome alone or liposomes prepared from a mixture of PC and PI (3:1) were used. Lower lipolysis rates (10–80%

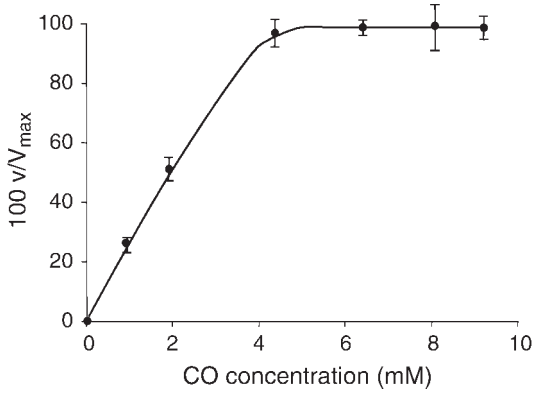

Fig. 3. Effects of CO concentration on the rate of hydrolysis of CO dispersion by HSL. Activity is expressed as a percentage of the maximum activity (100 v/V_{max}). Assay conditions were the same as in the experiments presented in Fig. 1 with BSA in the reaction medium. The error bars correspond to the kinetic recordings obtained in three independent experiments.

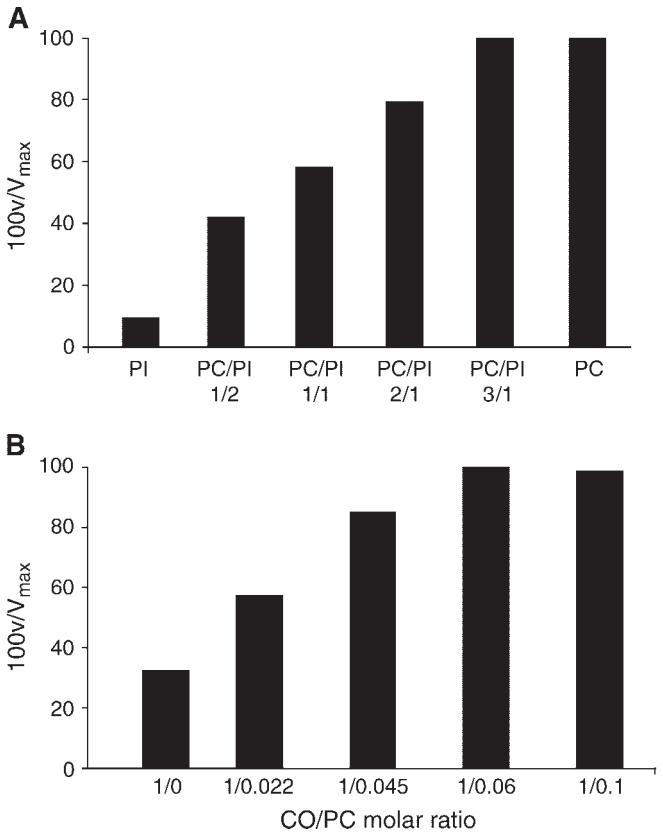

Fig. 4. Effects of the presence of phospholipids on the hydrolysis rates of CO dispersion. A: CO dispersed in gum arabic (GA) and phosphatidylinositol (PI), phosphatidylcholine (PC), or mixtures of PI and PC at various molar ratios was subjected to hydrolysis by HSL. B: CO dispersed in GA and PC at various molar ratios. Assay conditions were the same as described for Fig. 2. Activity is expressed as a percentage of the maximum activity (100 v/V_{max}).

of the maximum activity) were observed with liposomes consisting of either PI alone or a mixture of PC and PI with a PC/PI ratio of ≤2 (Fig. 4A). To determine the optimum CO/PC molar ratio, increasing concentrations of PC were used. As shown in Fig. 4B, HSL activity increased with increasing amounts of PC and reached a plateau at a CO/PC molar ratio of ~1:0.1. In the absence of PC, HSL was catalytically active toward the CO emulsion and expressed ~35% of its maximum activity (Fig. 4B). Based on these results, a CO/PC molar ratio of 1:0.1 was used in the kinetic experiments described below.

The ability of HSL to efficiently hydrolyze CO dispersion was further investigated by analyzing the lipolysis products using the TLC-FID technique (see Materials and Methods). Calibration curves of the FID mass detection data were drawn up using known amounts of CO, cholesterol, and oleic acid and fitted by a power law equation (FID response vs. weight of lipid, $y = ax^b$) using amounts of lipid ranging from 0 to 10 μg (data not shown). The cholesterol and oleic acid contents were quantified using this calibration curve in the 0.1 to 2 μg lipid range, where the response was linear. Upon enzymatic lipolysis of CO, an increase in FFA and cholesterol occurred (data not shown). Based on FFA and cholesterol quantification (mass determination

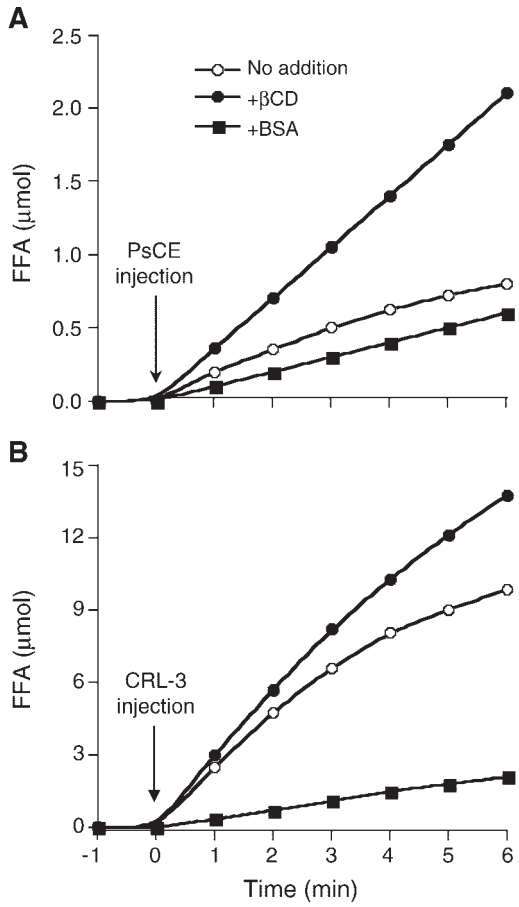

Fig. 5. Kinetic recording of the hydrolysis of CO dispersion by PsCE (A) or CRL-3 (B) using the pH-stat technique. The experiments were carried out at 37°C in a final assay volume of 10 ml containing 0.25 ml of CO dispersion, 0.25 mM Tris-HCl buffer, 150 mM NaCl, and 2 mM $CaCl_2$. BSA (0.6 μM final concentration) or β-CD (3 mM final concentration) was added to the assay system before enzyme injection. PsCE or CRL-3 was added after recording the background hydrolysis level for 1–5 min. The kinetic recordings shown here are typical of those obtained in three independent experiments.

followed by conversion into moles), the specific activity of HSL was estimated to be 22 U/mg. This value is comparable to that measured with the pH-stat method (18 U/mg). These values are of the same order of magnitude as the 15 U/mg obtained by Fredrikson et al. (12) using radiolabeled CO emulsified with PC/PI (3:1, w/w) as a substrate for purified rat adipose tissue HSL.

The rate of hydrolysis of the CO dispersion was also measured using other commercially available cholesteryl ester-hydrolyzing enzymes. We compared the kinetic recordings obtained during the hydrolysis of CO dispersion by PsCE (**Fig. 5A**) and CRL-3 (Fig. 5B). As can be seen from Fig. 4, the highest activities were measured in the presence of β-CD (3 mM final concentration) with both enzymes, and linear kinetics were recorded for at least 6 min with PsCE (Fig. 5A) and for 3 min with CRL-3 (Fig. 5B). Maximum specific activities of 100 ± 5 and 27 ± 3 U/mg were obtained with PsCE and CRL-3, respectively. It is worth noting that by replacing β-CD with BSA before the enzyme injection, the specific activities were reduced to an average of 25 and 7 U/mg with PsCE and CRL-3, respectively. We checked that the combined addition of β-CD and BSA did not increase the hydrolysis rate above the value measured in the presence of β-CD alone (data not shown). Similar inhibitory effects of proteins such as BSA on lipases have been observed both in a monolayer system (35) and with TAG emulsions (36–39). Gargouri et al. (39) reported that the lipase-inhibiting proteins isolated from soybean were highly surface-active and decreased the interfacial tension at the tributyrin-water and triolein-water interfaces. These authors provided evidence that the inhibitory effects of proteins on pancreatic lipase are attributable to their ability to interact with lipids and to modify the quality of the substrate-water interface (38).

The CO-hydrolyzing enzymes used in the present study (HSL, PsCE, CRL-3, and PCE) are known as true lipases, able to catalyze the hydrolysis of long-chain TAGs (1, 11, 40–42). The maximum specific activities of HSL, PsCE, CRL-3, and PCE toward CO dispersion and olive oil emulsions are summarized in **Table 1**. It can be seen from this table that the rates of olive oil hydrolysis by PsCE, CRL-3, and PCE are higher than those of CO hydrolysis. In contrast, the rate of hydrolysis of CO by HSL was approximately four to five times higher than the rate measured with olive oil (Table 1). Previous findings (11, 12, 43) have indicated that the rate of HSL activity toward CO was approximately twice that recorded with TAG. It is worth not-

TABLE 1. Specific activities of various lipases using CO dispersion and olive oil emulsion as substrates

	Specific Activity					
Enzyme/Substrate	HSL	PsCE	CRL-3	PCE	HPL	DGL
			U/mg			
Olive oil	4 ± 1	700 ± 50	100 ± 5	12 ± 2	4,200 ± 100	140 ± 10
CO	18 ± 2	100 ± 5	27 ± 3	3 ± 1	0	0
CO/olive oil ratio	4.5	0.14	0.27	0.25	0	0

CO, cholesterol oleate; CRL-3, *Candida rugosa* lipase-3; DGL, dog gastric lipase; HPL, human pancreatic lipase; HSL, hormone-sensitive lipase; PCE, cholesterol esterase from bovine pancreas; PsCE, cholesterol esterase from *Pseudomonas* species. Each kinetic assay was performed in a thermostated (37°C) vessel in a final assay volume of 10 ml containing 0.25 ml of the substrate emulsion and 9.75 ml of 0.25 mM Tris-HCl buffer, pH 7.5, 150 mM NaCl, and 2 mM $CaCl_2$. Additives such as BSA (0.6 μM final concentration) in the case of HSL or sodium taurodeoxycholate (4 mM final concentration) in the case of PCE were added to the pH-stat reaction medium when measuring their catalytic activities. When measuring the catalytic activities of PsCE and CRL-3, β-cyclodextrin (3 mM final concentration) was added in the pH-stat reaction medium. The catalytic activities of HPL and DGL were assayed as described in Materials and Methods.

ing that no cholesterol esterase activity was measured with either HPL or DGL when CO dispersion was used as the substrate (Table 1).

HSL has been described as an intracellular enzyme with a broad substrate specificity, hydrolyzing long-chain TAG, DAG, and MAG as well as cholesteryl esters and retinyl esters (for review, see 1). We (11) and others (12) have reported that the activity of HSL on DAG was ~10-fold higher than on long-chain TAG. In the present study, the activity of HSL on CO was found to be approximately four to five times higher than on long-chain TAG (Table 1). This relatively higher turnover of HSL on CO observed in vitro adds further molecular insight regarding the physiological importance of HSL in cholesteryl ester catabolism in vivo (6, 44). Thus, HSL could be considered more as a cholesteryl ester hydrolase than as a TAG lipase. Previous data have shown that targeted disruption of the HSL gene in mice resulted in complete abolition of HSL activity on cholesteryl esters, whereas a significant level of residual TAG lipase activity was still observed in white adipocytes (6, 44). In addition, Haemmerle et al. (44) have reported that HSL deficiency in mice results in the accumulation of DAG in various tissues. An adipose TAG lipase was identified recently and proposed to be responsible for the initial step in TAG catabolism (45). HSL and adipose TAG lipase may coordinately catabolize stored TAG in adipose tissue in mammals (45).

Together, these findings suggest that HSL may be the rate-limiting enzyme controlling the cellular catabolism of DAG in adipose tissue and that of cholesteryl esters in macrophage foam cells and steroidogenic tissues releasing the cholesterol required for adrenal steroidogenesis. In addition, the results of this study should make it possible to greatly improve our knowledge of the kinetics of HSL activity and that of other cholesterol esterases.

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