Pancreatic Bile Salt-Dependent Lipase Activity in Serum of Normolipidemic Patients

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ABSTRACT: Bile salt-dependent lipase (BSDL, E.C. 3.1.1.-) is a digestive enzyme secreted by the pancreatic acinar cell. Once in the duodenum, the enzyme, upon activation by primary bile salts, hydrolyzes dietary lipid esters such as cholesteryl esters and lipid-soluble vitamin esters. This enzyme is partially transferred from the duodenum or pancreas to the circulation where it has been postulated to exert a systemic action on atheroma-generating oxidized-low density lipoprotein (LDL). In the present study, sera from 40 healthy normolipidemic volunteers were used to investigate the possible linkage between circulating BSDL, lipids, and lipoproteins. We showed, firstly, that pancreatic-like BSDL activity can be detected in these sera. Secondly, BSDL activity increased significantly with the level of LDL-cholesterol and was also positively linked to the serum concentration of Apo B100 and Apo A-I. Thirdly, we also established that BSDL was associated with LDL, in part by a specific interaction with Apo B100, while no interaction was found with Apo A-I. No linkage with other recorded parameters (triglycerides, phospholipids, and high density lipoprotein-cholesterol) was detected. Because an increase in LDL-cholesterol represents an important risk factor for atheroma, the concomitant increase in BSDL, which can metabolize atherogenic LDL, suggests for the first time that this circulating enzyme may exert a positive effect against atherosclerosis.

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Pancreatic bile salt-dependent lipase (BSDL), also referred to as carboxy ester lipase or bile salt-dependent cholesterol ester hydrolase, is a 100 kDa enzyme which hydrolyzes cholesteryl and lipid-soluble vitamin esters, triglycerides, and (lyso)phospholipids (1,2). Except for the latter substrates, bile salts are a prerequisite for hydrolysis (3). Although the enzyme was secreted into the duodenum where it exerted its digestive function, BSDL has been shown to transit throughout the enterocyte (4). As a consequence, BSDL is found in the blood plasma of humans (5) where lipoproteins are possible targets for the enzyme. Nevertheless, BSDL activity in serum cannot be measured on cholesteryl esters or triglycerides, owing partly to the competitive effect of circulating lipoproteins (6). It has effectively been shown that BSDL has the capability to hydrolyze low density lipoprotein (LDL)-cholesterol esters in the presence of a concentration of bile salts achievable in plasma (7,8). Of physiological importance is the fact that BSDL reduces the atherogenicity of oxidized LDL by decreasing its lysophospholipid content (9). Evidence has accumulated to suggest that oxidized LDL is a key component in endothelial injury (10). Once formed by the endothelium, oxidized LDL may directly injure the vascular tissue and induce migration of monocytes and T lymphocytes in the intima (11). Oxidized LDL is enriched in lysophosphatidylcholine, which acts as a chemoattractant that recruits monocytes to the subintimal space (12). Once monocytes enter the subendothelial space, oxidized LDL may participate in the activation of monocytes into macrophages. These latter cells internalize LDL through scavenger receptors (13) and can oxidize LDL through several pathways (14). During this process, fatty acids undergo peroxidation which, in turn, yields a variety of reactive molecules, which can become covalently crosslinked to the apolipoprotein (Apo) B100 moiety of LDL particles. This could further exacerbate the atherogenic process. As a result, antioxidants such as probucol and vitamin E have been suggested as a means of preventing atherosclerotic lesions. Given the role attributed to lysophosphatidylcholine in the promotion of atherosclerosis, it has been proposed that BSDL could serve as a protective factor against the deleterious effects of oxidized LDL observed in atherosclerosis (9). Consequently, the aim of this study was twofold: (i) an attempt to accurately record BSDL activity in serum, and (ii) an investigation of whether the activity of circulating BSDL was linked to lipids and/or Apo present in serum.

EXPERIMENTAL PROCEDURES

Patients. Sera from 40 patients (27 women, 13 men, age range of 21–85 years) were used in this study. None had liver or pancreatic disease or was undergoing insulin treatment nor was taking corticosteroids, oral contraceptives or lipid-lowering agents. All were normoalbuminemic (>540 µmol/L). Blood (citrated) samples were drawn at 9:00 a.m. after an
overnight fast while subjects had rested for at least 20 min. The study protocol was approved by our Ethics Committee, and informed consent was obtained from all subjects.

Immunoprecipitation. Polyclonal antibodies (pAb L64) specific for human pancreatic BSDL were obtained, purified, and, when required, immobilized on agarose beads as described previously (15). Twenty µL of a suspension of immobilized pAb L64 (corresponding to 100 µg of antibodies) was added (assays) to 100 µL of serum sample and incubated for 4 h at 25°C under agitation. Beads were then pelleted by centrifugation at 10,000 × g for 20 min. The esterolytic activity was recorded in the supernatant. Controls were performed under the same conditions, omitting immobilized antibodies. Under these conditions, the difference in activity between control and assay was representative of the BSDL activity in serum.

Enzyme assay. Esterolytic activity of serum samples was spectrophotometrically determined using 4-nitrophenyl hexanoate as substrate (16) at 404 nm in a thermostated cell at 30°C. The substrate was dissolved in 0.2 M Tris/HCl buffer, pH 7.4 containing NaCl (150 mM). Assays were performed in the absence or presence of sodium taurocholate (4 mM) as activator of BSDL. Under these conditions, the difference of measured activity was representative of the bile salt-activated esterolytic activity present in serum samples. Diisopropyl fluorophosphate (DFP)-inhibited esterolytic activity of serum was obtained after sample incubation (1 h at 25°C) in the presence of 4 mM DFP (stock solution 1 M in dry isopropyl alcohol) or of vector alone. Then the esterolytic activity of serum was recorded in the presence of bile salts as described before. The difference in activity before and after DFP treatment represents the DFP-inhibited esterolytic activity. Unless otherwise stated, DFP-inhibited esterolytic activity represents the activity of BSDL (see below).

Polyacrylamide gel electrophoresis (PAGE) and Western blotting. PAGE in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed according to Laemmli (17). Replicas of SDS-PAGE on nitrocellulose membranes were obtained after electrotransfer. These replicas were then electroblotted. PAGE in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed according to Laemmli (17).

BSDL iodination. BSDL was purified from human pancreatic juice as previously described (3); then 10 µg of the pure protein was iodinated with 125I-Na by the chloramine-T method. The radiolabeled BSDL (8 mCi/mg protein) was chromatographed on a Sepharose 6B column (1 × 60 cm), equilibrated, and eluted with 10 mM sodium phosphate buffer (pH 7.4) before use. During the iodination process, no BSDL degradation could have occurred because radiolabeled BSDL comigrates with the native BSDL on the Sepharose 6B column, and more than 87% of radiolabeled BSDL was precipitable with trichloroacetic acid. Stored at 4°C, this preparation remained stable for at least one month.

Binding of BSDL to lipoproteins. Isolated lipoproteins (500 µL; concentration adjusted to the initial plasma concentration) were incubated for 15 min at 37°C in the presence of 400 ng of 125I-BSDL (4 pmole, 600 ± 35 cpm/ng of BSDL) in 10 mM Tris/HCl buffer pH 7.4 (containing 154 mM NaCl, 1 mM EDTA, and 0.01% NaN3). At the end of the incubation period, the mixture was chromatographed on a fast protein liquid chromatography-Superose 6 column (Pharmacia) (2 × 60 cm) eluted (0.5 mL/min) with the dialysis buffer. Radioactivity and optical density at 280 nm were monitored in each fraction. A blank experiment was performed by gel filtration under the same conditions of 400 ng of 125I-BSDL added to 20 µg of pure BSDL (40 µg/mL) as a vector.

Interaction between BSDL and Apo. Sepharose-immobilized BSDL was equilibrated and suspended in 10 mM Tris/HCl buffer pH 7.4 (1 mM EDTA; 0.15 M NaCl), adjusted to the same final amount of gel corresponding to 1 nmole of Sepharose-immobilized Apo B100 or to 5 nmoles of Sepharose-immobilized Apo A-I and pelleted. Immobilized-Apo were finally suspended in 1 mL of buffer and supplemented with approximately 1 pmole of 125I-BSDL and with increasing amounts (from 0 up to 0.50 pmole) of unlabeled BSDL. Tubes were then incubated overnight at 4°C under gentle agitation. The tubes were quickly centrifuged on a bench-top centrifuge, gel pellets were washed several times with the incubation buffer, and the remaining radioactivity of the pellet suspended in counting liquid was recorded. For corrections for nonspecific binding were performed by using adequate amounts of control gel and treated under the same conditions.

Other procedures. Serum concentrations of phospholipids, triacylglycerol, cholesterol, and glucose were measured in duplicate by enzymatic methods (Boehringer Mannheim, Ger-