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Activation of bile salt dependent lipase by (lyso)phosphatidic acid and platelet activating factor

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ABSTRACT

The activity of breast milk BSDL was assayed with or without phospholipids as extra-intestinal effector candidates. Phosphatidic acid, lysophosphatidic acid and platelet activating factor but not phosphatidylcholine and lysophosphatidylcholine stimulated BSDL activity at least as efficiently as taurocholate. The apparent dissociation constants of PA and LPA at saturating concentrations of three different substrates were between 0.1 and 13.4 μM and that of PAF was below or equal to 200 pM. Kinetic data suggested the existence of at least one binding site for each of these effectors. PA, LPA and PAF are likely extra-intestinal modulators of BSDL activity.

1. Introduction

Bile salt dependent lipase (EC 3.1.1.13) is an esterase active on various lipids, including esters of glycerol, cholesterol and lipid soluble vitamins, but unlike other lipases its activity on water insoluble substrates is stimulated by primary bile salts [1–3]. In humans, BSDL is secreted by pancreas and mammary glands, typically representing about 4% of adult pancreatic juice proteins and less than 1% of breast milk proteins [4–6]. BSDL seems to play a major role in the intestinal fat digestion at early life stages, before the lipolytic system is mature [7–9]. Besides this digestive function, growing data points out that this enzyme could have an important extra-intestinal function: BSDL was found to be able to traverse the intestinal mucosa towards blood circulation and was even retrieved in urines of healthy subjects [10–12]; it is secreted by endothelial cells and monocytes–macrophages [13,14] and several studies supported its involvement in the metabolism of lipoproteins, with impact on the cholesterol pools [15–17]. In link with this, it was reported that BSDL is or could be involved in pathophysiological processes, including atherosclerosis and inflammation [18–23].

However, the enzymatic activity of BSDL in the extra-intestinal compartments has never been clearly linked to whatever extra-intestinal function. The concentration of bile acids in plasma was reported to be 10 μM, or higher at postprandial conditions [15], which is at least two orders of magnitude lower than the corresponding intestinal concentration of bile acids, typically above 4 mM [24]. One therefore may suppose that bile acids are not major extra-intestinal BSDL activators and suspects the existence of other BSDL effector(s). So far, the activity of neutral cholesterol esterase from human aorta macrophages on cholesteryl-oleate in liposomes was found to be stimulated by phosphatidylserine, phosphatidylinositol, cardiolipin and phosphatidic acid but not by phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine or sphingomyeline [25]. Also, the activity of BSDL from human pancreas on *para*nitrophenyl-butyrate was stimulated by phosphatidylserine, phosphatidylinositol or phosphatidic acid, but not by phosphatidylcholine [26].

Consequently, we screened putative extra-intestinal activators of BSDL using pure BSDL from breast milk [6] and as substrates: *para*nitrophenyl-butyrate ($PNPC_4$), *para*nitrophenyl-laurate ($PNPC_{12}$), and cholesteryl-oleate ($ChoC_{18}$). Taurocholate (TC) served as a reference BSDL activator while testing phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE) phosphatidic acid (PA), lysophosphatidic acid

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(LPA) and platelet activating factor (PAF). The results obtained were discussed with respect to the extra-intestinal function of BSDL.

2. Materials and methods

2.1. Materials

PAF (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), LPA (oleyl-L-α-lysophosphatidic acid), LPC (oleyl-*sn*-glycero-3-phosphocholine), $PNPC_4$, $ChoC_{18}$, horseradish peroxidase and cholesterol oxidase were purchased from Sigma–Aldrich. TC and $PNPC_{12}$ were provided by Fluka-Biochemika and *para*nitrophenol by Biomedicals (Illkirch, France). PC (1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine), PE (1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine) and PA (1,2-dioleoyl-*sn*-glycero-3-phosphatidic acid) were purchased from Larodan Fine Chemicals (Malmö, Sweden). The enzyme was freshly purified to homogeneity from breast milk as described elsewhere [6].

2.2. Solubilization of substrates and ligands

TC was solubilized by gentle vortexing in the enzyme assay buffer Tris/HCl (0.1 M, pH 7.4, 0.15 M NaCl). Stock solutions of phospholipids, lysophospholipids and PAF were prepared in chloroform/methanol (1:1), then bubbled with nitrogen and stored at −20 °C. For use, appropriate amounts of these lipids were dried in 1.5 ml Eppendorf tubes using Speed Vacuum system, and then dissolved in the enzyme assay buffer by mean of vigorous vortexing and subsequent sonication (for 5 min). Appropriate dilutions were made for screening the effects of tested lipids. The substrates $PNPC_4$, $PNPC_{12}$ and $ChoC_{18}$ were dissolved in ethanol and diluted accordingly. Ethanol has no effect on BSDL activity under these experimental conditions [27].

2.3. Enzyme assay

In the case of paranitrophenyl esters, liberation of PNP was continuously monitored at 405 nm using microplate reader. Reaction took place in 200 μl at 37 °C for 3 min in the presence or absence of each BSDL candidate effector. The reaction started by addition of BSDL (20 μl) using multichannel pipette. Under these conditions, the molar extinction coefficient of PNP was 8300 M^{-1} cm^{-1}, which is slightly lower than that (11 500 M^{-1} cm^{-1}) previously reported [28]. Activity on cholesteryl-oleate was measured according to coupled cholesterol oxidase and peroxidase activities [29]. The final product (mono-imino-*p*-benzoquinone) was monitored continuously during 5 min at 500 nm. Free cholesterol was used instead of cholesteryl-oleate for the calibration curves.

2.4. Kinetic approach

The initial velocity expressed as μM min^{-1} corresponded to the slopes of the stationary phases of the kinetic plots, with correlation coefficients above 0.98. The spontaneous hydrolysis of $PNPC_4$ and that $PNPC_{12}$ were subtracted from those monitored in the presence of BSDL. The catalytic constant (k_{cat}, (s^{-1})) was determined using 100 kDa as molecular mass for BSDL [6].

In a first set of experiments, the effects of putative BSDL effectors were screened within large interval of concentration and using saturating concentrations of $PNPC_4$, $PNPC_{12}$ and cholesteryl-oleate. For each putative effector, the concentrations the effects of which fit the Michaelis–Menten kinetic model were used for calculation of k_{cat} and of apparent dissociation constant (K_d), using GraphPad prism program. In a second set of experiments, the kinetic parameters of BSDL (k_{cat} and K_m) with $PNPC_{12}$ as substrate were determined in the absence or presence of sub-micellar concentrations the already identified BSDL activators.

Each of putative BSDL activators was incubated alone in the reaction medium with each of the three substrates but no noticeable product release was observed, except the spontaneous decay of $PNPC_4$ and $PNPC_{12}$. The kinetic experiments were repeated at least four times and each replicate was used for determination of the apparent kinetic constants of BSDL.

2.5. Statistical analysis

Statistical analyses of data were performed using the ANOVA test of XLSTAT 2010 (Addinsoft, Paris, France). The model $Y = \alpha + \beta_l \text{treatment} + \epsilon_i$, was used to analyse the treatment effect (with effector candidate) for each substrate independently. The statistical model $Y = \alpha + \beta_l \text{treatment} + \chi_j \text{substrate} + \delta_{ij} \text{treatment} * \text{substrate} + \epsilon_{kij}$ was used to analyse the interaction treatment∗substrate for the variables K_m, K_d and k_{cat}, where α is the mean effect, β, χ and δ are the adjusted coefficients of the fixed effects in the model and ε is the random error associated with *k*th observation of the *i*th treatment and the *j*th substrate. Contrasts were computed using least significant difference Fisher's test to compare the difference between each treatment and the difference between each interaction. The treatment effects for each substrate and the interaction "treatment∗substrate" were considered statistically significant at $P < 0.05$.

3. Results and discussions

3.1. Screening BSDL putative effectors

As expected, BSDL performed basal activity on the two artificial substrates $PNPC_4$ and $PNPC_{12}$ (Fig. 1) and the stimulating effect of TC on the hydrolysis of these two substrates is similar to that previously reported [6,28]. In addition, PA, LPA and PAF exhibited stimulating effects on the same substrates at least as efficiently as TC. Conversely, PE, PC and LPC exhibited only slight effects, if any, on BSDL activity on $PNPC_4$ and $PNPC_{12}$. These data agree with those previously obtained for PA and PC with $PNPC_4$ as substrate [26].

The kinetic plots of BSDL activators fitted a rectangular hyperbola with high correlation coefficient ($R^2 > 0.95$), with the exception of PA with $PNPC_4$ as substrate ($R^2 = 0.87$) and of LPA with $PNPC_{12}$ as substrate ($R^2 = 0.91$). The kinetic constants thus obtained are summarized in Table 1. The catalytic constant of BSDL on $PNPC_4$ varied from 416 to 3680 s^{-1} following the stimulating rank LPA > PA > TC > PAF = PE, and from 173 to 396 s^{-1} with $PNPC_{12}$ as substrate following the stimulating rank PA = LPA > PAF > TC = PE. The apparent dissociation constants (K_d) of PA and LPA were quite similar whether the substrate was $PNPC_4$ or $PNPC_{12}$ (4.7–13.4 μM); those of TC and PE were different to each other depending on the substrate in the interval 12–200 μM. More interestingly, K_d of PAF was found to be much lower than those of PA, LPA, TC and PE: 200 pM and 80 pM with $PNPC_4$ and $PNPC_{12}$ as substrates, respectively.

No BSDL basal activity was observed with cholesteryl-oleate as substrate (Fig. 2), in conformity with the kinetic behaviour of BSDL on cholesterol esters [1,28]. Here again, the kinetic plots obey Michaelis–Menten–Henri model at the lower concentrations of effectors (see inserts). This means that at these concentrations, BSDL performs homogeneous rather than interfacial catalysis, although some sigmoidal tendency at low TC concentrations was observed. For all the BSDL activators, the saturation plateau running at maximal velocity was followed by a deceleration phase