Chemistry and Physics of Lipids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

# IR spectroscopy analysis of pancreatic lipase-related protein 2 interaction with phospholipids: 2. Discriminative recognition of various micellar systems and characterization of PLRP2-DPPC-bile salt complexes

Eduardo Mateos-Diaz[a], Priscila Sutto-Ortiz[a,b], Moulay Sahaka[a], Deborah Byrne[c], Hélène Gaussier[a], Frédéric Carrière[a,*]

[a] Aix-Marseille Université, CNRS, UMR7282 Enzymologie Interfaciale et Physiologie de la Lipolyse, Marseille, France
[b] Biotecnología Industrial, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. (CIATEJ), Zapopan, Jalisco, México
[c] Aix-Marseille Université, CNRS, FR3479 Institut de Microbiologie de la Méditerranée, Marseille, France

ARTICLE INFO

*Keywords:*
Enzyme
FTIR spectroscopy
Lipids
Lipid digestion
Lipolysis
Phospholipase

ABSTRACT

The interaction of pancreatic lipase-related protein 2 (PLRP2) with various micelles containing phospholipids was investigated using pHstat enzyme activity measurements, differential light scattering, size exclusion chromatography (SEC) and transmission IR spectroscopy. Various micelles of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and lysophosphatidylcholine were prepared with either bile salts (sodium taurodeoxycholate or glycodeoxycholate) or Triton X-100, which are substrate-dispersing agents commonly used for measuring phospholipase activities. PLRP2 displayed a high activity on all phospholipid-bile salt micelles, but was totally inactive on phospholipid-Triton X-100 micelles. These findings clearly differentiate PLRP2 from secreted pancreatic phospholipase A2 which is highly active on both types of micelles. Using an inactive variant of PLRP2, SEC experiments allowed identifying two populations of PLRP2-DPPC-bile salt complexes corresponding to a high molecular weight 1:1 PLRP2-micelle association and to a low molecular weight association of PLRP2 with few monomers of DPPC/bile salts. IR spectroscopy analysis showed how DPPC-bile salt micelles differ from DPPC-Triton X-100 micelles by a higher fluidity of acyl chains and higher hydration/H-bonding of the interfacial carbonyl region. The presence of bile salts allowed observing changes in the IR spectrum of DPPC upon addition of PLRP2 (higher rigidity of acyl chains, dehydration of the interfacial carbonyl region), while no change was observed with Triton X-100. The differences between these surfactants and their impact on substrate recognition by PLRP2 are discussed, as well as the mechanism by which high and low molecular weight PLRP2-DPPC-bile salt complexes may be involved in the overall process of DPPC hydrolysis.

## 1. Introduction

The substrate specificity of lipolytic enzymes is strongly dependent on the supramolecular organization of the lipid substrates present in membranes, monolayers, micelles, vesicles or oil-in-water emulsions. Various kinetic models have been proposed to describe the mode of action of these enzymes including lipases and phospholipases. Among them, the Verger-De Haas'model is the adaptation of the Michaelis-Menten-Henri kinetic model to a two-dimensional space representing the lipid-water interface, combined with a first step of reversible enzyme adsorption at this interface (Verger et al., 1973a). It has been established and used in connection with the development of (phospho) lipase assays based on lipid monomolecular films and surface tensiometry (Verger and De Haas, 1973). The "surface dilution model" of Deems-Eaton-Dennis is based on the use of mixed micelles (Deems et al., 1975) and it was further adapted to liposomal dispersions, with the definition of the "scooting" and "hopping" modes of enzyme action (Gelb et al., 1995; Jain and Berg, 1989; Jain et al., 1995). All these models include an initial step of enzyme association with a supramolecular structure made by the susbtrate itself (liposomes, oil drops) or in which the substrate is embedded. The surface dilution model was for instance established to describe the action of cobra venom

*Abbreviations:* DLS, dynamic light scattering; DPPC, 1,2-di-palmitoyl phosphatidylcholine; EggPC, egg yolk phosphatidylcholine; FTIR, fourier transform infrared spectroscopy; FWHM, full width at half maximum; GPLRP2, guinea pig pancreatic lipase-related protein 2; LUV, large unilamellar vesicles; lyso-C16-PC, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; NaGDC, sodium glycodeoxycholate; NaTDC, sodium taurodeoxycholate; PLRP2, pancreatic lipase-related protein 2; SEC, size exclusion chromatography; $sPLA_2$-IB, pancreatic phospholipase A2; TX100, Triton X-100

* Corresponding author at: CNRS, UMR7282 EIPL, 31 chemin Joseph Aiguier, 13402 Marseille cedex 20, France.
*E-mail address:* carriere@imm.cnrs.fr (F. Carrière).

https://doi.org/10.1016/j.chemphyslip.2017.11.012
Received 6 September 2017; Received in revised form 14 November 2017; Accepted 15 November 2017

phospholipase A2 on mixed micelles made of 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine (DPPC) and Triton X-100, a non-ionic surfactant (Deems et al., 1975). Studies on pancreatic phospholipase A2 have often been carried out using surfactants naturally found in the small intestine, bile salts (De Haas et al., 1968).

Since pancreatic lipase-related proteins type 2 (PLRP2) is also present in pancreatic juice (De Caro et al., 1998) and displays a phospholipase A1 activity, mixed phospholipid-bile salts micelles have also been used to characterize the phospholipase activity of PLRP2. In a previous study, we have shown using IR spectroscopy that the guinea pig PLRP2 (GPLRP2) interacts preferentially with phospholipid molecules organized in mixed micelles with sodium taurodeoxycholate (NaTDC), which correlates with the enzyme activity on this substrate, but it shows no interaction with the same phospholipids organized in large unilamellar or multilamellar vesicles (Mateos-Diaz et al., 2017b). We extend here this study by the characterization of various phospholipid micelles and their interaction with GPLRP2 using dynamic light scattering (DLS) to analyze particle sizes, size-exclusion chromatography (SEC) to estimate the molecular weight and hydrodynamic diameter of protein-lipid-surfactant complexes, and infrared (IR) spectroscopy to study the effects of surfactants and GPLRP2 on the acyl chains, interfacial region and polar head-group of phospholipids. As previously described, we use an inactive variant (S152G) of GPLRP2 in order to study the enzyme binding to lipid aggregates with no interference of lipid hydrolysis.

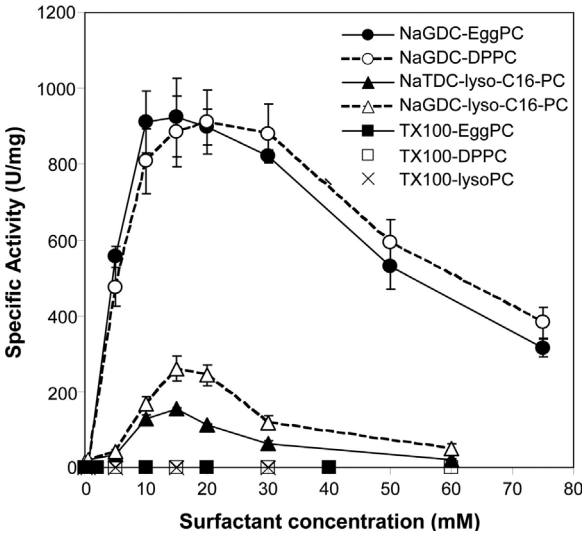


**Fig. 1.** Specific activity of GPLRP2 on egg yolk phosphatidylcholine (EggPC), 1,2-dipalmitoyl-sn-glycero-3-phospphocholine (DPPC) and 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-C16-PC) as function of the surfactant concentration. Substrate concentrations were 17, 16 and 10 mM for EggPC, DPPC and lyso-C16-PC, respectively. Surfactant was either NaTDC, NaGDC or TX100. Initial reaction rates were obtained from pHstat titrimetric assays at 37 °C and pH 8.0. Values are means ± SD (n = 3).

## 2. Materials and methods

### 2.1. Reagents

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-C16-PC) both > 99% purity were obtained from Echelon Biosciences Inc. Egg yolk phosphatidylcholine (EggPC) was obtained from Lipoid™. Sodium taurodeoxycholate (NaTDC), sodium glycoldeoxycholate (NaGDC), polyoxyethylene octyl phenyl ether or Triton X-100 (TX100), deuterium oxide ($D_2O$) 99.9%, benzamidine and dithiothreitol (DTT) were purchased from Sigma-Aldrich. Chloroform and methanol were from Carlo Erba. 2-(N-morpholino)-ethanesulfonic acid (MES), 2-Amino-2-(hydroxymethyl)propane-1,3-diol (Tris), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), calcium chloride ($CaCl_2$) and sodium chloride (NaCl) were obtained from Euromedex.

### 2.2. Production and purification of wild-type GPLRP2 and S152G variant

Recombinant wild-type GPLRP2 (WT) was produced in *Aspergillus orizae* and purified as previously described (Hjorth et al., 1993). GPLRP2 S152G variant construction, production and purification from *Pichia pastoris* cultures are described in the accompanying article (Mateos-Diaz et al., 2017b). Stock solutions of GPLRP2 WT and S152G variant at 7.5 mg/mL (160 μM) were prepared in 100 mM MES buffer, 150 mM NaCl and 5 mM $CaCl_2$ at pH 6 for activity measurements, DLS and SEC, as well as in the same buffer in $D_2O$ for IR experiments. A 10-fold dilution of these GPLRP2 stock solution (0.75 mg/mL, 16 μM) was used in all experiments.

### 2.3. Activity measurements of GPLRP2 on phospholipid-surfactant dispersions

Phospholipase and lysophospholipase activities of GPLRP2 were measured by automated titration of fatty acids released from stirred egg yolk phosphatidylcholine (EggPC), DPPC or lyso-C16-PC dispersions, using 0.1 N NaOH and a TTT80 Radiometer™ pHstat (Copenhagen). Each assay was performed in a thermostated (37 °C) vessel containing 1 mM buffer (Tris or MES for pH values around 8 and 6 respectively) with 150 mM NaCl and 5 mM $CaCl_2$. Routine assays were performed at pH 8 with a final substrate concentration of approximately 17, 16 and 10 mM for EggPC, DPPC and lyso-C16-PC respectively. One unit of enzymatic activity (U) corresponds to 1 μmol of free fatty acid released per min.

### 2.4. Preparation of phospholipid-surfactant micelles

DPPC-containing micelles for DLS and enzyme activity studies were prepared by dissolving first DPPC in chloroform:methanol 2:1 (v/v) and then evaporating the solvent under vacuum to obtain a thin film containing the necessary amount of lipid to get a final concentration of 5%, w/v (*i.e.* 68 mM DPPC). To form phospholipid micelles, the DPPC film was dissolved in 100 mM MES buffer, 150 mM NaCl and 5 mM $CaCl_2$ at pH 6 containing 50 mM of either NaTDC, NaGDC or TX100. The final phospholipid-to-surfactant molar ratio was 1.36, except when various amounts of surfactants were tested for their effects on enzyme activity (Fig. 1). The dispersions were then treated as previously described to yield mono dispersed mixed micelles at room temperature (Mateos-Diaz et al., 2017b). Similar procedures were used for preparing lyso-C16-PC micelles at a final concentration of 5% w/v (*i.e.* 100 mM), either with pure lyso-C16-PC or in the presence of NaTDC at a phospholipid-to-bile salt molar ratio of 0.5, except that no prior dissolution in chloroform-methanol was required because of the fast and efficient dissolution of lyso-C16-PC in MES buffer.

### 2.5. Dynamic light scattering measurements

Dynamic light scattering (DLS) experiments were carried out using a Zetasizer Nano S (Malvern Instruments) as previously described (Mateos-Diaz et al., 2017b). In some experiments, the temperature was increased by 5 °C-intervals from 25 to 70° to observe the thermotropic behavior of micellar dispersions (see Fig. S1). Three measurements were taken at each temperature, each one consisting in 10–15 runs of 10 s. The scattering angle was 173°. For each micellar dispersion, the determination of the hydrodynamic diameter (DH) was based on the Einstein-Strokes relation to obtain the intensity averaged size distribution. A viscosity of 0.8878 cP and a refractive index of 1.332 (at 25 °C) were used for the dispersion medium, while a value of 1.49 was used as an approximation of refractive index for micelles (Gagos et al., 2001). The changes in viscosity and refractive index induced by the