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Ostrich pancreatic phospholipase A_2: Purification and biochemical characterization

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Abstract

Ostrich pancreatic phospholipase A_2 ($OPLA_2$) was purified from delipidated pancreases. Pure protein was obtained after heat treatment (70 °C), precipitation by ammonium sulphate and ethanol, respectively followed by sequential column chromatography on MonoQ Sepharose and size exclusion HPLC column. Purified $OPLA_2$, which is not a glycosylated protein, was found to be monomeric protein with a molecular mass of 13773.93 Da. A specific activity of 840 U/mg for purified $OPLA_2$ was measured at optimal conditions (pH 8.2 and 37 °C) in the presence of 4 mM NaTDC and 10 mM $CaCl_2$ using PC as substrate. This enzyme was also found to be able to hydrolyze, at low surface pressure, 1,2-dilauroyl-sn-glycero-3 phosphocholine (di C_{12}-PC) monolayers. Maximal activity was measured at 5–8 mN m^{-1}. The sequence of the first 22 amino-acid residues at the N-terminal extremity of purified bird PLA_2 was determined by automatic Edman degradation and showed a high sequence homology with known mammal pancreatic secreted phospholipases A_2.

Keywords: Phospholipase A_2; Phosphatidylcholine; Bile salts; Lipid monolayer

1. Introduction

Phospholipases A_2 (PLA_2, phosphatide-2-acylhydrolase; EC 3.1.1.4) catalyse the hydrolysis of the fatty ester in the 2-position of *3-sn*-phospholipide to release fatty acid and lysophospholipide and are found in both intracellular and secreted forms [1–5].

Several PLA_2 have been identified as based on their gene sequences. They were classified mainly into three groups: (i) cytosolic PLA_2 ($cPLA_2$); (ii) Ca^{2+}-independent intracellular PLA_2 ($iPLA_2$) and (iii) Ca^{2+}-dependent secreted PLA2 ($sPLA_2$). They differ from each other in terms of substrate specificity, Ca^{2+} requirement and lipid modification. There is also a PLA_2 class which hydrolyses the platelet-activating factor (PAF) and the oxidized lipids, called PAF acetylhydrolases (PAF-AH) [6,7].

The $sPLA_2$ group forms a growing family of dependent Ca^{2+} lipolytic enzyme with a conserved Ca^{2+} binding loop and His-Asp dyad at the catalytic site [8,9]. They are low molecular mass enzymes (14–18 kDa) with a rigid tertiary structure, having five to eight disulfide bonds, which confers both stability against proteolysis and denaturing conditions [6,7]. Eleven $sPLA_2$ have been identified in mice (groups IB, IIA, IIC, IID, IIE, IIF, V, X, III, XIIA and XIIB) that display distinct yet partial overlapping tissue distribution [8,9]. Humans contain all of these except IIC, which occurs as a pseudogene [6,9]. There has been intense interest in the role of $sPLA_2$ in the liberation of arachidonic acid from cellular phospholipids for the formation of the

Abbreviations: $DrPLA_2$, dromedary pancreatic phospholipase A_2; $sPLA_2$, secreted pancreatic phospholipase A_2; $OPLA_2$, ostrich pancreatic phospholipase A_2; $TPLA_2$, turkey pancreatic phospholipase A_2; PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; HPLC, high pressure liquid chromatography; AU, arbitrary units; DTT, dithiothreitol; EDTA, ethylene diamine tetraacetic acid; EGTA, ethylene glycol-bis (β-aminoethylether) *N*,*N*,*N*′,*N*′-tetraacetic acid; BSA, bovine serum albumine; NaDC, sodium deoxycholate; NaTDC, sodium taurodeoxycholate; PC, phosphatidylcholine; di C_{12}-PC, 1,2-dilauroyl-*sn*-glycero-3 phosphocholine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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eicasonoïd [7,10]. Also, given the molecular diversity of $sPLA_2$ and their distinct distribution patterns, it is likely that these enzymes are involved in other physiological responses besides inflammation. Some of these additional known functions include digestion of dietary phospholipids in the gastrointestinal track [11,12] and other $sPLA_2$ have bactericidal action [13–17] and release free fatty acids for formation of the permeability barrier of skin [18,19].

The group IB PLA_2 is known as the pancreatic type $sPLA_2$. In the pancreas, however, this enzyme is present mainly as an inactive precursor, $proPLA_2$, a single-chain zymogen which is converted by trypsin into the active enzyme [20]. The major physiological function of $sPLA_2$-IB has been thought to be digestion of glycerophospholipids in nutrients, given its abundance in digestive organs [20]. However, the group IB PLA_2 is also found in non-digestive tissues, including lung, spleen, gonad and kidney [10,21]. Receptors of this enzyme have been identified in various tissues, and group IB PLA_2 is reported to play a role in cell proliferation and hormone release via these receptors [19,21].

$sPLA_2$-IB have been isolated from pancreases of various mammal species [22–29]. In the contrast, no study has been performed on bird PLA_2, in the exception of some assays to purify the turkey PLA_2 ($TPLA_2$) [30]. However, the purified $TPLA_2$ was found totally inactive and all biochemical and enzymatic analysis were done with partially purified enzyme [30]. It is therefore interesting to study some catalytic and biochemical properties of a purified bird PLA_2 to gain more insights into their action mode on phospholipids. To our knowledge, we described in this paper for the first time, the high yield purification and biochemical characterization of an active thermostable PLA_2 from a bird pancreas: the ostrich. The enzymatic properties of pure $OPLA_2$ were checked.

2. Materials and methods

2.1. Materials

Trypsin (treated with L-1-tosylamino 2-phenylethyl chloromethylketone), benzamidine were from Fluka (Buchs, Switzerland), bovine serum albumine (BSA), sodium deoxycholate (NaDC), sodium taurodeoxycholate (NaTDC), phosphatidylcholine (PC) and di C_{12}-PC were from Sigma Chemical (St. Louis, USA); acrylamide and bis-acrylamide electrophoresis grade were from BDH (Poole, UK), marker proteins and MonoQ Sephacryl were from Pharmacia (Uppsala, Sweden); PVDF membrane and protein sequencer Procise 492 equipped with 140C HPLC system purchased from Applied Biosystems (Roissy, France); pH-stat was from Metrohm (Herisau, Switzerland).

2.2. Delipidation of pancreases

Pancreases from ostrich were collected immediately after slaughter and kept at −20 °C. Pancreases were collected from a local slaughterhouse (Nabeul, Tunisia). After decongelation, pancreases were cut into small pieces (1–2 cm^2) and delipidated according to the method described previously [31]. After delipidation, about 17 g of delipidated powder of pancreas were obtained from 100 g of fresh tissue.

2.3. Determination of phospholipase activity

The PLA_2 activity was measured titrimetrically at pH 8.2 and at 37 °C with a pH-stat, under the standard assay conditions described previously [6], using PC or egg yolk emulsions as substrate in the presence of 4 mM NaTDC and 10 mM $CaCl_2$. One unit of phospholipase activity was defined as 1 μmole of fatty acid liberated per min under standard conditions.

2.4. Hydrolysis of di C_{12}-PC monolayer

Measurements were performed with KSV-2000 Baro-Stat equipment (KSV-Helsinki). The principle of the method was described previously by Verger and de Haas [32]. It involves the use of a "zero-order" through with two compartments: a reaction compartment and a reservoir compartment, which were connected to each other by a small surface channel. The enzyme solution was injected into the subphase of the reaction compartment only when the lipid film covered both compartments. A mobile barrier, automatically driven by the Baro-Stat, moved back and forth over the reservoir to keep the surface pressure (π) constant, thus compensating for the substrate molecules that were removed from the film by enzyme hydrolysis. The surface pressure was measured on the reservoir compartment with a Wilhelmy plate (perimeter 3.94 cm) attached to an electromicrobalance, which was connected in turn to a microprocessor programmed to regulate the mobile-barrier movement. As showed previously [32], the sensitivity of the Wilhelmy plate was estimated to 0.2 mN m^{-1}.

The reaction compartment was stirred at 250 rpm using 2 cm magnetic stirrers. The reactions were performed at room temperature (25 °C). The reaction compartment has a surface of 120 cm^2 and a volume of 120 ml. The reservoir compartment was 147 mm wide and 249 mm long. The aqueous subphase was composed of 10 mM Tris–HCl buffer, pH 8.0, 150 mM NaCl, 21 mM $CaCl_2$, and 1 mM EDTA.

All the activity measurements were performed after 15 min of PLA_2 injection. Maximal activities were expressed as the number of moles of substrate hydrolyzed by unit time and unit surface of the reaction compartment of the "zero-order" trough for an arbitrary phospholipase concentration of 1 M.

2.5. Determination of protein concentration

Protein concentration was determined as described by Bradford et al. [33] using BSA ($E^{1\%}_{1\,cm} = 6.7$) as reference.

2.6. Oligosaccharides content

The presence of carboglycane chains in the pure $OPLA_2$ was checked by anthrone–sulfuric acid method using glucose as a standard [34].

2.7. Alkylation of Cys residues

The alkylation of Cys residues of phospholipase was realized as the technique of Okazaki et al. [35]. Twenty micrograms of $OPLA_2$ were denatured in 185 μl of 8 M guanidine hydrochloride, 65 μl of 1 M Tris–HCl, 4 mM EDTA (pH 8.5) and 80 mM DTT during 30 min at 60 °C. *S*-Pyridylethylation of cysteine residues of protein was performed by adding 4 μl of vinyl pyridine during 3 h at 25 °C. The modified enzyme was dialyzed against water and transferred on PVDF membrane for N-terminal sequencing.

2.8. Amino acid sequencing and mass spectrometry

The N-terminal sequence was determined by automated Edman's degradation, using an Applied Biosystems Protein Sequencer Procise 492 equipped with 140 C HPLC system [36]. The molecular mass of purified $OPLA_2$ was determined using matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

3. Results and discussion

3.1. Purification of $OPLA_2$

Ten grams of delipidated ostrich pancreas were suspended in 200 ml of buffer A: 10 mM Tris–HCl, 0.15 M NaCl, pH 8.5 and ground mechanically twice for 30 s at room temperature using the Waring Blendor System. Then, the mixture was stirred with a magnetic bar for 35 min at room temperature and centrifuged during 30 min at 12,000 rpm. The endogenous trypsin was found to be sufficient to achieve the PLA_2 activation, since the total PLA_2 activity obtained (2000 U) did not increase when trypsin was added at different ratios to the PLA_2 solution (data not shown).

3.1.1. Heat treatment

As has been established for dromedary and other mammal pancreatic PLA_2 [22–29], $OPLA_2$ present in the homogenate can tolerate, without any denaturation, the incubation at high temperature. The ostrich extract PLA_2 solution was incubated 10 min at 70 °C. After rapid cooling, insoluble denatured proteins were removed by centrifugation during 30 min at 12,000 rpm. We obtained a clear supernatant which contains 95% of starting amount of PLA_2.

3.1.2. Ammonium sulphate precipitation

Pancreatic extract from ostrich was brought to 70% saturation with solid ammonium sulphate under stirring conditions and maintained for 30 min at 4 °C. After centrifugation (30 min, 12,000 rpm), precipitate was resuspended in minimum of buffer A containing 2 mM benzamidine. Insoluble proteins were discarded by centrifugation (15 min, 12,000 rpm). The recovery of PLA_2 activity was of about 87.5%.

3.1.3. Ethanol fractionation

Resuspended precipitate was subjected to fractionation using ethanol. We added an equal volume of ethanol at 0 °C. Insoluble proteins were removed by centrifugation (30 min; 12,000 rpm) and the ethanol (4V/V) was added slowly to the supernatant bringing the alcohol concentration to 90% (v/v) at 0 °C. Precipitated proteins which contained about 76.5% of the starting amount of PLA_2 were collected and solubilized in minimum of buffer A containing 2 mM benzamidine. In the present study, we

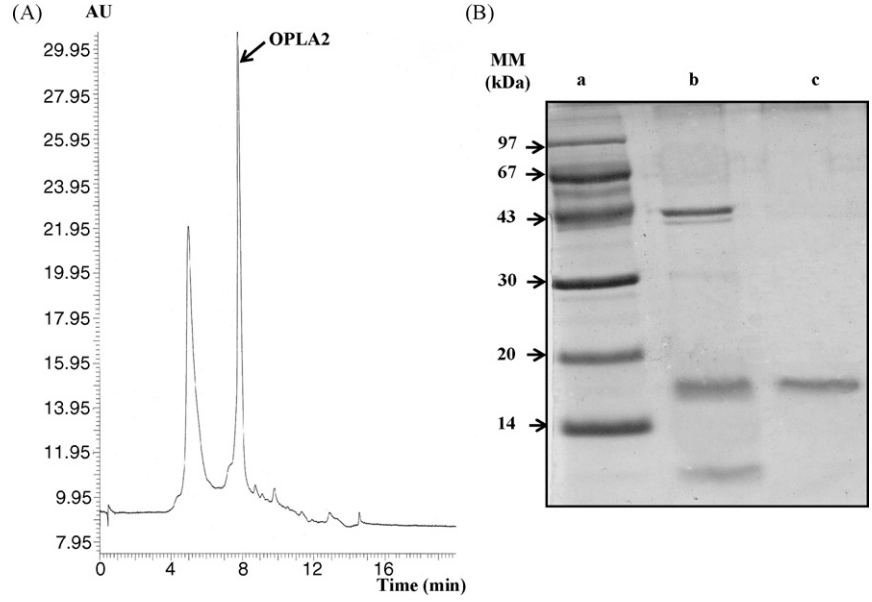

Fig. 1. Filtration on HPLC column and SDS-PAGE (15%) of $OPLA_2$. (A) Filtration on HPLC column Bio-sil SEC-125 (300 mm × 7.8 mm) equilibrated in 0.1 M phosphate buffer pH 6.8 containing 0.15 M NaCl, elution was performed at room temperature within 20 min with the same buffer at a flow rate of 1 ml/min. The effluent was monitored at 280 nm. AU: arbitrary units. (▬): pooled fractions. (B) SDS-PAGE (15%) of pure $OPLA_2$. Lane a, molecular mass markers (Pharmacia); lane b, 15 μg of $OPLA_2$ obtained after MonoQ chromatography and lane c, 5 μg of $OPLA_2$ eluted from HPLC filtration. The gel was stained with Comassie blue to reveal proteins.

Table 1
Flow sheet of the $OPLA_2$ purification

Purification step	Total activity (U)[a]	Total protein (mg)[b]	Specific activity (U/mg)	Yield (%)	Purification factor
Extraction (pH 8.5)	2000	660	3	100	1
Heat treatment (70 °C, 10 min)	1900	200	9.5	95	3.2
$(NH_4)_2SO_4$ precipitation	1750	158	11	87.5	3.7
Ethanol precipitation (50–90%)	1530	100	15.3	76.5	5.1
MonoQ Sepharose	1450	5.17	280	72.5	93.33
Filtration (HPLC)	1200	1.42	840	60	280

[a] 1 Unit: μmole of fatty acid released per min using PC emulsion as substrate in the presence of 4 mM NaTDC and in the presence of 10 mM $CaCl_2$. Measurements shown here are typical of those obtained in three independent experiments.
[b] Proteins were estimated by Bradford method [33]. The experiments were conducted four times.

have found that this step is of paramount importance to eliminate the last traces of lipids and to facilitate the $OPLA_2$ purification. The resulting sample was dialyzed over night against 10 mM Tris–HCl buffer pH 7.9 containing 10 mM NaCl, 2 mM $CaCl_2$ and 2 mM benzamidine (buffer B).

3.1.4. Anionic exchange and gel filtration chromatography

The dialyzed sample was poured into a column of MonoQ Sepharose (2.6 cm × 20 cm) equilibrated with buffer B. Under these conditions, the enzyme did not adsorb to the anionic support and was eluted during the wash by the same buffer at 45 ml/h. The fractions containing PLA_2 activity were pooled and lyophilized. The recovery of $OPLA_2$ after the MonoQ step was of about 95% with a specific activity of 280 U/mg. SDS-PAGE analysis of the pooled fraction of this MonoQ Sepharose chromatography are given in Fig. 1B and showed that $OPLA_2$ was contaminated by two major proteins with molecular masses of about 43 and 10 kDa. To remove these contaminants, the lyophilized proteins were resuspended in 2 ml of 0.1 M phosphate buffer pH 6.8 containing 0.15 M NaCl (buffer C). Fifty micrograms of this enzyme solution was loaded on size exclusion HPLC column Bio-sil SEC-125 (300 mm × 7.8 mm) equilibrated in buffer C. Elution was performed with phosphate buffer at 1 ml/min. The phospholipase emerged 7.5 min after injection (Fig. 1A). The fractions containing the PLA_2 activity were pooled and analyzed on SDS-PAGE (Fig. 1B). Only one band was revealed for $OPLA_2$. The molecular mass of $OPLA_2$ estimated by gel filtration on HPLC column Bio-sil SEC-125 (300 mm × 7.8 mm) was 14 kDa (data not shown). However, mass spectrometry analysis indicated that $OPLA_2$ has a molecular mass of 13773.93 Da (data not shown). Altogether, these results suggest that bird PLA_2 is monomeric protein as it was found for dromedary and all mammals PLA_2. Pure PLA_2 was lyophilized and conserved at −20 °C.

The presence of carbohydrate chains in pure PLA_2 molecule was checked and indicates that the purified protein is not glycosylated (data not shown).

The purification flow sheet given in Table 1 shows that the specific activity of pure $OPLA_2$ reached 840 and 700 U/mg, when PC and egg yolk emulsions were used as substrates, respectively, at pH 8.2, 37 °C and in the presence of 4 mM NaTDC and 10 mM $CaCl_2$. It is clear that ostrich pancreas is an ideal starting bird material for a high yield (60%) purification of PLA_2. This yield is five times higher than that of dromedary PLA_2 ($DrPLA_2$) [29]. In the case of turkey, the purified enzyme was totally inactive at the last step of its purification. Furthermore, the specific activity in the PC and egg yolk is higher than that of the $DrPLA_2$ (600 U/mg), whereas the specific activity of $TPLA_2$ has not been determined. Moreover, the procedure described here is more rapid than the ones used to purify mammal pancreatic phospholipases. In fact, ostrich PLA_2 was purified after only two chromatography steps, whereas in the case of dromedary

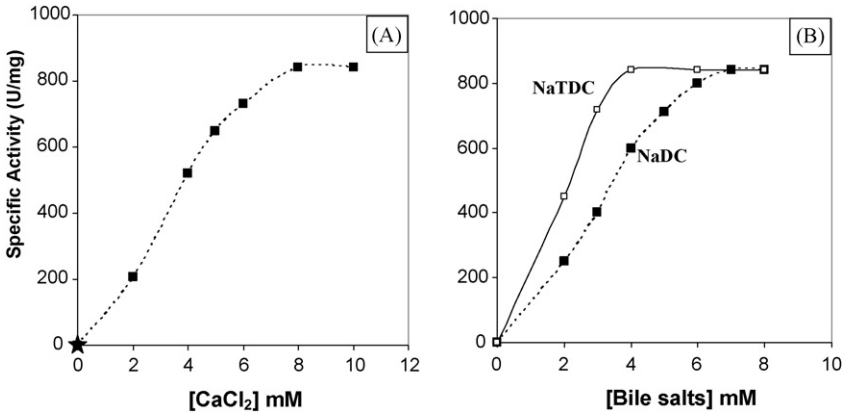

Fig. 2. (A) Effect of the concentration of Ca^{2+} on $OPLA_2$ activity. Enzyme activity was measured at various concentrations of Ca^{2+} using PC emulsion as substrate at pH 8.2 and at 37 °C in the presence of 4 mM NaTDC. The star indicates the phospholipase activity measured in the absence of $CaCl_2$ and in the presence of 10 mM EDTA or EGTA. (B) Effect of increasing concentration of bile salts: NaTDC (■) and NaDC (□) on $OPLA_2$ activity. PLA_2 activity was measured using PC emulsion as substrate at pH 8.2 and at 37 °C in the presence of 10 mM Ca^{2+}.

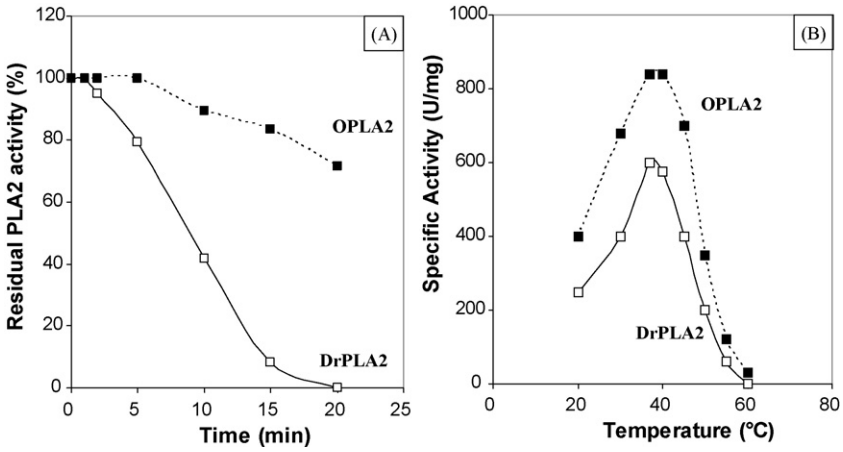

Fig. 3. Effects of temperature on $OPLA_2$ and $DrPLA_2$ stability (A) and activity (B). The enzyme activity was tested at various temperatures using PC emulsion as substrate in the presence of 10 mM Ca^{2+} and 4 mM NaTDC.

or porcine PLA_2 four chromatography steps are needed (two ionic exchange and two gel filtration chromatography steps) [20,29].

3.2. Enzymatic properties of the purified $OPLA_2$

3.2.1. Ca^{2+} dependence

Several studies have provided evidence that Ca^{2+} is essential for both, catalysis and enzyme binding to the substrate [37,38]. In this study, we measured the $OPLA_2$ activity at pH 8.2 and at 37 °C using PC emulsion as a substrate in the presence of increasing concentrations of Ca^{2+}. Our results show that PLA_2 activity cannot be determined in the presence of 10 mM of chelators such as EDTA or EGTA. In the absence of chelators, the specific activity of $OPLA_2$ increases to reach 840 U/mg measured on PC at 8 mM of Ca^{2+} (Fig. 2A). Similar result was obtained when egg-yolk emulsion was used as substrate. In agreement with previous findings with mammals' pancreatic PLA_2 [22–30], our study also attempted to show that bird PLA_2 require the presence of Ca^{2+} to trigger the hydrolysis of phospholipids emulsion.

3.2.2. Bile salts dependence

In order to investigate the effect of bile salts on $OPLA_2$ activity, the rate of hydrolysis of PC by $OPLA_2$ with various concentrations of bile salts, at pH 8.2 and at 37 °C, was studied. As shown in Fig. 2B, sodium Taurodeoxycholate (NaTDC) and sodium deoxycholate (NaDC) were specifically required for $OPLA_2$ activity. The maximum phospholipase activity was observed in the presence of 4 mM NaTDC or 7 mM NaDC. These observations corroborate with previous findings with mammals pancreatic PLA_2 [22–30].

3.2.3. Effect of temperature on $OPLA_2$ activity and stability

As has been established for many mammal pancreatic PLA_2s [22–29], PLA_2 purified from bird pancreas can tolerate the incubation at high temperature for long time. However, $OPLA_2$ was found, more resistant against temperature than the mammal ones. As shown in Fig. 3A, pure $OPLA_2$ maintained about 80% of its activity after 20 min incubation at 70 °C. For further comparison, we reported in the same Fig. 3A the results obtained with $DrPLA_2$. As we can see, the $DrPLA_2$ loses its full activity

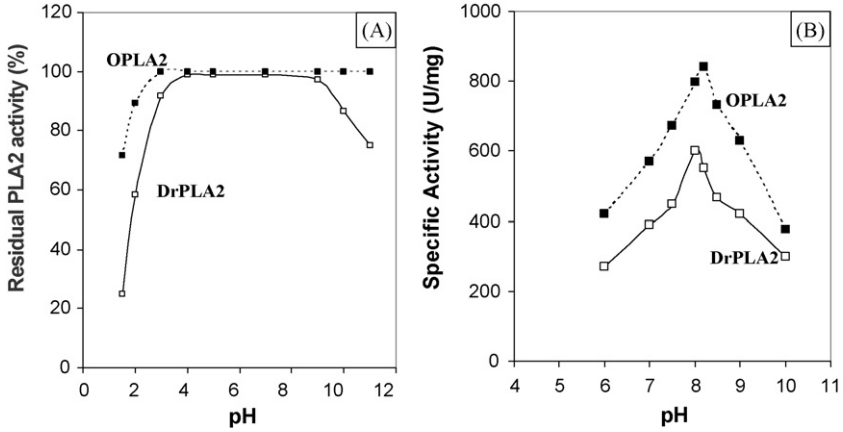

Fig. 4. Effects of pH on $OPLA_2$ and $DrPLA_2$ stability (A) and activity (B). The enzyme activity was tested at various pH using PC emulsion as substrate in the presence of 10 mM Ca^{2+} and 4 mM NaTDC.

when incubated under the same conditions at 70 °C. In the same manner, the $TPLA_2$ is not stable at high temperature since it is completely denaturated after incubation at 60 °C during a few minutes [30].

Fig. 3B shows that the highest activity of $OPLA_2$ was measured at 37–40 °C using PC emulsion as substrate in the presence of 10 mM Ca^{2+} and 4 mM NaTDC. Comparable results were obtained with mammal PLA_2 described so far [22–30].

3.2.4. Effect of pH on $OPLA_2$ activity and stability

In contrast to $DrPLA_2$, taken as a model of mammal PLA_2, which is not stable at pH less than 3, the pure $OPLA_2$ maintained more than 70% of its activity when incubated at pH 1.5 (Fig. 4A). Moreover, the $OPLA_2$ was found to be active between pH 2 and 11 during 10 min of incubation. However, the $TPLA_2$ loses its full activity when incubated at pH less than 5 for few minutes [30]. The maximal activity of $OPLA_2$ was measured at 8.2 (Fig. 5B) using PC emulsion as substrate in the presence of 8 mM Ca^{2+} and 4 mM NaTDC. Similar results were obtained with $DrPLA_2$ and $TPLA_2$ which require the presence of bile salts to trigger the hydrolysis of phospholipids [29,30].

3.2.5. Variations with surface pressure of the $OPLA_2$ activity

It is well established that the venom $sPLA_2$, which are very toxic, are able to hydrolyse phospholipids monolayer films even at high surface pressure (30–40 mN m^{-1}) and this allow them to attack biological membrane [39]. In order to check the ability of bird PLA_2 to penetrate and to hydrolyze phospholipids monolayer films, we reported the rate of hydrolysis of di C_{12}-PC films as a function of the surface pressure for the $OPLA_2$ (Fig. 5). To allow comparison, we reported in the same figure results obtained with $DrPLA_2$. As can be seen from Fig. 5, both bird and mammal pancreatic PLA_2 present a maximum activity with bell-shaped surface pressure curves. Based on the classification established by Verheij et al. [39], $OPLA_2$ and $DrPLA_2$ belong to group III of PLA_2 which includes some microbial and pancreatic PLA_2. Fig. 5 also shows that $OPLA_2$ hydrolyzes di C_{12}-PC monolayer less efficiently than $DrPLA_2$ does. The maximal ratio about 3.5 was found between catalytic activities of $OPLA_2$ and $DrPLA_2$, measured at their surface pressure optima (5 and 10 mN m^{-1}, respectively).

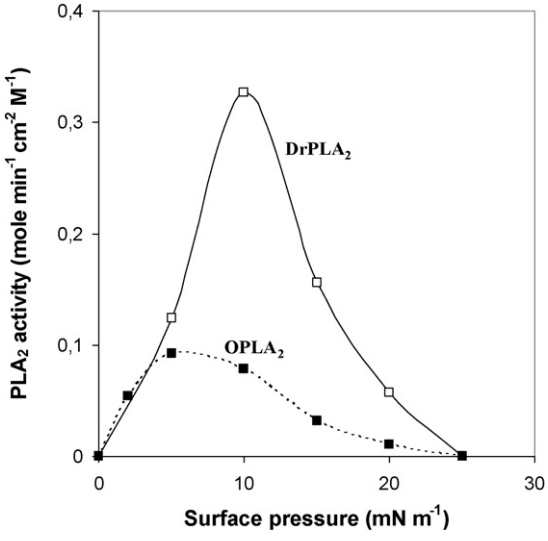

Fig. 5. Variation with surface pressure in $OPLA_2$ (■) and $DrPLA_2$ (□) activities measured on di C12-PC. Specific activity is expressed as the number of moles of substrates hydrolyzed by unit time and unit surface of the reaction compartment of the "zero-order" for an arbitrary lipase concentration of 1 M in the aqueous subphase of this compartment. Activity measurements were performed after 15 min of PLA_2 injection under di C_{12}-PC monomolecular films. The kinetic experiments were recorded as described in Section 2. PLA_2, final concentration in the reaction compartment (0.2 nM). Buffer: 10 mM Tris–HCl, pH 8.2, 150 mM NaCl, 21 mM $CaCl_2$, and 1 mM EDTA.

Table 2
Alignment of the N-terminal sequence of $OPLA_2$ with dromedary, bovine, pig, human and turkey pancreatic phospholipases

	1	5	10	15	20	
Ostrich	**AVWQF**	**REMIK**	**CTIPP**	**SDDLL**	**DF**	This study
Dromedary	**AL̲WQF**	**R*D*MIK**	**C*K*IP*D***	**S*SP*LL**	**DF**	[29]
Bovine	**AL̲WQF**	***NG*MIK**	**CKIP*S***	**S*EP*LL**	**DF**	[40]
Pig	**AL̲WQF**	**R*S*MIK**	**CTIP*G***	**SD*P*LL**	**DF**	[40]
Human	**AVWQF**	**R*K*MIK**	**C*V*IP*G***	**SD*P*F̲L**	E**Y̲**	[41]
Turkey	**AL̲F*E*F**	**R*S*MIK**	**CTIP*G***	**SD*PE*L**	**D**	[30]

For comparison, bold, underlined, and italic symbols of amino acids residues indicate identical, homologous, or different, respectively.

3.3. N-terminal sequence analysis of $OPLA_2$

Purified $OPLA_2$ was denaturated, reduced and alkylated as described in Section 2 and dialysed against distilled water. The NH_2-terminal sequencing of the $OPLA_2$ allowed unambiguously the identification of 22 residues of pure enzyme. The same sequences were obtained when the pure $OPLA_2$ was transferred without alkylation on a PVDF membrane. Result presented in Table 2 shows the N-terminal sequence, of $OPLA_2$, together with those of turkey [30], dromedary [29], pig [40], bovine [40] and human [41] PLA_2. N-terminal sequence of bird PLA_2 exhibits a high degree of homology with N-terminal sequences of mammal ones.

4. Conclusion

OPLA2 was purified to homogeneity from delipidated pancreases. The pure enzyme was not a glycosylated protein with a molecular mass of 13773.93 Da. The maximal PLA2 activity was measured at pH 8.2 and at 37–40 °C. Besides, this enzyme was found to be able to penetrate into phospholipids monolayer films. Pure OPLA2 maintained of about 80% of its activity after incubation during 20 min at 70 °C and it was found to be stable even at pH lower than 2. Moreover, bile salts and Ca^{2+} were required for pure PLA2 activity. Bird and mammal pancreatic PLA_2 share a high amino acid N-terminal sequences homology. However, further investigations are needed to better establish the relation structure–functions of the bird phospholipases which allowed us to distinguish the mammals from the bird ones.

Acknowledgments

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