Prostatic acid phosphatase degrades lysophosphatidic acid in seminal plasma

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Abstract **Lysophosphatidic acid (LPA) is a lipid mediator with multiple biological activities and is detected in various biological fluids, including human seminal plasma. Due to its cell proliferation stimulatory and anti-apoptotic activities, LPA has been implicated in the progression of some cancers such as ovarian cancer and prostate cancer. Here, we show that prostatic acid phosphatase, which is a non-specific phosphatase and which has been implicated in the progression of prostate cancer, inactivates LPA in human seminal plasma. Human seminal plasma contains both an LPA-synthetic enzyme, lysoPLD, which converts lysophospholipids to LPA and is responsible for LPA production in serum, and its major substrate, lysophosphatidylcholine. In serum, LPA accumulated during incubation at 37 °C. However, in seminal plasma, LPA did not accumulate. This discrepancy is explained by the presence of a strong LPA-degrading activity. Incubation of LPA with seminal plasma resulted in the disappearance of LPA and an accompanying accumulation of monoglyceride showing that LPA is degraded by phosphatase activity present in the seminal plasma. When seminal plasma was incubated in the presence of a phosphatase inhibitor, sodium orthovanadate, LPA accumulated, indicating that LPA is produced and degraded in the fluid. Biochemical characterization of the LPA-phosphatase activity identified two phosphatase activities in human seminal plasma. By Western blotting analysis in combination with several column chromatographies, the major activity was revealed to be identical to prostatic acid phosphatase. The present study demonstrates active LPA metabolism in seminal plasma and indicates the possible role of LPA signaling in male sexual organs including prostate cancer.**

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Abbreviations: PA, phosphatidic acid; LPA, lysophosphatidic acid; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; LPs, lysophospholipids; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; lysoPLD, lysophospholipase D; ATX, autotaxin; PS-PLA_1, phosphatidylserine-specific PLA_1; mPA-$PLA_1\alpha$, membrane-associated phosphatidic acid-selective $PLA_1\alpha$; $sPLA_2$, secretory PLA_2; GPCR, G protein-coupled receptor; EDG, endothelial cell differentiating gene; LCAT, lecithin-cholesterol acyltransferase; PAP, prostatic acid phosphatase; AP, alkaline phosphatase; PLAP, placental alkaline phosphatase; ESI, electrospray ionization; MS, mass spectrometry; G3P, glycerol-3-phosphate; MG, monoglyceride; SM, sphingomyeline; LPP, lipid phosphate phosphatase; C1P, ceramide-1-phosphate; S1P, sphingosine-1-phosphate

Keywords: Lysophosphatidic acid; Seminal plasma; LysoPLD; Lysophosphatidylcholine; Prostatic acid phosphatase

1. Introduction

Lysophosphatidic acid (LPA) is a simple lipid consisting of a phosphate, a glycerol, and a fatty acid [1,2]. In spite of its simple structure, LPA evokes various cellular responses in various cell types including cellular proliferation, prevention of apoptosis, cell migration, cytokine and chemokine secretion, platelet aggregation, smooth muscle contraction, and neurite retraction. LPA also induces transformation and cellular proliferation of smooth muscle cells [3]. Thus, LPA has been implicated in certain human diseases such as arteriosclerosis [3] and cancer cell invasion [4]. Most of these LPA actions are mediated by G protein-coupled receptors (GPCR) that are specific for LPA. At least four GPCRs have been identified so far (LPA_1/EDG2, LPA_2/EDG4, LPA_3/EDG7, and the recently identified GPR23/p2y9/LPA_4) [5]. The former three receptors share about 50% identities with each other at the amino acid level and form part of the endothelial differentiation gene (EDG) family. Studies of LPA receptor-null mice suggest that the receptors are needed for normal development [5].

LPA has been detected extracellularly in various biological fluids such as serum [6], saliva [7], follicular fluid [8], hen egg white [9] and ascites from ovarian cancer patients [10]. Among them, serum is the best characterized source of LPA [6,11]. Two major pathways for LPA production are known at present. LPA can be converted from pre-existing lysophospholipids (LPs) [1,11] or from phosphatidic acid (PA) generated by agonist stimulation in various types of cells [2]. We recently showed that LPA in serum is produced from LPs such as lysophosphatidylcholine (LPC) by the action of a plasma enzyme called lysophospholipase D (lysoPLD) (which is also called autotaxin, ATX/NPP2) [11–13]. In addition, LPs can be supplied by multiple routes [11]. In serum and plasma, LPC is produced from phosphatidylcholine (PC) on lipoprotein by a plasma enzyme called lecithin-cholesterol acyltransferase (LCAT). In activated platelets, and possibly other types of cells, LPs are produced from the corresponding diacyl phospholipids by phospholipase A-type (PLA) enzymes, such as secretory PLA_2 ($sPLA_2$) and phosphatidylserine-specific PLA_1 (PS-PLA_1). The mechanisms of LPA production in other biological fluids (saliva and ascites)

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are poorly understood. However, in the case of follicular fluid and hen egg white, LPA may be produced from LPC by lysoPLD activities [8,9].

Recently, we examined LPA activity in various biological fluids by measuring the LPA-induced increase in the intracellular concentration of calcium ion in three types of recombinant Sf9 insect cells, each expressing one of the LPA receptors [14,15]. Using this system, we found that seminal plasma contains LPA activity that is selective specific for LPA_3 [16]. LPA_3 is predominantly expressed in male and female sexual organs such as testis, prostate, uterus and oviduct [14,17], indicating a potential role of LPA_3 in reproductive organs. In this study, which is part of a series of studies to elucidate synthetic pathways of LPA in vivo, we attempted to clarify the enzymes involved in the LPA production in human seminal plasma. In the course of this study, we noticed that seminal plasma contains strong LPA-degrading activity in addition to LPA-producing activity. Here, we identify several enzymes that participate actively in LPA metabolism in seminal plasma.

2. Materials and methods

2.1. Materials

Mouse anti-human prostatic acid phosphatase (PAP) and mouse anti-placental alkaline phosphatase (PLAP) were purchased from PROGEN (Brisbane, Australia) and Lab Vision Corporation (Fremont, CA), respectively. 1-Oleoyl (18:1)-LPA, 1-oleoyl-LPC, and 1-myristoyl (14:0)-LPC were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). L-Homoarginine hydrochloride was purchased from Sigma (St. Louis, MO). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Generation of lysoPLD-specific monoclonal antibodies

A polypeptide (amino acid numbers 58–182 of human ATX) was expressed in *Escherichia coli* as a GST-fusion protein using the pGEX-4T vector (Amersham Bioscience). The protein was purified using GSH–Sepharose according to the manufacturer's protocol. The GST-fusion protein was used to immunize rats (WKY/Izm strain) into the hind footpads with Freund's complete adjuvant. The enlarged medial iliac lymph nodes from the rats were used for cell fusion with mouse myeloma cells, PAI. The antibody-secreting hybridoma cells were selected by screening with an enzyme-linked immunosorbent assay, immunofluorescence and Western blotting. We established three antibody-secreting hybridoma cell lines (2A12, 3D1 and 4F1).

2.3. Western blotting

Protein samples were separated by SDS–PAGE and transferred to nitrocellulose membranes using the Bio-Rad protein transfer system. The membranes were blocked with Tris-buffered saline containing 5% (w/v) skimmed milk and 0.05% (v/v) Tween 20, incubated with anti-human ATX/lysoPLD monoclonal antibodies and then treated with goat anti-rat IgG-horseradish peroxidase. Proteins bound to the antibodies were visualized with an enhanced chemiluminescence kit (ECL, Amersham Biosciences).

2.4. Preparation of various human biological fluids

Blood samples were drawn from healthy volunteers (aged 22–45). Human serum was prepared by allowing the blood to clot for 30 min at 37 °C and then centrifuging it at $2000 \times g$ for 20 min at 4 °C to remove the clot. Human seminal fluid, saliva, urine, sweat, and tear fluid were collected from healthy volunteers (aged 22–40). All samples were centrifuged to remove cell components and the resulting supernatants were immediately stored at −80 °C to prevent additional formation of LPA. Human seminal fluid was kept at room temperature for 30 min after collection. Seminal plasma was prepared by centrifuging the seminal fluid at $500 \times g$ for 10 min at 4 °C to remove the sperm. Cerebrospinal fluid was kindly donated by Dr. Hyeun-Wook Chang (College of Pharmacy, Yeungnam University, Gyonsan, Republic of Korea).

2.5. Quantification of LPA and LPC

Concentrations of LPA and LPC were determined by an enzyme-linked colorimetric method as described previously [18,19]. Glycerol 3-phosphate (G3P) was measured by a method similar to the method for measuring LPA as described [19]. The detection limits of our colorimetric assay for LPA and LPC are ~100 nM and ~5 μM, respectively.

2.6. LysoPLD assay

LysoPLD activity was assayed with an enzyme-based colorimetric method as described previously [12]. Briefly, samples (1–50 μl) were incubated with 2 mM 14:0-LPC in the presence of 100 mM Tris–HCl (pH 9.0), 500 mM NaCl, 5 mM $MgCl_2$, and 0.05% Triton X-100 for 1 h at 37 °C. The liberated choline was detected by an enzymatic photometric method using choline oxidase (Asahi Chemical, Tokyo, Japan), horseradish peroxidase (Toyobo, Osaka, Japan), and TOOS reagent (*N*-ethyl-*N*-(2-hydroxy-3-sulfoproryl)-3-methylaniline, Dojin, Tokyo, Japan) as a hydrogen donor.

2.7. Phosphatase assay toward LPA

Samples (1–20 μl) were incubated with 2 mM LPA (18:1) in a buffer containing 100 mM Tris–HCl, pH 7.4, at 37 °C for 60 min in a total volume of 40 μl. The production of monoglyceride was determined using Triglyceride E-test (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's protocol.

2.8. Mass spectrometry (MS)

MS spectra were obtained essentially as described previously [20] using a Quattro Micro tandem quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ion source (ESI). Lipid extracts from human seminal plasma were reconstituted in 2:1 chloroform/methanol (usually 100–300 μmol/l phosphorus); 2 μl of sample was injected per run. The samples were introduced by means of a flow injector into the ESI chamber, at the flow rate of 4 μl/min. The eluting solvent used was acetonitrile/methanol/water (2:3:1) containing 0.1% ammonium formate (pH 6.4). The mass spectrometer was operated in the positive and negative ion scan modes. The nitrogen drying gas flow rate was 12 liters/min and its temperature was 80 °C. Essentially, the capillary voltage was set at 3.7 kV and the cone voltage was set at 30 V, both in the positive and negative ion modes. For MS/MS experiments, $3–4 \times 10^4$ Torr of argon was used as collision gas, and a collision energy of 30–40 V was used for obtaining fragment ions for precursor ions.

2.9. Column chromatography

Seminal plasma (200 μl) was loaded onto a Superdex 200 gel filtration column (Amersham Biosciences) and the LPA-phosphatase activity was eluted with 100 mM Tris–HCl, pH 7.4. The pooled fractions containing LPA-phosphatase activities (peak II in Fig. 4A) were applied to a Bioassist Q anion exchange column (TOSOH, Kanagawa, Japan), which had been equilibrated with 100 mM Tris–HCl, pH 7.4, and the activities were eluted with a linear gradient of NaCl (0–2 M). Both columns were operated at 4 °C using ÄKTA (Amersham Biosciences).

3. Results

3.1. LysoPLD and LPC in human seminal plasma

To examine the mechanism underlying LPA production in human seminal plasma, we first tried to detect the lysoPLD that is responsible for LPA production in serum or incubated plasma. We found that among the various biological fluids tested, seminal plasma, cerebrospinal fluid, and serum had the highest lysoPLD activities (Fig. 1A). Unlike the serum and plasma samples, whose lysoPLD activities varied little, the seminal plasma samples had dramatically different activities (Fig. 1A). The activities were especially dependent on the interval between collections of seminal fluid and were propor-

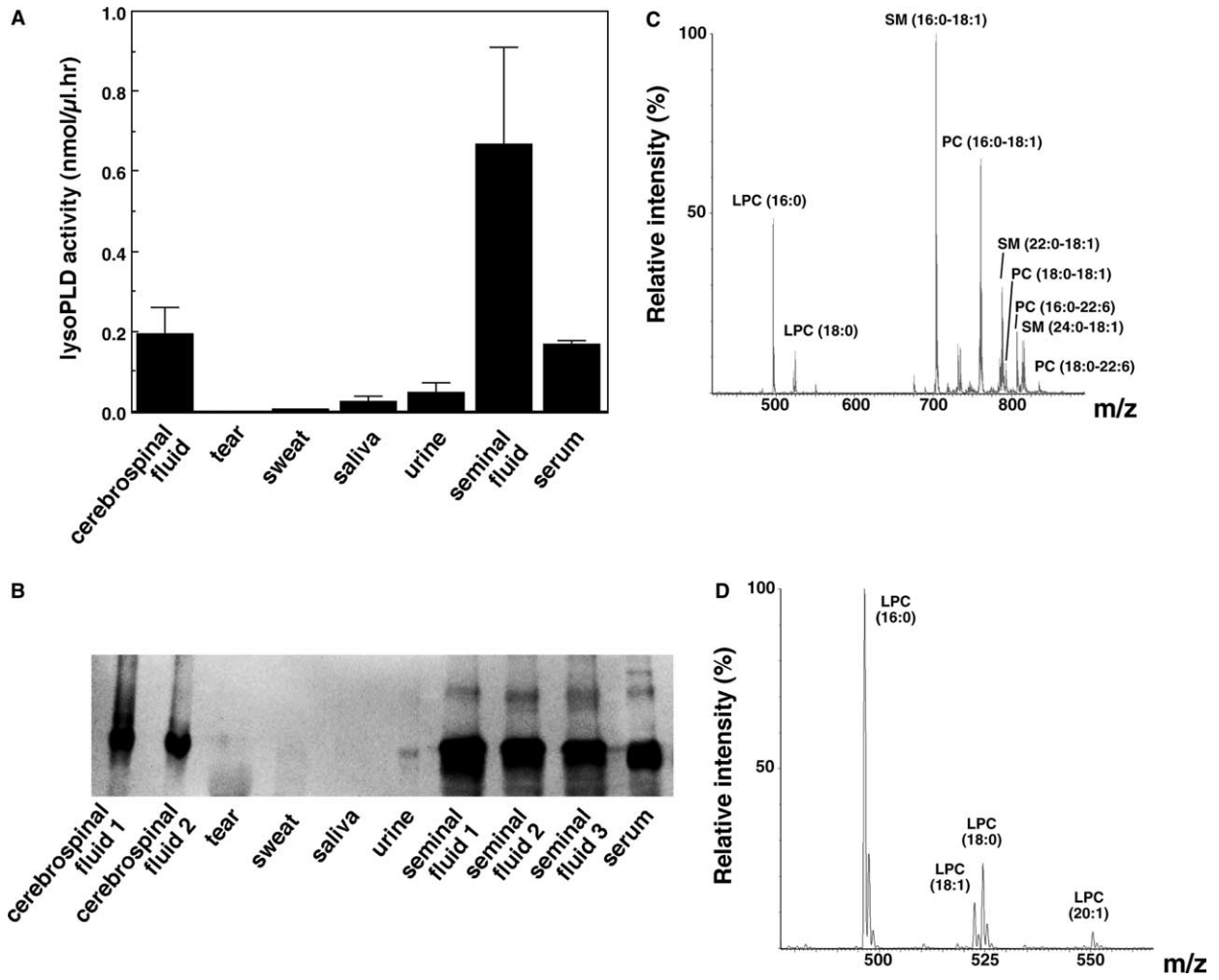

Fig. 1. Both lysoPLD and LPC are detected in seminal fluid. (A) LysoPLD activities of various human biological fluids (cerebrospinal fluid, tear, sweat, saliva, urine, seminal fluid, and serum) were determined using 14:0-LPC as a substrate. These data represent the means ± S.D. of three independent samples. (B) Detection of lysoPLD protein in various human biological fluids. Samples (each 5 μl) were subjected to Western blot analysis using anti-human lysoPLD monoclonal antibody (3D1). (C) Lipids of human seminal fluid were extracted by the method of Bligh & Dyer as described under Section 2 and then subjected to lipid analysis using ESI/MS. The lipid extract was directly infused into the ESI ion source using an Ultimate HPLC system (LC Packings) at a flow rate of 4 μl/min. (D) Magnification of the positive ion spectra in the mass region $m/z = 475$–560. The indicated molecular species were identified using tandem mass spectrometry.

tional to the protein concentration (data not shown), indicating that, in seminal fluid, lysoPLD is produced and released like other proteins. Western blot analysis using the anti-human lysoPLD monoclonal antibody established in this study confirmed that lysoPLD expression is high in seminal plasma (Fig. 1B). We also examined the expression of lysoPLD in sperm by Western blotting and found that it was expressed weakly in the cells (data not shown). LPC, a substrate of lysoPLD, was reported to be present in seminal plasma [21]. We confirmed the presence of LPC in human seminal plasma by MS. The MS analysis showed that phosphatidylcholine (PC), sphingomyeline (SM), and LPC are the major phospholipids in seminal plasma (Fig. 1C), confirming previous reports [21]. The major LPC species, 16:0-LPC and 18:0-LPC, were detected in seminal plasma with minor LPC components such as 18:1-LPC, 20:1-LPC (Fig. 1D). The LPC level in seminal plasma was found to be 8–19 μM using a recently developed enzyme-based colorimetric method. We also examined other phospholipases potentially involved in serum LPA production. In agreement with a previous report [22], we detected a high level of $sPLA_2$-IIA in human seminal plasma (data not shown). However, we did not detect phosphatidylserine-specific PS-PLA_1 [23] nor membrane-associated PA-selective PLA_1 (mPA-PLA_1) [24] (data not shown). Thus, it is reasonable to speculate that LPA in seminal plasma is produced from LPC by lysoPLD, as was observed in serum and incubated plasma.

3.2. Degradation of LPA in seminal plasma

LPA was found to rapidly accumulate in serum or plasma when these samples were incubated for a long period [1,11] and lysoPLD was found to be responsible for the LPA accumulation [11]. We, therefore, tested whether LPA accumulates in seminal plasma after a long incubation at 37 °C. However, unlike serum and plasma, seminal plasma did not accumulate LPA (Fig. 2A). To determine whether LPA is rapidly degraded in seminal plasma, we incubated LPA in the presence of seminal plasma. LPA was found to rapidly degrade in the

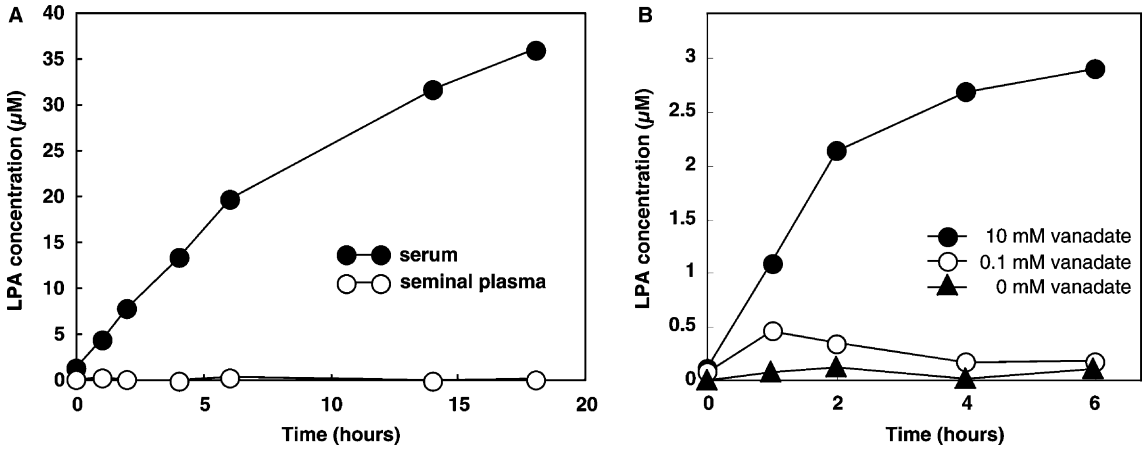

Fig. 2. Accumulation of LPA in incubated serum and seminal plasma. Human serum and seminal plasma were incubated at 37 °C for the indicated times (A). LPA concentrations were determined colorimetrically (see Section 2). In the case of seminal plasma, seminal plasma was incubated both in the presence and absence of sodium orthovanadate (B).

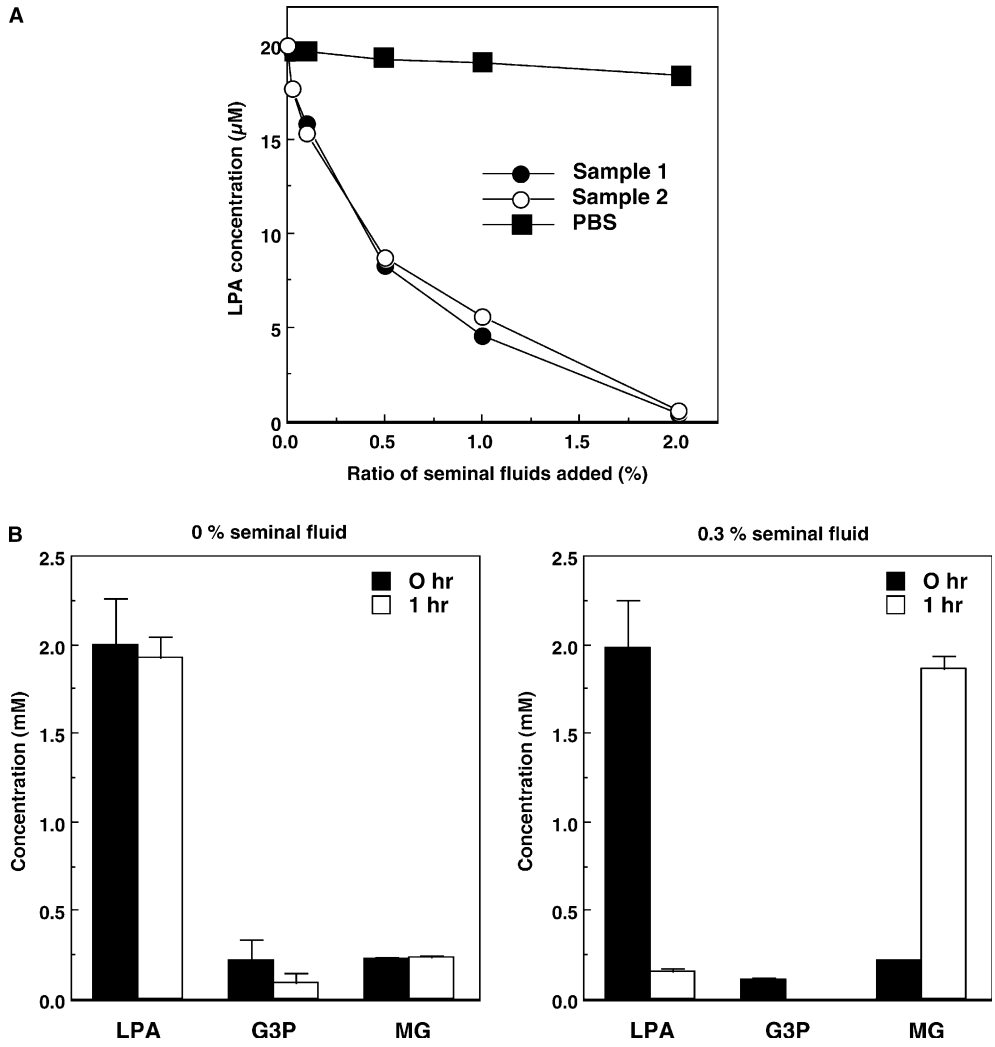

Fig. 3. Phosphatase activity is responsible for LPA degradation in seminal fluid. (A) LPA (20 μM) was incubated at 37 °C in the presence or absence of various concentrations of human seminal fluid. After 30 min, LPA concentration was determined by a colorimetric assay. (B) LPA (18:1; 2 mM) was incubated at 37 °C for 1 h in the presence and absence of 0.3% (v/v) human seminal fluid. The concentrations of LPA, G3P and MG were determined by an enzyme-linked colorimetric assay as described in Section 2. These data represent the means ± S.D. of three independent samples.

presence of seminal plasma. Indeed, 20 μM LPA completely disappeared in 30 min when LPA was incubated with 2% (v/v) seminal plasma at 37 °C (Fig. 3A).

3.3. LPA is degraded by phosphatase in seminal plasma

We, thus, focused on the mechanism of LPA degradation in seminal plasma. To know how LPA is degraded in seminal

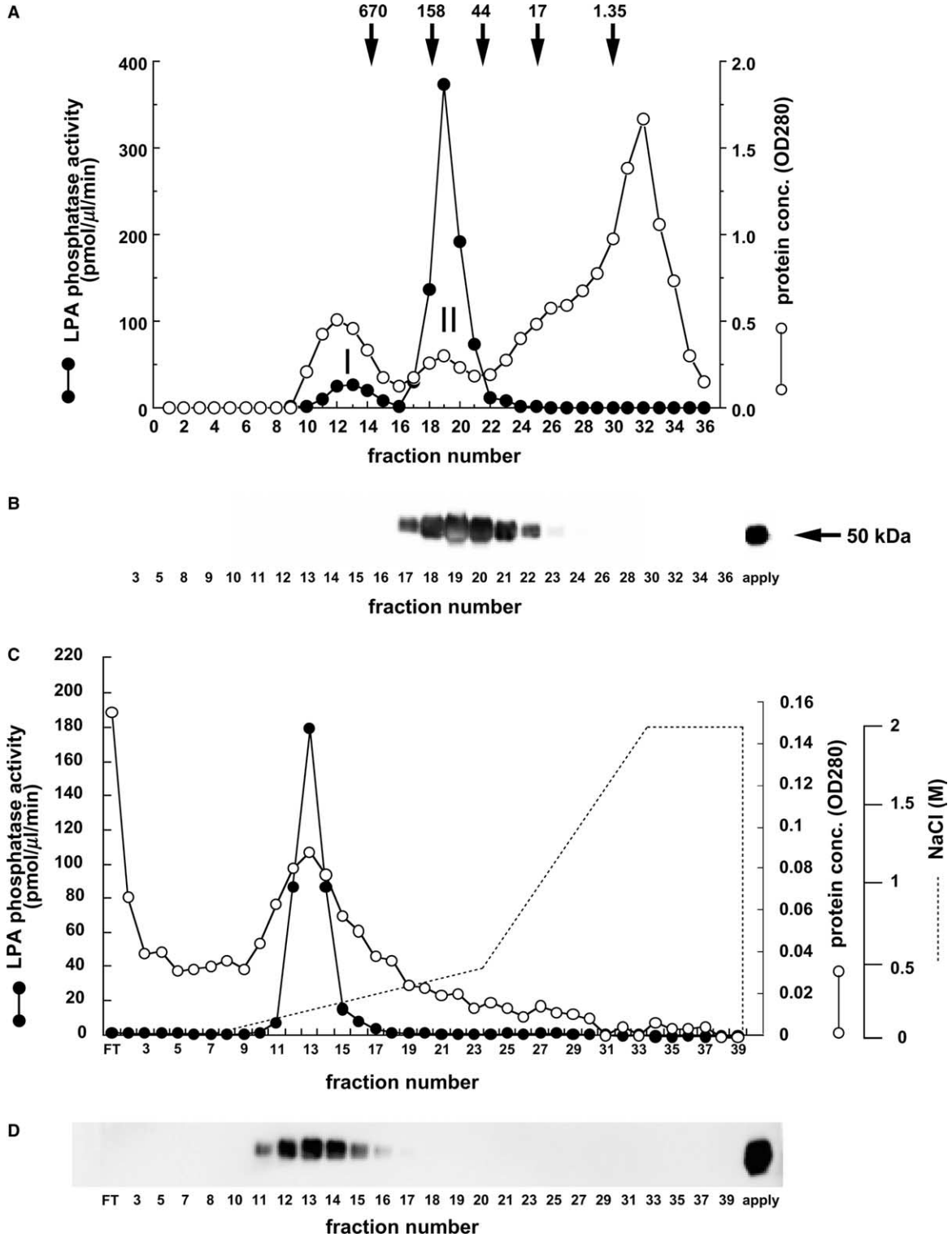

Fig. 4. Elution profiles of LPA-phosphatase activity and human prostatic acid phosphatase on gel filtration (A, B) and anion exchange column chromatography (C, D). (A, B) Human seminal plasma (200 μl) was subjected to Superdex 200 gel filtration column chromatography. For each fraction, phosphatase activity toward LPA was determined (A). Presence of PAP was also determined by Western blot analysis using mouse anti-human PAP monoclonal antibodies (B). (C, D) The active fractions of gel filtration column chromatography (fractions 17–22 in (A)) were further subjected to Bioassist Q anion exchange column chromatography and proteins were eluted by a linear gradient of sodium chloride. For each fraction, phosphatase activity toward LPA (C) and the presence of PAP (D) were determined.

plasma, we analyzed the products of the reaction. For this purpose, LPA was incubated in the presence of 0.3% (v/v) of seminal plasma and LPA and possible degradation products such as G3P and monoglyceride (MG) were analyzed by colorimetric assay. As shown in Fig. 3B, incubation of LPA with seminal plasma resulted in a decrease in LPA and a concomitant accumulation of MG but not G3P. This clearly indicates that LPA is degraded by phosphatase activity in seminal plasma. We, therefore, tested the effect of a phosphatase inhibitor on LPA accumulation in seminal plasma. We chose orthovanadate that is often used as an inhibitor for a broad range of phosphatases [25]. When we incubated seminal plasma in the presence of 10 mM sodium orthovanadate, we did observe an accumulation of LPA (Fig. 2B). The presence of LPA after incubation in the presence of orthovanadate was also confirmed by a bioassay in which recombinant LPA_3 activation was evaluated by transient intracellular calcium mobilization (data not shown). Thus, we conclude that phosphatase activity is responsible for LPA degradation in seminal plasma and that LPA is actually produced and degraded in seminal plasma.

3.4. *Biochemical characterization of LPA-phosphatase in seminal plasma*

To identify the phosphatase activity toward LPA, we next biochemically characterized the LPA-phosphatase in seminal plasma. Seminal plasma was subjected to gel filtration chromatography and the fractions were assayed for LPA phosphatase activity. One major peak (Fig. 4A, peak II) and one minor peak (peak I) were obtained, with estimated molecular weights of 150 kDa and 650 kDa, respectively. The activity of the major peak was highest in the pH range 4–7 (data not shown).

In seminal plasma, several isoforms of phosphatase have been detected and identified. Western blot analysis using a monoclonal antibody against human PAP [26] detected a 55-kDa band that co-migrated with LPA-phosphatase activity on both the gel filtration column (Fig. 4A and B) and a Bioassist Q column (Fig. 4C and D). This indicates that PAP is the major phosphatase that degrades LPA in human seminal plasma. Neither the anti-PAP antibody nor a monoclonal antibody against PLAP [27] reacted with the minor phosphatase peak (peak I in Fig. 4A) (data not shown).

Alkaline phosphatase (AP, EC 3.1.3.1), an enzyme which catalyzes hydrolysis of organic phosphate at basic pH values, has been reported in human seminal fluid [28]. Four APs, each encoded by a different gene, have been identified [28]. These APs (tissue non-specific, intestinal, placental, and placental-like AP) can be distinguished by their sensitivities to heat and certain L-amino acids. Heating is reported to destroy non-placental enzymes, while leaving placental and placental-like enzymes essentially unaffected [28]. Heat treatment (for 15 min at 56 °C or for 10 min at 65 °C) reduced the LPA-phosphatase activity of peak I by only 25–30% while it nearly abolished the activity of peak II (Fig. 5A). L-Phenylalanine and L-leucine are reported to inhibit the intestinal, placental and placental-like isoenzymes [25]. L-Homoarginine is reported to inhibit the tissue non-specific isoenzymes [25]. While L-leucine significantly reduced the phosphatase activity of peak I by 75%, L-phenylalanine and L-homoarginine did not markedly reduce the phosphatase activity of both peaks (Fig. 5B). Tartrate, which is an effective inhibitor of PAP [29], markedly reduced the phosphatase activity values of both peaks (Fig. 5B). The phosphatase activity of peak II was more sensitive to EDTA than that of peak I (Fig. 5B). The different temperature, leucine and EDTA sensitivities of the two LPA-phosphatase activities indicate that these activities are due to different isozymes. And the fact that the sensitivities of peak I activity are not consistent with those of any of the four known APs indicates that the peak I activity is due to a novel phosphatase isozyme.

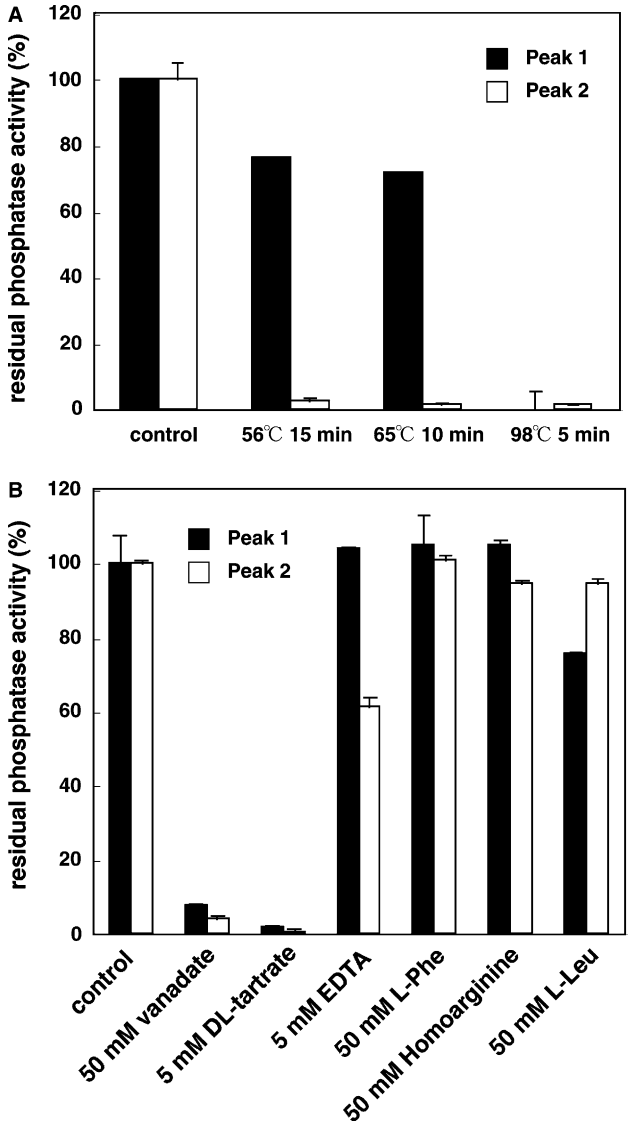

Fig. 5. Biochemical characterization of two LPA-phosphatases detected in human seminal fluid (A) Heat sensitivity of the two LPA-phosphatase activities detected in seminal plasma. Fractions containing LPA-phosphatase activities eluted from gel filtration column chromatography (peaks I and II) were heat-treated at the indicated temperature for the indicated period. The remaining phosphatase activities of the samples were then determined. These data represent the means ± S.D. of three independent experiments. (B) Effects of several phosphatase inhibitors on the two LPA-phosphatase activities. The LPA-phosphatase activities of the two peaks (peaks I and II on gel filtration column chromatography) were determined in the presence of inhibitors at the indicated concentrations. These data represent the means ± S.D. of three independent experiments.

4. Discussion

4.1. LysoPLD is involved in LPA production in various biological fluids including seminal plasma

We previously showed that serum LPA is produced from LPs such as LPC by the action of a plasma enzyme called lysoPLD [11]. Interestingly, lysoPLD is identical to a cell motility-stimulating factor, ATX [12,13]. ATX has been implicated in cancer invasion and metastasis, because it is sometimes overexpressed in various cancer tissues [30]. The present study was undertaken as continuing studies to examine a role of lysoPLD/ATX in LPA production in vivo. Our Western blot analysis demonstrated that lysoPLD/ATX is widely distributed in various biological fluids such as cerebrospinal fluid and seminal plasma in addition to serum (Fig. 1B). Interestingly, LPC, a major substrate of lysoPLD/ATX, was detected in these biological fluids (Fig. 1C and D). Thus, lysoPLD/ATX may be involved in LPA production in various biological fluids other than serum and plasma. It is particularly interesting to note that the expression of lysoPLD/ATX is extremely high in seminal plasma (Fig. 1A and B). We previously showed that LPA is present in seminal plasma, because seminal plasma induces transient $[Ca^{2+}]_i$ in LPA_3-expressing insect Sf9 cells [16]. We detected LPC in seminal plasma (Fig. 1C and D), but hardly detected LPA by our enzymatic colorimetric assay (Fig. 2A). The minimum concentrations of LPA in our colorimetric assay and bioassay for LPA-detecting system are ~100 and ~10 nM, respectively. This explains why we could detect LPA-activity only by bioassay.

4.2. Active LPA metabolism in seminal plasma

The LPA level in seminal plasma was low, but increased when seminal plasma was incubated in the presence of sodium orthovanadate, a classical phosphatase inhibitor (Fig. 2B). The level of choline, a reaction product of lysoPLD from LPC, in seminal plasma is extremely high (~mM order) [31]. Strong phosphatase activity against LPA was detected in seminal plasma (Fig. 3A). All these results support the hypothesis that LPA is continuously produced and degraded in seminal plasma. Because the major LPC species in seminal plasma were 16:0- and 18:0-LPC (Fig. 1D), PLA_2 may be responsible for the LPC production in seminal plasma. We detected PC (Fig. 1C) and $sPLA_2$IIA (data not shown) in seminal plasma, which could be potential sources of LPC in seminal plasma.

4.3. Potential role of LPA in seminal plasma

Seminal fluid is derived from at least three organs; prostate, seminal vesicle and testis. Fluids from each organ are mixed just before the ejaculation. It is clear that PAP is derived from the prostate [29], while it has not been determined where lysoPLD and LPC are derived from mRNA of lysoPLD/ATX is detected both in prostate and testis in both humans and mice, indicating that these organs are potential sources of lysoPLD/ATX. Interestingly, lysoPLD activity, LPC content and LPA-phosphatase activity differ in various samples. It should be noted that lysoPLD activity in seminal plasma from same individuals is dependent on the intervals between seminal fluid collections (data not shown). In addition, the ratios of LPC and lysoPLD differ among the samples. These results indicate that lysoPLD and LPC are derived from different organs.

The physiological role of LPA in seminal fluid is not known. So far, at least four LPA receptors have been identified and characterized [5]. These LPA receptors are highly expressed in several male sexual organs such as testis and prostate and in female sexual organs such as uterus, oviduct and ovary. LPA in seminal fluid may have a role in such male organs. In addition, it may have a role in female organs such as uterus and oviduct after semen is ejaculated. Interestingly, Kunikata et al. [32] reported that LPA stimulates mouse ovum transport by contracting oviductual smooth muscle mediated by a pertussus toxin-sensitive GPCR, possibly LPA_{1-4}. Recently, Yue et al. [33] demonstrated that mice with transgenic overexpression of one isoform of an LPA-degradation enzyme, lipid phosphate phosphatase-1 (LPP-1), display multiple organotypic deficits including abnormal testis development that results in a severely impaired spermatogenesis. This also implies that LPA has a role in signaling in sexual organs.

4.4. Potential role of PAP in prostate and prostate cancer

LPA has been shown to induce the growth and survival of cancer cells [4]. Thus, LPA has been implicated in cancer cell development, especially in prostate cancer cells [34,35]. Expression of LPA receptors (LPA_1, LPA_2 and LPA_3) and LPA-producing enzymes (lysoPLD and mPA-PLA_1) has been observed in prostate cells or prostate cancer cells. PAP (EC 3.1.3.2) is a non-specific phosphomonoesterase that is synthesized and secreted into seminal plasma under androgenic control [29]. The enzyme is a dimer of molecular weight around 100 kDa. PAP was formerly used as a marker for diagnosis and therapy control of prostate cancer [29]. However, its role as a prostate cancer marker has now been taken over by prostate-specific antigen. In this study, we identified PAP as the main LPA-phosphatase in seminal plasma, although a less active phosphatase was also detected (Fig. 4). The estimated molecular weight, heat stability, and inhibitory patterns by several phosphatase inhibitors of the minor phosphatase (Fig. 5) indicate that it is a novel phosphatase specific to LPA, which should be identified in further studies.

Lipid phosphate such as PA, ceramide 1-phosphate (C1P), sphingosine 1-phosphate (S1P) and LPA can be substrates for a family of enzymes called LPPs [36]. The LPPs can dramatically alter the balance between phosphate esters and their dephosphorylated products [36]. The LPPs are integral membrane proteins and the "ecto-activity" of the LPPs negatively regulates the extracellular actions of LPA and S1P [36]. We propose here that soluble enzymes also participate in regulating the extracellular actions of lipid phosphates and that PAP is one candidate for a degrading enzyme. It has been demonstrated that human prostate cancer cells show a decreased level of expression of the cellular form of the enzyme [37]. Several lines of evidence indicate that cellular PAP is critical for regulating proliferation and androgen responsiveness of human prostate cancer cells: (i) LNCaP cells that express cellular PAP have a slow growth rate, compared with PC-3 and DU-145 cells that lack endogenous PAP expression [38]. (ii) Introduction of cellular PAP into prostate cancer cells results in a decreased growth rate [38]. (iii) The cellular form of PAP is also involved in regulating the androgen-stimulated growth of prostate cells [39]. C-33 LNCaP cells that express PAP and

androgen receptor are responsive to androgen stimulation, whereas C-81 LNCaP and PC-3 prostate carcinoma cells that express functional androgen receptor but lack PAP expression are androgen-unresponsive. (iv) Androgen responsiveness of these cell lines can be restored by reintroducing cellular PAP expression [39]. Thus, we propose that PAP has a role in inhibiting the proliferation of prostate cells by negatively regulating the LPA level. The lower expression of PAP in prostate cancer may result in constitutive activation of LPA receptors and lead to the progression of prostate cancer. Further studies are needed to prove this hypothesis.

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References

[1] Tokumura, A. (1995) Prog. Lipid Res. 34, 151–184.
[2] Tigyi, G. and Parrill, A.L. (2003) Prog. Lipid Res. 42, 498–526.
[3] Yoshida, K., Nishida, W., Hayashi, K., Ohkawa, Y., Ogawa, A., Aoki, J., Arai, H. and Sobue, K. (2003) Circulation 108, 1746–1752.
[4] Mills, G.B. and Moolenaar, W.H. (2003) Nat. Rev. Cancer 3, 582–591.
[5] Anliker, B. and Chun, J. (2004) J. Biol. Chem. 279, 20555–20558.
[6] Tigyi, G. and Miledi, R. (1992) J. Biol. Chem. 267, 21360–21367.
[7] Sugiura, T., Nakane, S., Kishimoto, S., Waku, K., Yoshioka, Y. and Tokumura, A. (2002) J. Lipid Res. 43, 2049–2055.
[8] Tokumura, A., Miyake, M., Nishioka, Y., Yamano, S., Aono, T. and Fukuzawa, K. (1999) Biol. Reprod. 61, 195–199.
[9] Nakane, S., Tokumura, A., Waku, K. and Sugiura, T. (2001) Lipids 36, 413–419.
[10] Xu, Y., Gaudette, D.C., Boynton, J.D., Frankel, A., Fang, X.J., Sharma, A., Hurteau, J., Casey, G., Goodbody, A., Mellors, A. and Mills, G.B. (1995) Clin. Cancer Res. 1, 1223–1232.
[11] Aoki, J., Taira, A., Takanezawa, Y., Kishi, Y., Hama, K., Kishimoto, T., Mizuno, K., Saku, K., Taguchi, R. and Arai, H. (2002) J. Biol. Chem. 277, 39696–39702.
[12] Goto-Umezu, M., Kishi, Y., Taira, A., Hama, K., Dohmae, N., Takio, K., Yamori, T., Mills, G.B., Inoue, K., Aoki, J. and Arai, H. (2002) J. Cell Biol. 158, 227–233.
[13] Tokumura, A., Majima, E., Kariya, Y., Tominaga, K., Kogure, K., Yasuda, K. and Fukuzawa, K. (2002) J. Biol. Chem. 277, 39436–39442.
[14] Bandoh, K., Aoki, J., Hosono, H., Kobayashi, S., Kobayashi, T., Murakami, M.K., Tsujimoto, M., Arai, H. and Inoue, K. (1999) J. Biol. Chem. 274, 27776–27785.
[15] Bandoh, K., Aoki, J., Taira, A., Tsujimoto, M., Arai, H. and Inoue, K. (2000) FEBS Lett. 478, 159–165.
[16] Hama, K., Bandoh, K., Kakehi, Y., Aoki, J. and Arai, H. (2002) FEBS Lett. 523, 187–192.
[17] Contos, J.J. and Chun, J. (2001) Gene 267, 243–253.
[18] Kishimoto, T., Soda, Y., Matsuyama, Y. and Mizuno, K. (2002) Clin. Biochem. 35, 411–416.
[19] Kishimoto, T., Matsuoka, T., Imamura, S. and Mizuno, K. (2003) Clin. Chim. Acta 333, 59–67.
[20] Hiramatsu, T., Sonoda, H., Takanezawa, Y., Morikawa, R., Ishida, M., Kasahara, K., Sanai, Y., Taguchi, R., Aoki, J. and Arai, H. (2003) J. Biol. Chem. 278, 49438–49447.
[21] Schiller, J., Arnhold, J., Glander, H.J. and Arnold, K. (2000) Chem. Phys. Lipids 106, 145–156.
[22] Takayama, K., Hara, S., Kudo, I. and Inoue, K. (1991) Biochem. Biophys. Res. Commun. 178, 1505–1511.
[23] Nagai, Y., Aoki, J., Sato, T., Amano, K., Matsuda, Y., Arai, H. and Inoue, K. (1999) J. Biol. Chem. 274, 11053–11059.
[24] Sonoda, H., Aoki, J., Hiramatsu, T., Ishida, M., Bandoh, K., Nagai, Y., Taguchi, R., Inoue, K. and Arai, H. (2002) J. Biol. Chem. 277, 34254–34263.
[25] Crans, D., Simone, C., Saha, A. and Glew, R. (1989) Biochem. Biophys. Res. Commun. 165, 246–250.
[26] Svanholm, H. (1986) Acta Pathol. Microbiol. Immunol. Scand. 94, 7–12.
[27] Durbin, H., Tucker, D., Milligan, E., Bobrow, L., Warne, P., Pookim, Y. and Bodmer, W. (1988) Int. J. Cancer Suppl. 2, 50–58.
[28] Harris, H. (1989) Clin. Chim. Acta 186, 133–150.
[29] Ostrowski, W.S. and Kuciel, R. (1994) Clin. Chim. Acta 226, 121–129.
[30] Stracke, M.L., Clair, T. and Liotta, L.A. (1997) Adv. Enzyme Regul. 37, 135–144.
[31] Takatori, T., Tomii, S. and Tanaka, T. (1981) Forensic Sci. Int. 17, 79–84.
[32] Kunikata, K., Yamano, S., Tokumura, A. and Aono, T. (1999) Life Sci. 65, 833–840.
[33] Yue, J., Yokoyama, K., Balazs, L., Baker, D.L., Smalley, D., Pilquil, C., Brindley, D.N. and Tigyi, G. (2004) Cell Signal. 16, 385–399.
[34] Daaka, Y. (2002) Biochim. Biophys. Acta. 1582, 265–269.
[35] Sakamoto, S., Yokoyama, M., Zhang, X., Prakash, K., Nagao, K., Hatanaka, T., Getzenberg, R.H. and Kakehi, Y. (2004) Endocrinology 145, 2929–2940.
[36] Brindley, D., English, D., Pilquil, C., Buri, K. and Ling, Z. (2002) Biochim. Biophys. Acta 1582, 33–44.
[37] Lin, M., Lee, M., Zhou, X., Andressen, J., Meng, T., Johansson, S., West, W., Taylor, R., Anderson, J. and Lin, F. (2001) J. Urol. 166, 1943–1950.
[38] Lin, M., Garcia, A.R., Xia, X., Biela, B. and Lin, F. (1994) Differentiation 57, 143–149.
[39] Lin, M.F., Meng, T.C., Rao, P.S., Chang, C., Schonthal, A.H. and Lin, F.F. (1998) J. Biol. Chem. 273, 5939–5947.